

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Pregnancy exposure to air pollution and early childhood respiratory health in the Norwegian Mother and Child Cohort Study (MoBa)
AUTHORS	Madsen, Christian; Håberg, Siri; Magnus, Maria; Aamodt, Geir; Stigum, Hein; London, Stephanie J.; Nystad, Wenche; Nafstad, Per

VERSION 1 – REVIEW

REVIEWER	Ulrike Gehring Institute for Risk Assessment Sciences, Utrecht University The Netherlands
REVIEW RETURNED	24-Jan-2017

GENERAL COMMENTS	<p>Summary</p> <p>The paper describes the associations of maternal exposure to ambient air pollution (NO₂) during pregnancy with lower respiratory tract infections (LRTI) and wheeze up to the age of 18 months in the Norwegian MoBa cohort. Overall, there was no association between NO₂ exposure and the outcomes studied. However, wheezing was associated with NO₂ in girls, but not in boys.</p> <p>General comments</p> <p>This is an interesting paper based on a large dataset describing associations between LRTI and air pollution exposure at low levels of exposure. A major limitation of the paper in its current form is the cross-sectional analysis. Moreover, the discussion should be more focused (see specific comments below).</p> <p>Specific comments</p> <ul style="list-style-type: none">• Highlights, 3rd bullet: This suggests that longitudinal analyses have been performed. However, this is not the case. Two sets of cross-sectional analyses were performed for health outcomes during the first 6 months of life and between age 6 and 18 months. This needs to be corrected or preferably, cross-sectional analyses should be replaced by longitudinal analyses (e.g. GEEs), which are more appropriate in prospective cohort studies with repeated follow-ups.• Introduction, page 4, line 31ff, effects of maternal smoking during pregnancy and respiratory health . I suggest to focus on ambient air pollution here and delete text on smoking OR to motivate why this is relevant here.• Introduction, page 4, last paragraph, air pollution exposure assessment. I suggest to move the text on personal exposure assessment vs exposure modeling, strengths and limitations of the different approaches to the discussion. You may wish to mention also that personal exposure assessment usually not feasible in large population studies. Text on proximity to traffic can be deleted unless
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	<p>this is relevant for the comparison with other studies. In that case this should be moved to the discussion.</p> <ul style="list-style-type: none">• Introduction, page 6, 1st paragraph. I personally would skip the sentence saying that this is the first Norwegian study. This is not a very strong selling point. The fact that air pollution levels are low in Norway is a much better selling point.• Page 8, statistical analysis. The two sets of cross-sectional analyses at ages 6 months and 6-18 months should be replaced by longitudinal analyses of the (repeated measures) of the outcomes of interest making full use of the longitudinal data and accounting for the correlation between observations within subjects.• Page 8, statistical analysis. Parity is mentioned in the sentence about multiplicative interactions, but not in the sentence about stratified analyses, while all other variables are mentioned in both. This is inconsistent.• Page 8, statistical analysis. It is not clear to me how the authors accounted for the fact that some women contributed multiple pregnancies to the cohort. The observations from siblings are likely not independent. This needs to be taken into account in the analysis.• Page 8, statistical analysis. All analyses were adjusted for “area” (Oslo, Bergen, Akershus and Hordaland). To what extent do air pollution levels differ between areas? This should be clarified by adding descriptives of air pollution levels by study area. Since the study areas include both urban and rural areas, I would expect air pollution levels to be different. Consequently, adjustment for study area may result in overadjustment. Therefore, analyses with and without adjustment for study area should be presented and differences including potential overadjustment should be discussed.• Results. See my comment above. Please add descriptive analyses of air pollution levels by study area.• Discussion. The first part of the discussion, where results of the present study are compared with findings of other studies needs better structure. I suggest to start with studies that investigated associations of LRTI and/or wheeze with air pollution and focus on the ones that are comparable with regard to the age of the study participants. If these are few, also include relevant studies among older children. When referring to other studies, please make sure that the direction of the observed effects is always clear.• Since the focus of the present study is on LRTI, I suggest to remove text on other outcomes such as asthma and lung function from the discussion and focus on LRTI.• Page 10, last paragraph “Living outside the city...” I think this sentence needs to be revised as in its current form it compares risks of LRTI between urban and non-urban study areas while it should be about air pollution effects in urban and non-urban study areas – at least this is what I conclude from the sentence following that sentence. Please double check.• Same paragraph, the statement about higher mobility and less time spent at home needs to be supported by a reference.• The authors discuss exposure misclassification due to residential mobility. Differences in time-activity patterns may be another source of exposure misclassification when relying on residential addresses solely that needs to be discussed in more detail (currently it is only briefly mentioned on page 10). Do you have information about the percentage of women working outside their own home during pregnancy? Is information regarding work addresses available? If yes, do results change if work address exposures are included? If no, the lack of information on exposures at work and other addresses needs to be discussed.
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REVIEWER	Marisa Estarlich and Amparo Ferrero Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Madrid, Spain Epidemiology and Environmental Health Joint Research Unit, FISABIO–Universitat Jaume I–Universitat de València, Avenida de Catalunya 21, 46020 Valencia, Spain
REVIEW RETURNED	06-Feb-2017

GENERAL COMMENTS	<p>Major comments</p> <p>I think that the methodology for exposure assessment is not adequately clarified in the manuscript, the authors should explain it better. How many monitoring points were used? Which NO₂ levels were obtained? Which GIS variables were included in the LUR models? This data gives information about the "validity" of the air pollution measurements.</p> <p>Moreover, the authors should mention that the NO₂ levels were collected during 2010 and 2011 and GIS variables were collected in 2013 in the limitations section.</p> <p>I do not understand why the authors replaced the negative modelled exposures levels by 0.01. Is it the detection limit?</p> <p>The authors said that the correlations between exposures in different trimesters of pregnancy are very high. In any case, associations between exposures in different trimesters and respiratory health could have been explored in the study. Ritz and cols identified the importance of studying the critical periods of exposure (Ritz B, Wilhelm M. Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. <i>Basic Clin Pharmacol Toxicol.</i> 2008 Feb;102(2):182-90. doi: 10.1111/j.1742-7843.2007.00161.x.)</p> <p>The statistical methodology is not clear. Were the analyses adjusted by area or were the results from different centers meta-analysed? Regarding sensitivity analyses, it is worthwhile to note that stratified analyses are generally done subsequently to the interaction test. I.e., if interaction term is statistically significant you can proceed with stratified analyses. Otherwise, stratified analyses have not much sense.</p> <p>The authors restrict the analysis to pregnancies during the last period of the MoBa recruitment; I suppose that this is due to the problem of misclassification. The authors should discuss about this and include it in the limitations section.</p> <p>The authors comment "Area variable may reflect the spatial distribution of air pollution, and therefore a separate analysis was performed in adjusted model without the area variable". I suppose that you have said it is important to adjust the models by area since differences between areas exist. Are covariates different?</p>
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I think that would be also interesting to know the relationship between NO₂ exposure and LRTI of 0-18 . Furthermore, I am interested in the longitudinal analysis, that is, the authors could build a variable: 0: not LRTI, 1: LRTI only 0-6 months, 2: LRTI only 6-18, and 3: LRTI in both periods (incidence and persistent cases).

I do not understand this sentence: "Living outside the city was associated with higher risk of developing LRTI and wheeze, although the estimates were not statistically significant. This might be due to a higher misclassification of exposure in cities because of higher mobility and less staying at home. On the other hand, non-city population might have more homogenous exposure and spending more time close to home". What is the reason to make that claim? Do the authors have time-activity data?

The authors do not mention other limitations in the methodology of exposure assessment, such as not considering other air pollution sources in indoor air (e.g., gas cooking) or other relevant outdoor pollutants.

Minor comments:

Abstract:

LRTI is already defined in introduction section. It is not necessary to include it in the results section.

In the section "What this paper adds", English should be checked: Analyse the longitudinal effect of...? or Analyse longitudinally the effect...?

Introduction:

The authors have commented "Norway is characterized by relatively low levels of air pollution". They could mention the article published by Cyrus about NO₂ levels in different European areas.

"Josef Cyrus, Marloes Eeftens, Joachim Heinrich, Christophe Ampe, Alexandre Armengaud, Rob Beelen, Tom Bellander, Tímea Beregszaszi, Matthias Birk, Giulia Cesaroni, Marta Cirach, Kees de Hoogh, Audrey De Nazelle, Frank de Vocht, Christophe Declercq, Audrius Dédélé, Konstantina Dimakopoulou, Kirsten Eriksen, Claudia Galassi, Regina Grąulevičienė, Georgios Grivas, Olena Gruzieva, Annika Hagenbjörk Gustafsson, Barbara Hoffmann, Minas Iakovides, Alex Ineichen, Ursula Krämer, Timo Lanki, Patricia Lozano, Christian Madsen, Kees Meliefste, Lars Modig, Anna Mölter, Gioia Mosler, Mark Nieuwenhuijsen, Michael Nonnemacher, Marieke Oldenwening, Annette Peters, Sabrina Pontet, Nicole Probst-Hensch, Ulrich Quass, Ole Raaschou-Nielsen, Andrea Ranzi, Dorothee Sugiri, Euripides G. Stephanou, Pekka Taimisto, Ming-Yi Tsai, Éva Vaskóvi, Simona Villani, Meng Wang, Bert Brunekreef, Gerard Hoek, Variation of NO₂ and NO_x concentrations between and within 36 European study areas: Results from the ESCAPE study, Atmospheric Environment, Volume 62, December 2012, Pages 374-390, ISSN 1352-2310, <http://dx.doi.org/10.1016/j.atmosenv.2012.07.080>"

	Results: How many children surpassed NO2 boundary values established by European Directive (40 µg/m3)?
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REVIEWER	Ana Esplugues Cebrián Faculty of Nursing and Chiropractic Valencia University FISABIO– Universitat Jaume I –Universitat de València Joint Research Unit of Epidemiology and Environmental Health
REVIEW RETURNED	17-Feb-2017

GENERAL COMMENTS	<p>The role of prenatal ambient air pollution in the development of childhood respiratory chronic diseases in infants is still debated, and also the role of child sex. Authors used a large (N=17533) pregnancy cohort to analyze the effect of pregnancy exposure to air pollution (NO2) on respiratory symptoms in a birth cohort.</p> <p>Effect variables were obtained by questionnaire, and the estimates of air pollution exposure during pregnancy were based on the methodology developed for the ESCAPE project. But there are some methodological aspects that are not clear in the text.</p> <p>-Authors had not specified in the text if questionnaires were obtained by face to face interview, telephone, postal, etc.</p> <p>-Children were born from 2001 to 2009. Could you justify why exposure measurements were from 2010 (Oslo and Akershus) and 2011 (Bergen and Hordaland)? Methodology of exposure measurement should be better explained. Maybe you could introduce a supplementary table with the number of points with measurements, dates, period, type of dosimeter (active or passive?), medium values in each point, quality parameters (not detectable, etc.)</p> <p>-In addition to maternal education variable, authors consider other social variable as social class, income, etc. as adjustment variable?</p> <p>-In the same line, Have you considered breast feeding? Exclusive breastfeeding until the age of 4 months and partially thereafter was associated with a significant reduction of respiratory morbidity in infants. Ex: Pediatrics. 2010 Jul;126(1):e18-25. doi: 10.1542/peds.2008-3256. Epub 2010 Jun 21. Prolonged and exclusive breastfeeding reduces the risk of infectious diseases in infancy. Duijts L, Jaddoe VW, Hofman A, Moll HA.</p> <p>-Why you performed sensitivity analyses restricting to pregnancies during the last period of the MoBa recruitment? Is related to exposure? What is the reason?</p>
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REVIEWER	Mary B. Rice BIDMC Boston, MA USA
REVIEW RETURNED	21-Feb-2017

GENERAL COMMENTS	<p>This is a well-designed study with longitudinal questionnaire follow-up of a large Norwegian birth cohort. It is novel in that the study examines a population with relatively low exposure levels and examines odds of early life respiratory infection at multiple time points in the first two years of life, with adjustment of child- and maternal-level covariates. I have one major concern with the analytical approach: the authors have not addressed potential confounding by time-varying exposures. The study period spans 10 years, during which there has been a substantial decline in pollution levels. I have made specific suggestions below, under methods. This manuscript is reasonably well-written, but is in need of further English language editing.</p> <p>Introduction</p> <p>-The introduction could be tightened up and focused a bit better. The authors cover infections, wheeze, asthma, lung function and “respiratory diseases in childhood” as if they are all part of the same disease and pathogenesis. Since this manuscript is about LRTI and wheeze, I would suggest that the authors focus on those outcomes and the literature on air pollution and early life infection and wheeze. It might be worth mentioning that infection and wheeze are closely related in early life.</p> <p>-Technically croup is an upper respiratory infection because it involves inflammation of the upper respiratory tract</p> <p>Methods</p> <p>-The huge drop from 114 500 participants to 17 533 appears to be due to the fact that the 114 500 value includes all Norwegian participants (and not those living in the four study areas), but it is not clear. It might be more meaningful to give as the starting number the number of participants in the study areas and then to narrow that down to the 17 533 with non-missing exposure data.</p> <p>-I worry about confounding due to other time-varying exposures, as there has been a substantial decline in pollution exposure over time and the study spans a ten year interval (1999-2008). Although the exposure measurements all occurred in 2010 and 2011, the pollution models subsequently estimate yearly exposures and back-extrapolated to the date of pregnancy to introduce temporal</p>
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variability. The authors have not adjusted for date of birth, which is a major concern, as other time-varying exposures could explain differences in risk of infection or wheeze over time. At a minimum, I suggest that the authors adjust for date in their primary models.

-As an additional test for temporal confounding, I suggest the authors perform a meta-analysis, comparing pregnancy exposure and respiratory outcomes among participants within the same year of birth, then combining the results using meta-analytic techniques. That way the associations are only examined within-year.

-The discussion of the NO₂ models does not discuss whether there was any validation of the modeled estimates. Did the authors compare the estimates from the land use regression model to any ground monitor measurements to obtain a cross-validation R²?

-The statistical analysis section is very brief and would benefit from more explanation of why the authors made certain decisions. Why did the authors run so many stratified analyses, and then also run a relatively large number of multiplicative interactions on the same variables, but adding maternal parity? In general, I recommend testing first for interaction and then reporting the effect estimates by the interaction term if there is statistical interaction. Is there any biological rationale for testing for differential susceptibility by these particular variables? Running many tests for interaction without an a priori rationale or expectation is likely to yield chance findings.

-Why did the authors restrict analyses to pregnancies during the last period of recruitment?

-Logistic regression for a very common outcome (e.g. 40.6% had wheeze) will result in OR's that are much larger than the RR. Authors can consider running a relative risk regression (e.g. Poisson distribution with robust standard error; see Zou Am J Epidemiol 2004 and Spiegelman Am J Epidemiol 2005).

Results

-The authors report stratified results by sex, but there is no evidence of statistical interaction by sex for any of the outcomes examined. Although the OR for LRTI at age 6-18 months does not cross the null for girls, I highly question the conclusion that there is truly a biologic difference here between girls and boys.

-I do not understand the sentence "This was also the case for crude analyses in a sample of children with available information on all covariates." This crude analysis was not described in the methods. Don't all the children have available covariate information? I am not sure what the authors are referring to.

Discussion

-Again, I have serious concerns about suggesting a differential effect in girls, when there was no statistical evidence of effect modification

	<p>by sex</p> <p>-The discussion jumps into a number of citations of other air pollution studies, including PM2.5 studies, which is confusing as that is a different pollutant. Given that this study modeled NO2, consider starting with a discussion of NO2 pollution and where it comes from (geared to an audience without air pollution expertise).</p> <p>-I have the same concern about the second paragraph of the discussion as the introduction: it jumbles together studies of lung function, asthma and eczema. There is enough literature on early childhood wheeze and infection to be more focused than that and help place the study findings in context.</p>
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REVIEWER	Kent E. Pinkerton University of California, Davis USA
REVIEW RETURNED	22-Feb-2017

GENERAL COMMENTS	<p>The manuscript by Madsen and colleagues, "Pregnancy exposure to air pollution and early childhood respiratory health", is an impressive study to examine possible associations between exposure to moderate levels of air pollution during pregnancy and early childhood lower respiratory tract infections (LRTI) and wheezing in a subgroup of 17,533 participants from the Norwegian Mother and Child Cohort Study (MoBa). The authors report no statistically significant associations between moderate levels of NO2 exposure during pregnancy and respiratory health outcomes for children during early childhood. However, stratified analysis of the data suggests girls between 6 to 18 months of age may be at higher risk for developing LRTI due to prenatal air pollution exposure of the mother.</p> <p>The study is well-designed and executed, but some questions remain that should be addressed by the authors.</p> <p>Specific Comments:</p> <ol style="list-style-type: none"> 1. The authors should clarify why only NO2 exposure levels were measured in this study. There are numerous air pollutants to which one can be exposed during pregnancy. Why do the authors only use this single criteria air pollutant for study? 2. Please justify the importance of NO2 as an indoor air pollutant. 3. Moderate levels of exposure to NO2 is a rather vague designation for exposure. The actual range of NO2 levels should be provided, along with an explanation of how one deals with the variation of exposure conditions among individuals. 4. The bullet points under "What this paper adds" are quite helpful to provide a concise overview of the study.
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	<p>5. Does this represent a negative study that can provide relevance to other publications in the literature?</p> <p>6. Nice introduction.</p> <p>7. Introduction: The authors state "...children are exposed to tobacco smoke or higher levels of ambient air pollution are more prone to respirator disorders." What are "higher levels of ambient air pollution: for children?</p> <p>8. Introduction: The authors state "Some studies report associations of prenatal air pollution exposure with respiratory infections and decreased lung function (references 18-20). Other studies have found no such association for LRTI and lung function in early childhood (references 21-22). For these references showing disparities in findings, there is no explanation of what is the air pollution. Please clarify.</p> <p>9. Introduction: the authors state they used "...LUR modeled exposure to traffic-related pollutant NO2 built for specific areas of Norway..." What about other pollutants such as particulate matter, SO2, CO, etc.? Please clarify why only NO2 is being investigated.</p> <p>10. How significant is traffic-related air pollution (TRAP) in Norway?</p> <p>11. Methods: Good. Large population base for study is excellent.</p> <p>12. Please clarify the rationale for the selection of symptoms (RSV, bronchiolitis, pneumonia for LRTI, along with wheezing/tightness of chest.</p> <p>13. Results: Logical and well-presented</p> <p>14. Discussion: Why increased risk of developing LRTI in girls, but not boys? Why this specific age range from 6 to 18 months of age? The authors begin to address this issue, but the arguments are rather weak and not well-established.</p> <p>15. Discussion: The authors state "Living outside the city was associated with higher risk of developing LRTI and wheeze...".Is the air quality outside the city different? If so, how is it different?</p> <p>16. Discussion: Final sentence of discussion: The authors state: "...no statistically significant associations for moderate levels of pregnancy NO2 exposure and childhood..."</p> <p>Might be more clearly stated as "...no statistically significant associations for moderate levels of exposure to NO2 during pregnancy and childhood..."</p>
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VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

General comments

This is an interesting paper based on a large dataset describing associations between LRTI and air pollution exposure at low levels of exposure. A major limitation of the paper in its current form is the cross-sectional analysis. Moreover, the discussion should be more focused (see specific comments below).

Answer: We comment on the limitation of the design in Q1 below. The introduction and the discussion is, as suggested, revised to be more focused on LRTI and air pollution per se.

Specific comments

Q1: Highlights, 3rd bullet: This suggests that longitudinal analyses have been performed. However, this is not the case. Two sets of cross-sectional analyses were performed for health outcomes during the first 6 months of life and between age 6 and 18 months. This needs to be corrected or preferably, cross-sectional analyses should be replaced by longitudinal analyses (e.g. GEEs), which are more appropriate in prospective cohort studies with repeated follow-ups.

Answer: The prospective design of this cohort underlines that the collection of exposure data (thru the questionnaires) is collected at several stages before disease manifestation. We therefore consider this design to be longitudinal since exposure is modelled at residency at time of pregnancy and thus before the outcomes. The study design used in our manuscript, is also used in several other MoBa papers (including Haberg et al 2007 which we use a reference (#16) to smoking and early childhood respiratory health). Furthermore, we have not considered the LRTI outcomes as part of the same risk scenario. Several of the other reviewers have suggested to include “year of birth” in the adjusted models. We have done this in the present version of the manuscript. This did not change the overall interpretation of the associations between the exposure and the outcomes.

The Highlights section was replaced with a “Strengths and limitations of this study” section (as recommended by the Editor).

Q2: Introduction, page 4, line 31ff, effects of maternal smoking during pregnancy and respiratory health. I suggest to focus on ambient air pollution here and delete text on smoking OR to motivate why this is relevant here.

Answer: We agree and have added additional text and a new reference to motivate why the link between smoking and respiratory outcomes is relevant here.

Q3: Introduction, page 4, last paragraph, air pollution exposure assessment. I suggest to move the text on personal exposure assessment vs exposure modeling, strengths and limitations of the different approaches to the discussion. You may wish to mention also that personal exposure assessment usually not feasible in large population studies. Text on proximity to traffic can be deleted unless this is relevant for the comparison with other studies. In that case this should be moved to the discussion.

Answer: Good suggestion. We have moved the paragraph as recommended. In addition, we have deleted the text on proximity to traffic and included that personal exposure assessment is usually not feasible in large population studies like MoBa.

Q4: Introduction, page 6, 1st paragraph. I personally would skip the sentence saying that this is the first Norwegian study. This is not a very strong selling point. The fact that air pollution levels are low in Norway is a much better selling point.

Answer: We agree that the fact that this study is from areas with low air pollution levels is a better way of presenting the paper. The sentence referring to this as the first Norwegian study is deleted.

Q5: Page 8, statistical analysis. The two sets of cross-sectional analyses at ages 6 months and 6-18

months should be replaced by longitudinal analyses of the (repeated measures) of the outcomes of interest making full use of the longitudinal data and accounting for the correlation between observations within subjects.

Answer: See comments under Q1 above.

Q6: Page 8, statistical analysis. Parity is mentioned in the sentence about multiplicative interactions, but not in the sentence about stratified analyses, while all other variables are mentioned in both. This is inconsistent.

Answer: We agree that this is inconsistent since “parity” was analysed in the stratified analyses. “Parity” is now included in the text as part of the stratified analysis.

Q7: Page 8, statistical analysis. It is not clear to me how the authors accounted for the fact that some women contributed multiple pregnancies to the cohort. The observations from siblings are likely not independent. This needs to be taken into account in the analysis.

Answer: Each women only contributes with one pregnancy (multiple births have been removed), and only singleton births are included. Additional text has been added under the Methods section to clarify this.

Q8: Page 8, statistical analysis. All analyses were adjusted for “area” (Oslo, Bergen, Akershus and Hordaland). To what extent do air pollution levels differ between areas? This should be clarified by adding descriptives of air pollution levels by study area. Since the study areas include both urban and rural areas, I would expect air pollution levels to be different . Consequently, adjustment for study area may result in overadjustment. Therefore, analyses with and without adjustment for study area should be presented and differences including potential overadjustment should be discussed.

Answer: We agree. The main analysis (Table 2) is now changed so that the adjusted model does not include “area”.

Q9: Results. See my comment above. Please add descriptive analyses of air pollution levels by study area.

Answer: Table 1 has been updated to reflect air pollution distribution in the study areas.

Q10: Discussion. The first part of the discussion, where results of the present study are compared with findings of other studies needs better structure. I suggest to start with studies that investigated associations of LRTI and/or wheeze with air pollution and focus on the ones that are comparable with regard to the age of the study participants. If these are few, also include relevant studies among older children. When referring to other studies, please make sure that the direction of the observed effects is always clear.

Answer: As the title suggest, this is a study on early childhood respiratory outcomes per se. We have tried to modify the introduction and discussion to reflect the most relevant papers on this topic. We hope that the current version is providing a sufficient overview of early respiratory outcomes as measured here (LRTI and Wheeze).

Q11: Since the focus of the present study is on LRTI, I suggest to remove text on other outcomes such as asthma and lung function from the discussion and focus on LRTI.

Answer: We agree, and have made changes to the discussion as recommended.

Q12: Page 10, last paragraph “Living outside the city...” I think this sentence needs to be revised as in its current form it compares risks of LRTI between urban and non-urban study areas while it should be about air pollution effects in urban and non-urban study areas – at least this is what I conclude from the sentence following that sentence. Please double check.

Answer: We agree, and the sentence has been revised in the manuscript to several comments from

the different reviewers.

Q13: Same paragraph, the statement about higher mobility and less time spent at home needs to be supported by a reference.

Answer: We agree, and additional text and a reference has been added.

Q14: The authors discuss exposure misclassification due to residential mobility. Differences in time-activity patterns may be another source of exposure misclassification when relying on residential addresses solely that needs to be discussed in more detail (currently it is only briefly mentioned on page 10). Do you have information about the percentage of women working outside their own home during pregnancy? Is information regarding work addresses available? If yes, do results change if work address exposures are included? If no, the lack of information on exposures at work and other addresses needs to be discussed.

Answer: We added more text in the discussion as recommended above.

Reviewer: 2

General comments

The authors study the associations between exposure to air pollution during pregnancy and early childhood lower respiratory tract infections and wheezing. The importance of study is that the NO₂ levels were moderate.

Major comments

Q1: I think that the methodology for exposure assessment is not adequately clarified in the manuscript, the authors should explain it better. How many monitoring points were used? Which NO₂ levels were obtained? Which GIS variables were included in the LUR models? This data gives information about the "validity" of the air pollution measurements.

Answer: The exposure assessment and its methodology is clarified in a separate paper which is already included as a reference in the manuscript (Panasevich et al 2016). Additional text is provided with more information on the adjusted R² of these models.

Q2: Moreover, the authors should mention that the NO₂ levels were collected during 2010 and 2011 and GIS variables were collected in 2013 in the limitations section.

Answer: We agree, and have included this several places in text, including in the limitations section.

Q3: I do not understand why the authors replaced the negative modelled exposures levels by 0.01. Is it the detection limit?

Answer: As already specified in the manuscript, this is a value assigned to negative modelled values, or modelled values below 0. In order to avoid the unlikely scenario of negative values, we assigned these negative values with a low value (0.01). This low value does not change the fact that these addresses are low exposed from traffic-related air pollution. We have added text to clarify this point and a reference (Panasevich et al 2016).

Q4: The authors said that the correlations between exposures in different trimesters of pregnancy are very high. In any case, associations between exposures in different trimesters and respiratory health could have been explored in the study. Ritz and cols identified the importance of studying the critical periods of exposure (Ritz B, Wilhelm M. Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. *Basic Clin Pharmacol Toxicol*. 2008 Feb;102(2):182-90. doi: 10.1111/j.1742-7843.2007.00161.x.)

Answer: We agree that critical periods of pregnancy is important since different stages of development could make the foetus more vulnerable for air pollution exposure. Still, as described in the manuscript, the modelled exposure is highly correlated between the trimesters. Thus, trimester-specific models would not provide more information as compared to the whole pregnancy exposure

per se.

Q5: The statistical methodology is not clear. Were the analyses adjusted by area or were the results from different centers meta-analysed?

Answer: The overall analysis was adjusted for area as supported by a previous paper on this cohort (Panasevich et al 2016, see reference #30 in the current version of the manuscript). This overall analysis was not based on a meta-analysis since we had full access to do a complete analysis. The area-variable was found to be representative of both the spatial distribution of air pollution per se (as a proxy), but also a potential proxy for unmeasured factors that could vary between each study area, factors that could influence the outcome variables (see Panasevich et al 2016).

Q6: Regarding sensitivity analyses, it is worthwhile to note that stratified analyses are generally done subsequently to the interaction test. I.e., if interaction term is statistically significant you can proceed with stratified analyses. Otherwise, stratified analyses have not much sense.

Answer: The sensitivity test were provided to show potential conditions that could result in different overall associations. In this model: a) change of residency during pregnancy and b) pregnancies closer in time to the exposure assessment and GIS-variables included in the exposure modelling. The stratified analyses were posthoc and is thus more exploratory. This has now been included in the text. The motivation for presenting area-specific results is due to different models for each area. This information could be useful for the reader in relation to the previously published method paper by Panasevich et al 2016. Also see answer to Q5 above.

Q7: The authors restrict the analysis to pregnancies during the last period of the MoBa recruitment; I suppose that this is due to the problem of misclassification. The authors should discuss about this and include it in the limitations section.

Answer: Yes, correct. We have included a discussion of this in the limitations section.

Q8: The authors comment "Area variable may reflect the spatial distribution of air pollution, and therefore a separate analysis was performed in adjusted model without the area variable". I suppose that you have said it is important to adjust the models by area since differences between areas exist. Are covariates different?

Answer: In a previous study, area was found to be an important factor in attenuating the associations between exposure and birth outcome in MoBa (see Panasevich et al 2016 – reference #30). A general discussion of this is found in this paper.

Q9: I think that would be also interesting to know the relationship between NO₂ exposure and LRTI of 0-18 . Furthermore, I am interested in the longitudinal analysis, that is, the authors could build a variable: 0: not LRTI, 1: LRTI only 0-6 months, 2: LRTI only 6-18, and 3: LRTI in both periods (incidence and persistent cases).

Answer: The combined variable "LRTI 0-18 months" yields the following results in the adjusted analysis: 1.04 (0.93, 1.16). Building a combined variable examining the incidence and persisted cases as suggested yields the following results using mlogit:

Reference group: no LRTI (N=18.880)

Group 1: only LRTI 0-6 months (N=517) RR: 0.98 (0.98, 1.21)

Group 2: only LRTI 6-18 months (N=1.520) RR: 1.07 (0.94, 1,22)

Group 3: LRTI at both 0-6 months and 6-18 months (N=311) RR: 1.02 (0.77, 1.35)

Combining group 1 and 2 above (incidence) gives: 1.04 (0.93, 1.17). This is not included in the present manuscript, but we are open for including this.

Q10: I do not understand this sentence: "Living outside the city was associated with higher risk of

developing LRTI and wheeze, although the estimates were not statistically significant. This might be due to a higher misclassification of exposure in cities because of higher mobility and less staying at home. On the other hand, non-city population might have more homogenous exposure and spending more time close to home". What is the reason to make that claim? Do the authors have time-activity data?

Answer: The sentence has been revised in the manuscript as suggested by several of the reviewers.

Q11: The authors do not mention other limitations in the methodology of exposure assessment, such as not considering other air pollution sources in indoor air (e.g., gas cooking) or other relevant outdoor pollutants.

Answer: Gas cooking has not been widely spread in Norway (as compared to European countries). Electricity has been far more widespread. The Norwegian Mother and Child Study (MoBa) does not include indoor sampling at the residential addresses at the time around each pregnancy. Thus, we use outdoor traffic-pollution exposure on residence as a proxy for residential exposure and thus "personal exposure".

Minor comments:

Abstract:

Q12: LRTI is already defined in introduction section. It is not necessary to include it in the results section.

Answer: We have changed this in the methods section as suggested.

Q13: In the section "What this paper adds", English should be checked:

Analyse the longitudinal effect of...? or Analyse longitudinally the effect...?

Answer: We appreciate the suggestions. However, the section was deleted as suggested by the Editor.

Introduction:

Q14: The authors have commented "Norway is characterized by relatively low levels of air pollution". They could mention the article published by Cyrus about NO₂ levels in different European areas. "Josef Cyrus, Marloes Eeftens, Joachim Heinrich, Christophe Ampe, Alexandre Armengaud, Rob Beelen, Tom Bellander, Tímea Beregszászi, Matthias Birk, Giulia Cesaroni, Marta Cirach, Kees de Hoogh, Audrey De Nazelle, Frank de Vocht, Christophe Declercq, Audrius Dédélé, Konstantina Dimakopoulou, Kirsten Eriksen, Claudia Galassi, Regina Gražulevičienė, Georgios Grivas, Olena Gruzjeva, Annika Hagenbjörk Gustafsson, Barbara Hoffmann, Minas Iakovides, Alex Ineichen, Ursula Krämer, Timo Lanki, Patricia Lozano, Christian Madsen, Kees Meliefste, Lars Modig, Anna Mölter, Gioia Mosler, Mark Nieuwenhuijsen, Michael Nonnemacher, Marieke Oldenwening, Annette Peters, Sabrina Pontet, Nicole Probst-Hensch, Ulrich Quass, Ole Raaschou-Nielsen, Andrea Ranzi, Dorothee Sugiri, Euripides G. Stephanou, Pekka Taimisto, Ming-Yi Tsai, Éva Vaskövi, Simona Villani, Meng Wang, Bert Brunekreef, Gerard Hoek, Variation of NO₂ and NO_x concentrations between and within 36 European study areas: Results from the ESCAPE study, Atmospheric Environment, Volume 62, December 2012, Pages 374-390, ISSN 1352-2310, <http://dx.doi.org/10.1016/j.atmosenv.2012.07.080>"

Answer: Good suggestion. We have included this reference in the manuscript.

Results:

Q15: How many children surpassed NO₂ boundary values established by European Directive (40 µg/m³)?

Answer: A total of 27 children had annual residential address levels of NO₂ equal to or above 40 µg/m³. This has been included in the manuscript.

Reviewer: 3

General comment

The role of prenatal ambient air pollution in the development of childhood respiratory chronic diseases in infants is still debated, and also the role of child sex. Authors used a large (N=17533) pregnancy cohort to analyze the effect of pregnancy exposure to air pollution (NO₂) on respiratory symptoms in a birth cohort.

Effect variables were obtained by questionnaire, and the estimates of air pollution exposure during pregnancy were based on the methodology developed for the ESCAPE project. But there are some methodological aspects that are not clear in the text.

Q1: Authors had not specified in the text if questionnaires were obtained by face to face interview, telephone, postal, etc.

Answer: We have now included in the text that the questionnaires were postal.

Q2: Children were born from 2001 to 2009. Could you justify why exposure measurements were from 2010 (Oslo and Akershus) and 2011 (Bergen and Hordaland)? Methodology of exposure measurement should be better explained. Maybe you could introduce a supplementary table with the number of points with measurements, dates, period, type of dosimeter (active or passive?), medium values in each point, quality parameters (not detectable, etc.) -In addition to maternal education variable, authors consider other social variable as social class, income, etc. as adjustment variable?

Answer: The methodology of the sampling was explained in a previously published paper (Panasevich et al 2016). Still, we have included some additional text to clarify this in the present manuscript. It is correct that we have included maternal education as a proxy for socioeconomic status (SES). We also tested for maternal income. Maternal income was medium correlated with maternal education ($r=0.36$). This variable was not significant in itself in the models.

Q3: In the same line, Have you considered breast feeding? Exclusive breastfeeding until the age of 4 months and partially thereafter was associated with a significant reduction of respiratory morbidity in infants. Ex: Pediatrics. 2010 Jul;126(1):e18-25. doi: 10.1542/peds.2008-3256. Epub 2010 Jun 21. Prolonged and exclusive breastfeeding reduces the risk of infectious diseases in infancy. Duijts L, Jaddoe VW, Hofman A, Moll HA.

Answer: We did not consider breastfeeding in this study since there is no obvious reason how breast feeding could confounding any association between air pollution exposure and respiratory morbidity since breast feeding could not influence air pollution exposure per se

Q4: Why you performed sensitivity analyses restricting to pregnancies during the last period of the MoBa recruitment? Is related to exposure? What is the reason?

Answer: The reason for restricting to the last period of the MoBa recruitment is related to the modelling. The air pollution measurement (for spatial distribution) was collected in 2010 and 2011, and the GIS-variables was collected in 2013. The models was then developed for the spatial distribution of traffic-related air pollution, with a temporal-adjustment using data from local fixed monitoring stations. The sensitivity analyses was performed as a test to see if the associations would be different for pregnancies closer in time to the NO₂ and GIS-variables, as compared to the overall model. Additional text have been provided to clarify this sensitivity test.

Reviewer: 4

This is a well-designed study with longitudinal questionnaire follow-up of a large Norwegian birth cohort. It is novel in that the study examines a population with relatively low exposure levels and examines odds of early life respiratory infection at multiple time points in the first two years of life, with adjustment of child- and maternal-level covariates. I have one major concern with the analytical approach: the authors have not addressed potential confounding by time-varying exposures. The study period spans 10 years, during which there has been a substantial decline in pollution levels. I

have made specific suggestions below, under methods. This manuscript is reasonably well-written, but is in need of further English language editing.

Introduction

Q1: The introduction could be tightened up and focused a bit better. The authors cover infections, wheeze, asthma, lung function and “respiratory diseases in childhood” as if they are all part of the same disease and pathogenesis. Since this manuscript is about LRTI and wheeze, I would suggest that the authors focus on those outcomes and the literature on air pollution and early life infection and wheeze. It might be worth mentioning that infection and wheeze are closely related in early life.

Answer: We agree and have modified the introduction in agreement with the suggestions above.

Q2: Technically croup is an upper respiratory infection because it involves inflammation of the upper respiratory tract

Answer: Yes, we agree and croup is now removed from the text.

Methods

Q3: The huge drop from 114 500 participants to 17 533 appears to be due to the fact that the 114 500 value includes all Norwegian participants (and not those living in the four study areas), but it is not clear. It might be more meaningful to give as the starting number the number of participants in the study areas and then to narrow that down to the 17 533 with non-missing exposure data.

Answer: Most of this information have been provided in a previous published paper (Panasevich et al 2016). However, we agree that some clarification is needed. We have included the number of participants from the four study areas and details on how we arrive at the number included in the study.

Q4: I worry about confounding due to other time-varying exposures, as there has been a substantial decline in pollution exposure over time and the study spans a ten year interval (1999-2008). Although the exposure measurements all occurred in 2010 and 2011, the pollution models subsequently estimate yearly exposures and back-extrapolated to the date of pregnancy to introduce temporal variability. The authors have not adjusted for date of birth, which is a major concern, as other time-varying exposures could explain differences in risk of infection or wheeze over time. At a minimum, I suggest that the authors adjust for date in their primary models.

Answer: We agree and have adjusted the models with year of birth. This did not change the adjusted models substantially. Also, the sensitivity models using only pregnancies in the last part of the period, showed no difference compared to the full model.

Q5: As an additional test for temporal confounding, I suggest the authors perform a meta-analysis, comparing pregnancy exposure and respiratory outcomes among participants within the same year of birth, then combining the results using meta-analytic techniques. That way the associations are only examined within-year.

Answer: We have as suggested performed a meta-analysis to examine within-year associations. Overall, this did not give another interpretation than the sensitivity test were we restricted to birth years within the last period of the MoBa recruitment. The results of this meta-analysis is thus not included in the manuscript.

Q6: The discussion of the NO₂ models does not discuss whether there was any validation of the modeled estimates. Did the authors compare the estimates from the land use regression model to any ground monitor measurements to obtain a cross-validation R²?

Answer: The modelled exposure was validated in a separate paper (Panasevich et al 2016), which is referenced to in the method section. An additional comparison to fixed monitoring in the areas would

be problematic since these fixed monitoring stations already are included in the model (temporal-modeling). The adjusted R² for the models is now included in the manuscript.

Q7: The statistical analysis section is very brief and would benefit from more explanation of why the authors made certain decisions. Why did the authors run so many stratified analyses, and then also run a relatively large number of multiplicative interactions on the same variables, but adding maternal parity? In general, I recommend testing first for interaction and then reporting the effect estimates by the interaction term if there is statistical interaction. Is there any biological rationale for testing for differential susceptibility by these particular variables? Running many tests for interaction without an a priori rationale or expectation is likely to yield chance findings.

Answer: Several of the stratified analysis are exploratory and thus post hoc. We have included additional text in the Methods section to underline this in the current version of the manuscript. In addition, we have updated the Discussion section to avoid any mistakes: the primary planned analysis showed not associations between exposure and outcomes. Any reported post hoc analysis is exploratory.

Q8: Why did the authors restrict analyses to pregnancies during the last period of recruitment?

Answer: This was done as part of the sensitivity analysis to check if there was a difference in OR for the pregnancies closer in time to the sampled exposure and the GIS-variables. Both the exposure (sampling of spatial distribution in the areas during 2010-2011) and the GIS-variables (layers dated 2013) were collected after the MoBa cohort inclusion (1999-2008).

Q9: Logistic regression for a very common outcome (e.g. 40.6% had wheeze) will result in OR's that are much larger than the RR. Authors can consider running a relative risk regression (e.g. Poisson distribution with robust standard error; see Zou Am J Epidemiol 2004 and Spiegelman Am J Epidemiol 2005).

Answer: We agree, and have replaced the logistic regression analysis for with a relative risk regression (glm, using family=binomial + link=log). This resulted in very similar associations (see Table 2).

Results

Q10: The authors report stratified results by sex, but there is no evidence of statistical interaction by sex for any of the outcomes examined. Although the OR for LRTI at age 6-18 months does not cross the null for girls, I highly question the conclusion that there is truly a biologic difference here between girls and boys.

Answer: See answer to Q7 above.

Q11: I do not understand the sentence "This was also the case for crude analyses in a sample of children with available information on all covariates." This crude analysis was not described in the methods. Don't all the children have available covariate information? I am not sure what the authors are referring to.

Answer: Yes, all children had available covariate information. The sentence was vaguely formulated and merely pointed out that there was no difference between the crude and adjusted analysis for children included in the analysis (with available covariate information). The sentence is deleted to avoid further misunderstanding.

Discussion

Q12: Again, I have serious concerns about suggesting a differential effect in girls, when there was no statistical evidence of effect modification by sex.

Answer: See answer to Q7 above.

Q13: The discussion jumps into a number of citations of other air pollution studies, including PM2.5 studies, which is confusing as that is a different pollutant. Given that this study modeled NO2, consider starting with a discussion of NO2 pollution and where it comes from (geared to an audience without air pollution expertise).

Answer: We agree and have tried to change the discussion to better reflect this.

Q14: I have the same concern about the second paragraph of the discussion as the introduction: it jumbles together studies of lung function, asthma and eczema. There is enough literature on early childhood wheeze and infection to be more focused than that and help place the study findings in context.

Answer: We agree, and have tried to modify the text as suggested.

Reviewer: 5

General comments

The manuscript by Madsen and colleagues, "Pregnancy exposure to air pollution and early childhood respiratory health", is an impressive study to examine possible associations between exposure to moderate levels of air pollution during pregnancy and early childhood lower respiratory tract infections (LRTI) and wheezing in a subgroup of 17,533 participants from the Norwegian Mother and Child Cohort Study (MoBa). The authors report no statistically significant associations between moderate levels of NO2 exposure during pregnancy and respiratory health outcomes for children during early childhood. However, stratified analysis of the data suggests girls between 6 to 18 months of age may be at higher risk for developing LRTI due to prenatal air pollution exposure of the mother.

The study is well-designed and executed, but some questions remain that should be addressed by the authors.

Specific Comments:

Q1: The authors should clarify why only NO2 exposure levels were measured in this study. There are numerous air pollutants to which one can be exposed during pregnancy. Why do the authors only use this single criteria air pollutant for study?

Answer: We agree that there are several pollutants of interest for pregnancy outcomes. NO2 is strongly related to traffic-pollution, and has been shown to have a larger spatial variation as compared to e.g. PM2.5 and PM10. Particulate matter is because of its homogeneous spatial pattern, more difficult to model. We have added more text with references to why we used this pollutant.

Q2: Please justify the importance of NO2 as an indoor air pollutant.

Answer: Indoor and outdoor levels of NO2 has been shown to be highly correlated. Thus, we consider outdoor modelled exposure at residential address as a proxy for personal exposure for both outdoor and indoor exposures.

Q3: Moderate levels of exposure to NO2 is a rather vague designation for exposure. The actual range of NO2 levels should be provided, along with an explanation of how one deals with the variation of exposure conditions among individuals.

Answer: Moderate levels in this study is at best described as annual residential exposures of NO2 in the range up to 60.4 µg/m³. Most children had concentrations at home address equal to 13.6 (SD=6.9). We have included the mean and the range in the abstract and in the Results section. We have also included an explanation of how we dealt with the variation of exposure among the children.

Q4: The bullet points under "What this paper adds" are quite helpful to provide a concise overview of the study.

Answer: Good to hear, but this section is now deleted as suggested by the Editor, and replaced by a

“Strengths and Limitations of this study” section.

Q5: Does this represent a negative study that can provide relevance to other publications in the literature?

Answer: Yes, given the general interpretation of these data, we consider this as a negative study which can provide relevance to similar studies, especially studies from low polluted areas.

Q6: Nice introduction.

Answer: Good to hear.

Q7: Introduction: The authors state “...children are exposed to tobacco smoke or higher levels of ambient air pollution are more prone to respirator disorders.” What are “higher levels of ambient air pollution: for children?

Answer: Higher levels of ambient air pollution is here equal to concentrations above recommended levels, such as the EU or WHO standards. We have specified this in the text to clarify what we mean by “higher levels” per se.

Q8: Introduction: The authors state “Some studies report associations of prenatal air pollution exposure with respiratory infections and decreased lung function (references 18-20). Other studies have found no such association for LRTI and lung function in early childhood (references 21-22). For these references showing disparities in findings, there is no explanation of what is the air pollution. Please clarify.

Answer: We updated the text to show which air pollutant is used in these studies.

Q9: Introduction: the authors state they used “...LUR modeled exposure to traffic-related pollutant NO₂ built for specific areas of Norway...” What about other pollutants such as particulate matter, SO₂, CO, etc.? Please clarify why only NO₂ is being investigated.

Answer: See comments under Q1 above.

Q10: How significant is traffic-related air pollution (TRAP) in Norway?

Answer: Short episodes with peak concentrations above recommended levels is not uncommon in larger Norwegian cities. On average, the concentration levels are moderate compared to other European cities.

Q11: Methods: Good. Large population base for study is excellent.

Answer: Good to hear.

Q12: Please clarify the rationale for the selection of symptoms (RSV, bronchiolitis, pneumonia for LRTI, along with wheezing/tightness of chest.

Answer: These are commonly used diagnosis/symptoms used to define outcomes in this type of epidemiological studies. We have updated the text to reflect this.

Q13: Results: Logical and well-presented

Answer: Good to hear.

Q14: Discussion: Why increased risk of developing LRTI in girls, but not boys? Why this specific age range from 6 to 18 months of age? The authors begin to address this issue, but the arguments are rather weak and not well-established.

Answer: Several of the stratified analysis are exploratory and performed post hoc. We have included additional text in the current version of the manuscript to underline this as a general response to all reviewer.

Q15: Discussion: The authors state “Living outside the city was associated with higher risk of developing LRTI and wheeze...”. Is the air quality outside the city different? If so, how is it different?
 Answer: In a previous study, area was found to be an important factor in attenuating the associations between exposure and birth outcome in MoBa (see Panasevich et al 2016 – reference #30). A general discussion of this is found in this paper. The air pollution could be an issue, given other components that remain unmeasured here (ozone, PM from other local sources), but also differences in lifestyle factors etc. We can only speculate here – as added to the text.

Q16: Discussion: Final sentence of discussion: The authors state: “...no statistically significant associations for moderate levels of pregnancy NO2 exposure and childhood...”
 Might be more clearly stated as “...no statistically significant associations for moderate levels of exposure to NO2 during pregnancy and childhood...”
 Answer: We agree and have changed the text accordingly.

VERSION 2 – REVIEW

REVIEWER	Marisa Estarlich and Amparo Ferrero CIBERESP, FISABIO, SPAIN
REVIEW RETURNED	09-May-2017

GENERAL COMMENTS	<p>General comments The authors study the associations between exposure to air pollution during pregnancy and early childhood lower respiratory tract infections and wheezing. The importance of study is that the NO2 levels were moderate.</p> <p>Major comments Q1: I think that the methodology for exposure assessment is not adequately clarified in the manuscript, the authors should explain it better. How many monitoring points were used? Which NO2 levels were obtained? Which GIS variables were included in the LUR models? This data gives information about the "validity" of the air pollution measurements. Answer: The exposure assessment and its methodology is clarified in a separate paper which is already included as a reference in the manuscript (Panasevich et al 2016). Additional text is provided with more information on the adjusted R2 of these models.</p> <p>I think that some recap of the methodology used to estimate NO2 exposure is strictly needed in the Manuscript. It is essential to better understand the results. At least the number of sample points, number of campaigns... The authors could add it in the text or in supplemental material</p> <p>Q2: Moreover, the authors should mention that the NO2 levels were collected during 2010 and 2011 and GIS variables were collected in 2013 in the limitations section. Answer: We agree, and have included this several places in text, including in the limitations section. I thank the authors that they had added it in the text</p> <p>Q3: I do not understand why the authors replaced the negative modelled exposures levels by 0.01. Is it the detection limit? Answer: As already specified in the manuscript, this is a value assigned to negative modelled values, or modelled values below 0.</p>
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In order to avoid the unlikely scenario of negative values, we assigned these negative values with a low value (0.01). This low value does not change the fact that these addresses are low exposed from traffic-related air pollution. We have added text to clarify this point and a reference (Panasevich et al 2016). I think that the authors would indicate the number of addresses in that it happened

Q4: The authors said that the correlations between exposures in different trimesters of pregnancy are very high. In any case, associations between exposures in different trimesters and respiratory health could have been explored in the study. Ritz and cols identified the importance of studying the critical periods of exposure (Ritz B, Wilhelm M. Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. Basic Clin Pharmacol Toxicol. 2008 Feb;102(2):182-90. doi: 10.1111/j.1742-7843.2007.00161.x.)

Answer: We agree that critical periods of pregnancy is important since different stages of development could make the foetus more vulnerable for air pollution exposure. Still, as described in the manuscript, the modelled exposure is highly correlated between the trimesters. Thus, trimester-specific models would not provide more information as compared to the whole pregnancy exposure per se. The authors would indicate that they have analyzed these periods. Could you indicate that the results do not change by trimesters?

Q5: The statistical methodology is not clear. Were the analyses adjusted by area or were the results from different centers meta-analysed?

Answer: The overall analysis was adjusted for area as supported by a previous paper on this cohort (Panasevich et al 2016, see reference #30 in the current version of the manuscript). This overall analysis was not based on a meta-analysis since we had full access to do a complete analysis. The area-variable was found to be representative of both the spatial distribution of air pollution per se (as a proxy), but also a potential proxy for unmeasured factors that could vary between each study area, factors that could influence the outcome variables (see Panasevich et al 2016).

The authors could indicated why the area variable is also a potential proxy for unmeasured factors

Q6: Regarding sensitivity analyses, it is worthwhile to note that stratified analyses are generally done subsequently to the interaction test. I.e., if interaction term is statistically significant you can proceed with stratified analyses. Otherwise, stratified analyses have not much sense.

Answer: The sensitivity test were provided to show potential conditions that could result in different overall associations. In this model: a) change of residency during pregnancy and b) pregnancies closer in time to the exposure assessment and GIS-variables included in the exposure modelling. The stratified analyses were posthoc and is thus more exploratory. This has now been included in the text. The motivation for presenting area-specific results is due to different models for each area. This information could be useful for the reader in relation to the previously published method paper by Panasevich et al 2016. Also see answer to Q5 above.

I thank the authors clarification

Q7: The authors restrict the analysis to pregnancies during the last period of the MoBa recruitment; I suppose that this is due to the problem of misclassification. The authors should discuss about this and include it in the limitations section.

Answer: Yes, correct. We have included a discussion of this in the limitations section.

I thank the authors clarification

Q8: The authors comment "Area variable may reflect the spatial distribution of air pollution, and therefore a separate analysis was performed in adjusted model without the area variable". I suppose that you have said it is important to adjust the models by area since differences between areas exist. Are covariates different?

Answer: In a previous study, area was found to be an important factor in attenuating the associations between exposure and birth outcome in MoBa (see Panasevich et al 2016 – reference #30). A general discussion of this is found in this paper.

I think that the authors should clarified it in the text

Q9: I think that would be also interesting to know the relationship between NO₂ exposure and LRTI of 0-18 . Furthermore, I am interested in the longitudinal analysis, that is, the authors could build a variable: 0: not LRTI, 1: LRTI only 0-6 months, 2: LRTI only 6-18, and 3: LRTI in both periods (incidence and persistent cases).

Answer: The combined variable "LRTI 0-18 months" yields the following results in the adjusted analysis: 1.04 (0.93, 1.16). Building a combined variable examining the incidence and persisted cases as suggested yields the following results using mlogit:

Reference group: no LRTI (N=18.880)

Group 1: only LRTI 0-6 months (N=517) RR: 0.98 (0.98, 1.21)

Group 2: only LRTI 6-18 months (N=1.520) RR: 1.07 (0.94, 1,22)

Group 3: LRTI at both 0-6 months and 6-18 months (N=311) RR: 1.02 (0.77, 1.35)

Combining group 1 and 2 above (incidence) gives: 1.04 (0.93, 1.17). This is not included in the present manuscript, but we are open for including this.

I think that this analysis could be included. These results would give more force to the paper

Q10: I do not understand this sentence: "Living outside the city was associated with higher risk of developing LRTI and wheeze, although the estimates were not statistically significant. This might be due to a higher misclassification of exposure in cities because of higher mobility and less staying at home. On the other hand, non-city population might have more homogenous exposure and spending more time close to home". What is the reason to make that claim? Do the authors have time-activity data?

Answer: The sentence has been revised in the manuscript as suggested by several of the reviewers.

I thank the authors clarification

Q11: The authors do not mention other limitations in the methodology of exposure assessment, such as not considering other air pollution sources in indoor air (e.g., gas cooking) or other

relevant outdoor pollutants.

Answer: Gas cooking has not been widely spread in Norway (as compared to European countries). Electricity has been far more widespread. The Norwegian Mother and Child Study (MoBa) does not include indoor sampling at the residential addresses at the time around each pregnancy. Thus, we use outdoor traffic-pollution exposure on residence as a proxy for residential exposure and thus "personal exposure".

The models were adjusted for tobacco smoke? It is an important indoor pollutant.

Apart from gas cooking there are other sources that can contribute to increase levels of indoor atmospheric pollutants (such as NO₂), such as tobacco. All these sources can confuse the relationship you are measuring between NO₂ and respiratory. And therefore they should be considered at the models or at least recognized in limitations.

Minor comments:

Abstract:

Q12: LRTI is already defined in introduction section. It is not necessary to include it in the results section.

Answer: We have changed this in the methods section as suggested.

I thank the authors clarification

Q13: In the section "What this paper adds", English should be checked:

Analyse the longitudinal effect of...? or Analyse longitudinally the effect...?

Answer: We appreciate the suggestions. However, the section was deleted as suggested by the Editor.

I think it's correct

Introduction:

Q14: The authors have commented "Norway is characterized by relatively low levels of air pollution". They could mention the article published by Cyrus about NO₂ levels in different European areas. "Josef Cyrus, Marloes Eeftens, Joachim Heinrich, Christophe Ampe, Alexandre Armengaud, Rob Beelen, Tom Bellander, Tímea Beregszaszi, Matthias Birk, Giulia Cesaroni, Marta Cirach, Kees de Hoogh, Audrey De Nazelle, Frank de Vocht, Christophe Declercq, Audrius Dédélé, Konstantina Dimakopoulou, Kirsten Eriksen, Claudia Galassi, Regina Grąulevičienė, Georgios Grivas, Olena Gruzieva, Annika Hagenbjörk Gustafsson, Barbara Hoffmann, Minas Iakovides, Alex Ineichen, Ursula Krämer, Timo Lanki, Patricia Lozano, Christian Madsen, Kees Meliefste, Lars Modig, Anna Mölter, Gioia Mosler, Mark Nieuwenhuijsen, Michael Nonnemacher, Marieke Oldenwening, Annette Peters, Sabrina Pontet, Nicole Probst-Hensch, Ulrich Quass, Ole Raaschou-Nielsen, Andrea Ranzi, Dorothee Sugiri, Euripides G. Stephanou, Pekka Taimisto, Ming-Yi Tsai, Éva Vaskövi, Simona Villani, Meng Wang, Bert Brunekreef, Gerard Hoek, Variation of NO₂ and NO_x concentrations between and within 36 European study areas: Results from the ESCAPE study, Atmospheric Environment, Volume 62, December 2012, Pages 374-390, ISSN 1352-2310, <http://dx.doi.org/10.1016/j.atmosenv.2012.07.080>"

Answer: Good suggestion. We have included this reference in the

	<p>manuscript. I thank the authors your consideration</p> <p>Results: Q15: How many children surpassed NO₂ boundary values established by European Directive (40 µg/m³)? Answer: A total of 27 children had annual residential address levels of NO₂ equal to or above 40 µg/m³. This has been included in the manuscript.</p>
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REVIEWER	<p>Mary Rice Assistant Professor BIDMC, Harvard Medical School Boston MA USA</p>
REVIEW RETURNED	07-May-2017

GENERAL COMMENTS	<p>Major Comments:</p> <p>The authors have done a nice job responding to reviewer comments. The manuscript is more focused, and reporting results as RR (rather than OR) is appropriate. There is a need for some minor English language editing and some additional clarity in the methods section. Some minor concerns remain about how the results of the sex-stratified results are presented.</p> <p>Specific Comments:</p> <p>Abstract:</p> <ul style="list-style-type: none"> -The first sentence of the Results of the Abstract is long and awkward and has some grammatical errors (e.g. "not statistically significant associated"). It would read better if the authors first report the NO₂ levels in 1 sentence, then report in the second sentence on the associations. -What is the value given for NO₂ of 13.6. The mean? Median? It would help to repeat that this is a pregnancy average. -What are the RR values scaled by? An IQR increase in NO₂? -In the abstract, wherever the authors state "analysis" they are generally referring to multiple "analyses" -I suggest removing the use of "due to" air pollution in the conclusion. This strongly implies causality, which cannot be inferred. Better to say "associated with" <p>Strengths and Limitations</p> <ul style="list-style-type: none"> -I do not agree with some of the listed limitations. The fact that the authors did not identify statistically significant results is not a limitation of the paper, it is simply what was observed! Also, the fact that trimester-specific averages were highly correlated is true almost anywhere in the world and is not really a limitation of this paper. The same is true for exposures in early life in the year after birth. <p>Introduction</p> <ul style="list-style-type: none"> -the interest in associating pre-natal pollution exposure and child respiratory health is not "emerging". It has been of interest to epidemiologists for decades and there have been many studies on this topic -The introduction is more focused than it was before. However, the first paragraph does not make a clear link on why air pollution might increase risk of LRTI (there are mechanistic studies and prior epi studies that lend support to this hypothesis).
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	<p>-Why are the authors discussing lung function studies (2nd paragraph of intro)? This paper is focused on early life infection and wheeze. Although these may affect lung function later in life, I think the intro should focus on the outcomes of interest, especially given the relatively large number of possible studies to cite.</p> <p>Methods:</p> <p>-The comment that LRTI are important health problems does not belong in the methods section. Authors should state exactly how LRTI was defined (i.e. what question was asked), just as they did with wheeze.</p> <p>-Since the questionnaires were filled out at 6 months and 18 months, do the outcomes of wheeze and LRTI only count as a “yes” if they were not present at 6 months, but were reported on the 18 month questionnaire (i.e. incident wheeze/LRTI)? What about those who had LRTI or wheeze prior to 6 months? This is not clear as described in the methods.</p> <p>-Authors need to describe that they ran relative risk regression models with robust standard errors (otherwise these results cannot be replicated).</p> <p>Results:</p> <p>-More confusion about how outcomes were defined: “ 4.5% had LRTI at 6 months “ (this outcome was not specified in methods), “12% had LRTI” (when? Ever LRTI by 18 months?)</p> <p>-It helps to point out that these are “post hoc” analyses, but I still feel it is odd to report stratified results when there is no statistical evidence of effect modification. The authors should be clear about this up front (as many readers will assume there was evidence of effect modification if stratified results are reported). First state that there was no evidence of effect modification by sex and the other variables before presenting those stratified results. Was the test for interaction at least borderline significant for child sex (e.g. p value for interaction term between 0.05 and 0.1?)</p> <p>Discussion:</p> <p>-The authors report that they found a “sex difference” in the association between NO₂ and LRTI, but if the tests for interaction were not significant, they did not!</p> <p>-I do not follow what the authors are saying with “the chronicity of inflammatory process has more adverse effect on girls than on boys”</p> <p>-The authors mis-state that living in non-urban area was associated with a higher risk of LRTI and wheeze, but I believe the authors mean that associations between NO₂ and these outcomes were greater in the non-urban population.</p>
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REVIEWER	Kent Pinkerton University of California, Davis United States of America
REVIEW RETURNED	15-May-2017

GENERAL COMMENTS	Excellent revised manuscript. Adequately addresses all original reviewer concerns.
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VERSION 2 – AUTHOR RESPONSE

Reviewer: 4

Reviewer Name: Mary Rice

Institution and Country: Assistant Professor, BIDMC, Harvard Medical School, Boston MA USA

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below Major Comments:

The authors have done a nice job responding to reviewer comments. The manuscript is more focused, and reporting results as RR (rather than OR) is appropriate. There is a need for some minor English language editing and some additional clarity in the methods section. Some minor concerns remain about how the results of the sex-stratified results are presented.

Specific Comments:

Abstract:

-The first sentence of the Results of the Abstract is long and awkward and has some grammatical errors (e.g. "not statistically significant associated"). It would read better if the authors first report the NO₂ levels in 1 sentence, then report in the second sentence on the associations.

Answer: We apologize for awkward language. The abstract is now changed to reflect your suggestions. The text now reads:

"Moderate mean levels of NO₂ (13.6 µg/m³, range 0.01 to 60.4) exposure at residential address during pregnancy were not statistically associated with LRTI and wheezing. No association was found per 10 µg/m³ change in NO₂ exposure and LRTI before age 6 months (adjusted RR 0.99; 95% CI 0.84 to 1.17), or between 6-18 months of age (adjusted RR 1.05; 95% CI 0.94 to 1.16). Similar, we found no association per 10 µg/m³ change in NO₂ exposure and wheezing between 6-18 months of age (adjusted RR 1.02; 95% CI 0.97 to 1.07)."

-What is the value given for NO₂ of 13.6. The mean? Median? It would help to repeat that this is a pregnancy average.

Answer: This is pregnancy mean NO₂ concentration. We have changed the text so that it now reads:

"Moderate mean levels of NO₂ (13.6 µg/m³, range 0.01 to 60.4) exposure at residential address during pregnancy were not statistically associated with LRTI and wheezing."

-What are the RR values scaled by? An IQR increase in NO₂?

Answer: As already mentioned in the manuscript, per 10 µg/m³ change in NO₂. We have now also included this information at several places in the current manuscript.

-In the abstract, wherever the authors state "analysis" they are generally referring to multiple "analyses"

Answer: We apologize for the lack of precision in the language. The current manuscript is updated to reflect when we are referring to multiple analyses and not a single analysis.

-I suggest removing the use of "due to" air pollution in the conclusion. This strongly implies causality, which cannot be inferred. Better to say "associated with"

Answer: We agree. The final sentence in the conclusion now reads as follows:

"However, a post hoc analysis gave some support to the idea that girls may have higher risk for developing LRTI given prenatal air pollution exposure."

Strengths and Limitations

-I do not agree with some of the listed limitations. The fact that the authors did not identify statistically significant results is not a limitation of the paper, it is simply what was observed! Also, the fact that trimester-specific averages were highly correlated is true almost anywhere in the world and is not really a limitation of this paper. The same is true for exposures in early life in the year after birth.

Answer: We see your point of view and agree. The last two limitations have been replaced. The list now reads:

- “Large prospective cohort with data on lower respiratory tract infections, with additional linked data from medical birth registry.
- Land use regression modelled traffic exposure assessment at residential address using both spatial and temporal adjustment.
- The mean air pollution exposures explored in this study are relatively low.
- Not possible to identify pregnancies where the mothers continued to live at the same address during the whole study period.”

Introduction

-the interest in associating pre-natal pollution exposure and child respiratory health is not “emerging”. It has been of interest to epidemiologists for decades and there have been many studies on this topic

-The introduction is more focused than it was before. However, the first paragraph does not make a clear link on why air pollution might increase risk of LRTI (there are mechanistic studies and prior epi studies that lend support to this hypothesis).

Answer: The text in the introduction is changed to reflect the comments. We have included a new sentence in the first paragraph and it now reads:

“There is increasing evidence from both experimental and epidemiologic studies that the prenatal period is a critical window for harmful effects from different types of exposures on respiratory health.¹”

We have also updated the second paragraph and it now reads:

“The effect of air pollution exposure during pregnancy on respiratory health and allergic responses early in life has been examined by several studies with large heterogeneity.¹⁹⁻²⁴ Some studies report associations of prenatal air pollution exposure with LRTI in early childhood.¹⁹⁻²² Other studies have found no support for an association between air pollution exposure and LRTI in early childhood.^{23 24} In addition, there are animal exposure studies that have identified both anatomic/mechanical and immunological mechanisms by which air pollution exposure may increase susceptibility of the respiratory system to infections.^{25 26}”

-Why are the authors discussing lung function studies (2nd paragraph of intro)? This paper is focused on early life infection and wheeze. Although these may affect lung function later in life, I think the intro should focus on the outcomes of interest, especially given the relatively large number of possible studies to cite.

Answer: We have changed the manuscript to reflect the comments regarding lung function, and the manuscript is now focused on LRTI and wheeze. See our answer to previous comments above.

Methods:

-The comment that LRTI are important health problems does not belong in the methods section. Authors should state exactly how LRTI was defined (i.e. what question was asked), just as they did with wheeze.

Answer: We agree and have deleted the mentioned sentence in the method section. We have also updated the text as follows:

“The outcomes, LRTI and wheeze, were based on the maternal report from questionnaires filled when

children were 6 and 18 months of age. The questionnaires can be viewed at the MoBa website (<https://www.fhi.no/en/studies/moba/>). LRTIs included respiratory syncytial virus, bronchiolitis, bronchitis, and pneumonia. We classified hospitalization for any of these conditions as being hospitalized for LRTI at a) between 0-6 months of age, and b) between 6-18 months of age. Wheeze was defined as “wheezing/whistling in the chest” or “tightness in the chest” between 6 and 18 months of age. The outcomes were treated as dichotomous.”

-Since the questionnaires were filled out at 6 months and 18 months, do the outcomes of wheeze and LRTI only count as a “yes” if they were not present at 6 months, but were reported on the 18 month questionnaire (i.e. incident wheeze/LRTI)? What about those who had LRTI or wheeze prior to 6 months? This is not clear as described in the methods.

Answer: We apologize that this was unclear. The questionnaire at 6 months covered LRTI and wheeze between 0-6 months. We have updated the text so that it reflects that it is 0-6 months and not at 6 months. The text in the methods section now reads:

“We classified hospitalization for any of these conditions as being hospitalized for LRTI at a) between 0-6 months of age, and b) between 6-18 months of age. Wheeze was defined as “wheezing/whistling in the chest” or “tightness in the chest” between 6 and 18 months of age.”

-Authors need to describe that they ran relative risk regression models with robust standard errors (otherwise these results cannot be replicated).

Answer: Correct, and the text in the method section is now updated to reflect this:

“Generalized linear models was fitted to evaluate the associations between pregnancy NO₂ exposure and respiratory outcomes. Results are presented for crude and adjusted models as risk ratios (RR) with robust standard errors.”

Results:

-More confusion about how outcomes were defined: “ 4.5% had LRTI at 6 months “ (this outcome was not specified in methods), “12% had LRTI” (when? Ever LRTI by 18 months?)

Answer: We apologize for the misunderstanding. This is not a new outcome, and what we indicate is that by 6 months, a total of 4.5% had registered LRTI. Also see previous comment above concerning “0-6 months versus at 6 months”. We have updated the text to clarify this:

“A total of 4.5% of children had LRTI between 0-6 months of age, and 12% of children had LRTI between 6-18 months of age. A total of 40.6% of the children had wheezing symptoms between 6 and 18 months of age.”

-It helps to point out that these are “post hoc” analyses, but I still feel it is odd to report stratified results when there is no statistical evidence of effect modification. The authors should be clear about this up front (as many readers will assume there was evidence of effect modification if stratified results are reported). First state that there was no evidence of effect modification by sex and the other variables before presenting those stratified results. Was the test for interaction at least borderline significant for child sex (e.g. p value for interaction term between 0.05 and 0.1?)

Answer: There was a borderline significant interaction, and we have updated the text in the method section so that it now reads:

“There was no evidence of effect modification by the adjustment variables. Still, there was a borderline significant interaction for child sex. A stratified analysis post hoc, showed a consistent tendency for association of pregnancy NO₂ exposure with LRTI and wheeze in girls, but not in boys.”

Discussion:

-The authors report that they found a “sex difference” in the association between NO₂ and LRTI, but if the tests for interaction were not significant, they did not!

Answer: True enough, and we apologize for this statement. We have removed the term “sex difference” and the text in the discussion now reads as follows:

“In a post hoc analysis, we found a consistent tendency of prenatal NO₂ exposure association with LRTI: the association was present in girls, but not in boys. The tests of interaction was only borderline significant.”

-I do not follow what the authors are saying with “the chronicity of inflammatory process has more adverse effect on girls than on boys”

Answer: We have changed the sentence so that it now reads:

“As was reviewed by Casimir and colleagues, while boys outnumber girls in acute respiratory infections, girls had an overall enhanced inflammatory response.³⁸ This increased inflammatory response could have more adverse effects on girls than on boys.³⁸”

-The authors mis-state that living in non-urban area was associated with a higher risk of LRTI and wheeze, but I believe the authors mean that associations between NO₂ and these outcomes were greater in the non-urban population.

Answer: We have changed the sentence as suggested. It now reads:

“Associations between prenatal air pollution exposure and the respiratory outcomes was overall greater in the non-urban areas, although the estimates were not statistically significant.”

Reviewer: 2

Reviewer Name: Marisa Estarlich and Amparo Ferrero Institution and Country: CIBERESP, FISABIO, SPAIN Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below General comments The authors study the associations between exposure to air pollution during pregnancy and early childhood lower respiratory tract infections and wheezing. The importance of study is that the NO₂ levels were moderate.

Major comments

Q1: I think that the methodology for exposure assessment is not adequately clarified in the manuscript, the authors should explain it better. How many monitoring points were used? Which NO₂ levels were obtained? Which GIS variables were included in the LUR models? This data gives information about the "validity" of the air pollution measurements.

Answer: The exposure assessment and its methodology is clarified in a separate paper which is already included as a reference in the manuscript (Panasevich et al 2016). Additional text is provided with more information on the adjusted R² of these models.

I think that some recap of the methodology used to estimate NO₂ exposure is strictly needed in the Manuscript. It is essential to better understand the results.

At least the number of sample points, number of campaigns... The authors could add it in the text or in supplemental material

Answer: We have updated the section “Air pollution exposure” so that it now includes the number of sampling sites/sampling points. The text already includes information on number of campaigns (N=3, winter, summer and intermediate season).

“We measured the spatial distribution of air pollution for Oslo and Akershus in 2010, and for Bergen and Hordaland in 2011. Measurement campaigns included three rounds of approximately two weeks

duration with NO₂ measurements (during winter, summer and an intermediate season) within a one year period. Measurement sites (14 in Oslo, 36 in Akershus and 46 in Bergen/Hordaland) were selected to represent the range of residential exposure for each study area. In the analyses, we included sites with no missing data, and no geocoding mismatches.”

Q2: Moreover, the authors should mention that the NO₂ levels were collected during 2010 and 2011 and GIS variables were collected in 2013 in the limitations section.

Answer: We agree, and have included this several places in text, including in the limitations section. I thank the authors that they had added it in the text

Answer: Good to hear.

Q3: I do not understand why the authors replaced the negative modelled exposures levels by 0.01. Is it the detection limit?

Answer: As already specified in the manuscript, this is a value assigned to negative modelled values, or modelled values below 0. In order to avoid the unlikely scenario of negative values, we assigned these negative values with a low value (0.01). This low value does not change the fact that these addresses are low exposed from traffic-related air pollution. We have added text to clarify this point and a reference (Panasevich et al 2016).

I think that the authors would indicate the number of addresses in that it happened

Answer: We have included the number of addresses in the section “Air pollution exposure”. The text now reads:

“Negative modelled values were replaced with 0.01 to avoid the unlikely scenario of negative modelled exposure and keep these in the analyses as low exposed addresses (N=112).28“

Q4: The authors said that the correlations between exposures in different trimesters of pregnancy are very high. In any case, associations between exposures in different trimesters and respiratory health could have been explored in the study. Ritz and cols identified the importance of studying the critical periods of exposure (Ritz B, Wilhelm M. Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. *Basic Clin Pharmacol Toxicol*. 2008 Feb;102(2):182-90. doi: 10.1111/j.1742-7843.2007.00161.x.)

Answer: We agree that critical periods of pregnancy is important since different stages of development could make the foetus more vulnerable for air pollution exposure. Still, as described in the manuscript, the modelled exposure is highly correlated between the trimesters. Thus, trimester-specific models would not provide more information as compared to the whole pregnancy exposure per se.

The authors would indicate that they have analyzed these periods. Could you indicate that the results do not change by trimesters?

Answer: We have not analysed by trimester per se, since the trimester-specific exposures were highly correlated. Any further analyses of trimester-specific associations would be difficult to interpret. The trimester-specific exposures is already been included in the paper in Table 2 and show mean values and SD by area. We have moved some of the text from the introduction to the results section and included the range of correlation between trimester-specific NO₂ concentrations.

“Exposures by trimester and the whole pregnancy exposure were highly correlated ($r = 0.73$ to 0.85). We therefore decided to use only the average NO₂ exposure during the whole pregnancy as our exposure estimate in the analyses.”

Q5: The statistical methodology is not clear. Were the analyses adjusted by area or were the results from different centers meta-analysed?

Answer: The overall analysis was adjusted for area as supported by a previous paper on this cohort (Panasevich et al 2016, see reference #30 in the current version of the manuscript). This overall

analysis was not based on a meta-analysis since we had full access to do a complete analysis. The area-variable was found to be representative of both the spatial distribution of air pollution per se (as a proxy), but also a potential proxy for unmeasured factors that could vary between each study area, factors that could influence the outcome variables (see Panasevich et al 2016).

The authors could indicated why the area variable is also a potential proxy for unmeasured factors
Answer: We have updated the text under the method section so that it now reads as follows:

“Area variable was defined by the location of the address at delivery: Oslo, Akershus, Bergen and Hordaland. In a previous study, the area variable was found to be an important factor in attenuating the associations between exposure and birth outcome in MoBa28 These differences by area could be linked to more travel to and from work and to other activities, visiting central more polluted parts of the city by e.g. higher educated women and for city dwellers per se. This variable is included in the adjusted models since it previously has been reported to be a potential proxy for unmeasured factors that could vary between each study area and thus could influence the outcome variables within each separate area.”

Q6: Regarding sensitivity analyses, it is worthwhile to note that stratified analyses are generally done subsequently to the interaction test. I.e., if interaction term is statistically significant you can proceed with stratified analyses. Otherwise, stratified analyses have not much sense.

Answer: The sensitivity test were provided to show potential conditions that could result in different overall associations. In this model: a) change of residency during pregnancy and b) pregnancies closer in time to the exposure assessment and GIS-variables included in the exposure modelling. The stratified analyses were posthoc and is thus more exploratory. This has now been included in the text. The motivation for presenting area-specific results is due to different models for each area. This information could be useful for the reader in relation to the previously published method paper by Panasevich et al 2016. Also see answer to Q5 above.

I thank the authors clarification

Answer: Good to hear.

Q7: The authors restrict the analysis to pregnancies during the last period of the MoBa recruitment; I suppose that this is due to the problem of misclassification. The authors should discuss about this and include it in the limitations section.

Answer: Yes, correct. We have included a discussion of this in the limitations section.

I thank the authors clarification

Answer: Good to hear.

Q8: The authors comment “Area variable may reflect the spatial distribution of air pollution, and therefore a separate analysis was performed in adjusted model without the area variable”. I suppose that you have said it is important to adjust the models by area since differences between areas exist. Are covariates different?

Answer: In a previous study, area was found to be an important factor in attenuating the associations between exposure and birth outcome in MoBa (see Panasevich et al 2016 – reference #30). A general discussion of this is found in this paper.

I think that the authors should clarified it in the text

Answer: We have updated the text under the method section so that it now reads as follows:

“Area variable was defined by the location of the address at delivery: Oslo, Akershus, Bergen and Hordaland. In a previous study, the area variable was found to be an important factor in attenuating

the associations between exposure and birth outcome in MoBa28 This variable is included in the adjusted models since it previously has been reported to be a potential proxy for unmeasured factors that could vary between each study area and thus could influence the outcome variables within each separate area.”

Q9: I think that would be also interesting to know the relationship between NO₂ exposure and LRTI of 0-18 . Furthermore, I am interested in the longitudinal analysis, that is, the authors could build a variable: 0: not LRTI, 1: LRTI only 0-6 months, 2: LRTI only 6-18, and 3: LRTI in both periods (incidence and persistent cases).

Answer: The combined variable “LRTI 0-18 months” yields the following results in the adjusted analysis: 1.04 (0.93, 1.16). Building a combined variable examining the incidence and persisted cases as suggested yields the following results using mlogit:

Reference group: no LRTI (N=18.880)

Group 1: only LRTI 0-6 months (N=517) RR: 0.98 (0.98, 1.21) Group 2: only LRTI 6-18 months (N=1.520) RR: 1.07 (0.94, 1.22) Group 3: LRTI at both 0-6 months and 6-18 months (N=311) RR: 1.02 (0.77, 1.35)

Combining group 1 and 2 above (incidence) gives: 1.04 (0.93, 1.17). This is not included in the present manuscript, but we are open for including this.

I think that this analysis could be included. These results would give more force to the paper

Answer: We have updated the results section so that it now reads as follows:

“An analysis of the cumulative incidence of LRTI (0-18 months of age) was equal to an RR= 1.04 (95% CI 0.93, 1.17).”

Q10: I do not understand this sentence: "Living outside the city was associated with higher risk of developing LRTI and wheeze, although the estimates were not statistically significant. This might be due to a higher misclassification of exposure in cities because of higher mobility and less staying at home. On the other hand, non-city population might have more homogenous exposure and spending more time close to home". What is the reason to make that claim? Do the authors have time-activity data?

Answer: The sentence has been revised in the manuscript as suggested by several of the reviewers.

I thank the authors clarification

Answer: Good to hear.

Q11: The authors do not mention other limitations in the methodology of exposure assessment, such as not considering other air pollution sources in indoor air (e.g., gas cooking) or other relevant outdoor pollutants.

Answer: Gas cooking has not been widely spread in Norway (as compared to European countries). Electricity has been far more widespread. The Norwegian Mother and Child Study (MoBa) does not include indoor sampling at the residential addresses at the time around each pregnancy. Thus, we use outdoor traffic-pollution exposure on residence as a proxy for residential exposure and thus “personal exposure”.

The models were adjusted for tobacco smoke? It is an important indoor pollutant.

Apart from gas cooking there are other sources that can contribute to increase levels of indoor atmospheric pollutants (such as NO₂), such as tobacco. All these sources can confuse the relationship you are measuring between NO₂ and respiratory. And therefore they should be considered at the models or at least recognized in limitations.

Answer: Yes, the models are adjusted for smoking during pregnancy. This is included in the original manuscript in the tables, but we see that it is not so clearly stated in the text. We have therefore updated the text as follows under the method section:

“Multiplicative interactions were tested in the adjusted models between the continuous NO₂ pregnancy exposure variable and the following categorical variables: area, sex, smoking during pregnancy, parity, birth season and maternal atopy.”

Minor comments:

Abstract:

Q12: LRTI is already defined in introduction section. It is not necessary to include it in the results section.

Answer: We have changed this in the methods section as suggested.

I thank the authors clarification

Answer: Good to hear.

Q13: In the section “What this paper adds”, English should be checked:

Analyse the longitudinal effect of...? or Analyse longitudinally the effect...?

Answer: We appreciate the suggestions. However, the section was deleted as suggested by the Editor.

I think it's correct

Answer: Yes, it is.

Introduction:

Q14: The authors have commented “Norway is characterized by relatively low levels of air pollution”.

They could mention the article published by Cyrus about NO₂ levels in different European areas.

“Josef Cyrus, Marloes Eeftens, Joachim Heinrich, Christophe Ampe, Alexandre Armengaud, Rob Beelen, Tom Bellander, Timea Beregszaszi, Matthias Birk, Giulia Cesaroni, Marta Cirach, Kees de Hoogh, Audrey De Nazelle, Frank de Vocht, Christophe Declercq, Audrius Dédélé, Konstantina Dimakopoulou, Kirsten Eriksen, Claudia Galassi, Regina Gražulevičienė, Georgios Grivas, Olena Gruzjeva, Annika Hagenbjörk Gustafsson, Barbara Hoffmann, Minas Iakovides, Alex Ineichen, Ursula Krämer, Timo Lanki, Patricia Lozano, Christian Madsen, Kees Meliefste, Lars Modig, Anna Mölter, Gioia Mosler, Mark Nieuwenhuijsen, Michael Nonnemacher, Marieke Oldenwening, Annette Peters, Sabrina Pontet, Nicole Probst-Hensch, Ulrich Quass, Ole Raaschou-Nielsen, Andrea Ranzi, Dorothee Sugiri, Euripides G. Stephanou, Pekka Taimisto, Ming-Yi Tsai, Éva Vaskövi, Simona Villani, Meng Wang, Bert Brunekreef, Gerard Hoek, Variation of NO₂ and NO_x concentrations between and within 36 European study areas: Results from the ESCAPE study, Atmospheric Environment, Volume 62, December 2012, Pages 374-390, ISSN 1352-2310, <http://dx.doi.org/10.1016/j.atmosenv.2012.07.080>”

Answer: Good suggestion. We have included this reference in the manuscript.

I thank the authors your consideration

Answer: Good to hear.

Results:

Q15: How many children surpassed NO₂ boundary values established by European Directive (40 µg/m³)?

Answer: A total of 27 children had annual residential address levels of NO₂ equal to or above 40 µg/m³. This has been included in the manuscript.

I thank the authors clarification

Answer: Good to hear.

Reviewer: 5

Reviewer Name: Kent Pinkerton

Institution and Country: University of California, Davis, United States of America Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below Excellent revised manuscript. Adequately addresses all original reviewer concerns.

Answer: Thank you for positive feedback on our manuscript.

VERSION 3 - REVIEW

REVIEWER	Marisa Estarlich and Amparo Ferrero CIBER Epidemiology and Public Health (CIBERESP), Madrid, Spain, and the Joint Research Unit for Epidemiology and Environmental Health, FISABIO-Universitat de València-Universitat Jaume I, Valencia, Spain
REVIEW RETURNED	14-Jul-2017

The reviewer completed the checklist but made no further comments.

REVIEWER	Mary Rice BIDMC, USA
REVIEW RETURNED	27-Jul-2017

GENERAL COMMENTS	<p>Overall Comment: The authors have been reasonably responsive to the reviewer comments. I continue to have concerns about reporting effect modification by sex. Describing the stratified analysis as “post hoc” and “exploratory” does not resolve this issue. In this well-powered study, there was no statistical evidence of effect modification by sex. The statement in the abstract of an “increased risk for LRTIs 6 to 18 months in girls only” implies there is evidence of effect modification. Stratified analyses often yield spurious results.</p> <p>Additional Comments: -is it a strength or a limitation that the mean air pollution levels are low? I would say it is a strength and suggest rewriting this point slightly to make that point -I agree with reviewer 1 that the discussion on smoking in the introduction is not very relevant, especially since there is adequate data on air pollution and early life infection to motivate this study. The authors added a reference, but did not really change the text. I recommend removing the discussion of tobacco from the introduction. -Authors mention that there is “borderline significant interaction” by sex and heavily emphasize the finding of an OR with a CI that does not cross the null for girls at 6-18 months only. But as far as I can tell, these results are not provided in a table anywhere. What is</p>
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	<p>meant by borderline significant?</p> <p>-in the abstract, the sentence starting with “Similar,..” should be “Similarly,..” or just delete the word</p> <p>-the limitation “Not possible to identify pregnancies where the mothers continued to live at the same address during the whole study period.” Is a bit confusing to follow by someone who has not read the full manuscript. “Unable to account for any changes in address location and pollution exposure during pregnancy”</p> <p>-page 7 line 17 “retrospective” should be “retrospectively”</p>
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VERSION 3 – AUTHOR RESPONSE

Reviewer: 4

Reviewer Name: Mary Rice

Institution and Country: BIDMC, USA

Please state any competing interests or state ‘None declared’: No competing interests

Please leave your comments for the authors below Overall Comment:

The authors have been reasonably responsive to the reviewer comments. I continue to have concerns about reporting effect modification by sex. Describing the stratified analysis as “post hoc” and “exploratory” does not resolve this issue. In this well-powered study, there was no statistical evidence of effect modification by sex. The statement in the abstract of an “increased risk for LRTIs 6 to 18 months in girls only” implies there is evidence of effect modification. Stratified analyses often yield spurious results.

Answer: This seems to be a challenge with our manuscript. After several attempts, this is yet to be resolved. To resolve this issue, we have now removed all mentioning of effect modification by sex in the current version of the manuscript.

Additional Comments:

-is it a strength or a limitation that the mean air pollution levels are low? I would say it is a strength and suggest rewriting this point slightly to make that point

Answer: We have changed the sentence to (hopefully) reflect that low air pollution concentrations is considered a strength. The sentence now reads: “Cohort living in areas with low to moderate air pollution concentrations”. We hope that this is sufficient.

-I agree with reviewer 1 that the discussion on smoking in the introduction is not very relevant, especially since there is adequate data on air pollution and early life infection to motivate this study. The authors added a reference, but did not really change the text. I recommend removing the discussion of tobacco from the introduction.

Answer: We agree. The discussion on smoking in the introduction has been removed as suggested.

-Authors mention that there is “borderline significant interaction” by sex and heavily emphasize the finding of an OR with a CI that does not cross the null for girls at 6-18 months only. But as far as I can tell, these results are not provided in a table anywhere. What is meant by borderline significant?

Answer: This part of the manuscript is now removed. Still, borderline significant interaction is here a p-value between 0.05 and 0.10.

-in the abstract, the sentence starting with “Similar,..” should be “Similarly,..” or just delete the word

Answer: Hereby corrected in the current version of the manuscript.

-the limitation “Not possible to identify pregnancies where the mothers continued to live at the same address during the whole study period.” Is a bit confusing to follow by someone who has not read the full manuscript. “Unable to account for any changes in address location and pollution exposure during pregnancy”

Answer: We agree, and have changed the wording as suggested.

-page 7 line 17 "retrospective" should be "retrospectively"
Answer: Hereby corrected in the current version of the manuscript.