Author's response to reviews

Title: Residential traffic exposure and pregnancy-related outcomes in a prenatally recruited birth cohort

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Author's response to reviews: see over
Dear Professor Grandjean and Professor Ozonoff,

Thank you very much for reviewing our manuscript: “Residential traffic exposure and pregnancy-related outcomes in mother and child: The Generation R Study” (Manuscript 6655756103129274).

We are grateful for the extensive and useful comments of the reviewers. We have made changes in our manuscript in response to these comments. Most importantly, we have focused on a limited number of outcomes and exposures, to reduce the amount of data presented. In addition, we have omitted the evaluation of maternal sociodemographic risk factors for traffic exposure. Furthermore, we have included the results of various sensitivity analyses as Additional files. We feel that these suggested changes have improved the quality of the manuscript.

Attached and in response to the reviewers’ reports, you will find our specific responses to all the questions and comments from the reviewers. We hope you will find the revised manuscript acceptable for publication in Environmental Health.

Please contact us if further clarifications or modifications are required.

With kind regards,

Yours sincerely,

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Answers to the editor’s and reviewers’ comments have been inserted in blue. The sentences presented below in bold are adjusted in the revised manuscript, in response to the reviewers’ comments.

**EDITOR’S COMMENTS TO AUTHOR**

The title should include the study design e.g. X is a risk factor for Y: a case control study (Generation R is not informative in this regard). When you revise the text, please focus on key messages relevant to the international readership of Environmental Health. The Discussion is rather lengthy and would benefit from being tightened.

We have rewritten the title into “Residential traffic exposure and pregnancy-related outcomes in mother and child in a prenatally recruited birth cohort”. Furthermore, we have made shortened the Discussion, and, where possible, rephrased it more concise.

Please also ensure that your revised manuscript conforms to the journal style (http://www.ehjournal.net/info/instructions/). It is important that your files are correctly formatted.

On the title page, the academic degrees should be removed after the names and the colon and all information after it following the phrase Corresponding author. The email addresses should be formatted in sentence format separating the pairs with semi-colons. Please remove the page numbers and italics in the text. The Introduction should be moved to the top of the next page and changed to Background. The figure notation toward the bottom of page 13 should be moved after the references section. Please include a List of Abbreviations section before the Competing interests section and move these sections and those following to the top of the next page. The journal article titles and the volume numbers should be bolded. (See Instructions for Authors for more information.) The tables should be formatted in closed cell format with the horizontal and vertical lines visible. Whenever possible, please try to fit tables on one page in portrait format. Otherwise, please submit as additional files that will be linked into the final published article in the form supplied by the author, but will not be displayed within the paper. They will be made available in exactly the same form as originally provided.

We have made these adjustments to conform to the style of *Environment Health*. 
1. Comparable studies have been performed and are mentioned, however no discussion of size of study populations and powers of this study and comparable studies has been given. A table is suggested.

   We agree with the reviewer that we should elaborate on these topics in the manuscript. The following sentences were added in the beginning of the Statistical Analysis section: “Based on a population for analysis of 7,000 subjects and a proportion exposed of 10%, we were able to detect a difference of 0.11 SD (type I error of 5%, type II error of 20% (power 80%)) for a continuous normally distributed outcome. Previous studies on air pollution and birth weight showed reductions in birth weight ranging to 140 grams, which is equal to 0.3 SD.” Additionally, we adjusted the consideration of statistical power in the Discussion section in the following way: “Second, the sample size of our study was smaller than that of previous studies, which were based on birth certificate data and had sample sizes of 37,000-99,000 subjects. Our study had 7,339 participants. We were able to detect a difference of 0.11 SD in birth weight, which is smaller than effect sizes observed in previous studies. However, the power to detect a relationship between air pollution and some of the dichotomous outcome measures was lower compared to previous studies, especially for the analyses with pregnancy complications.”

2. The discussion should include validity of exposure assessment eg compared to monitoring and biomonitoring data as described eg in Pedersen et al (2009) Increased micronuclei and bulky DNA adducts in cord blood after maternal exposures to traffic-related air pollution Environ Res.109:1012-1020.

   The suggested reference on biomonitoring in a cross-sectional study (n=75) provides an interesting additional method to assess exposure in specific study designs. For our large prospective cohort study (n=7,339), however, these methods are too expensive and too much burden for study participants because many samples over many years would be required. Therefore, exposure models are often used for prospective cohort studies. We have added the following sentences to the first paragraph of the Background: “In large studies, assessing individual exposure to air pollution is often rather demanding for participants and requires extensive resources. Therefore, other approaches have been used to estimate exposure of individuals.” Furthermore, the reviewer is right that it is important to discuss the validity of the exposure assessment. In the manuscript, we have indicated that there are limitations to the exposure assessment method that we used. This is described in the following section: “Moreover, traffic measures may be viewed as crude estimates of air pollution. They do not take into account influencing factors such as type of traffic, emission factors, meteorology, and land cover data. Furthermore, they are based on annual averages and do not reflect seasonal, monthly or daily differences in air pollution levels. Ideally, these temporal variations would be taken into account in the exposure assessment, next to the spatial variability. This has been done by a few earlier pregnancy studies, some of them conducted in Europe, in which air pollution concentrations were modeled
and subsequently adjusted for temporal variation [33, 34, 51, 57]. Unfortunately, we were not able to take into account temporal variations in our air pollution exposure assessment, but we are planning to do this for future analyses.”

The use of biomarkers may be subject for a separate substudy, but we find those methods currently outside the scope of this paper.

3. Perspectives for future analyses of the hypothesis could be given.

For future research on this topic, we would recommend to use more refined exposure assessment estimates that capture both the spatial and temporal variation in exposure. We have addressed this point in the Conclusion of the Abstract and in the Discussion and Conclusion section of the manuscript.
Reviewer: 2
Comments to the Author

General comments
1. This is an interesting paper. In particular the analysis between air pollution exposure and pregnancy complications is innovative.

   Thank you.

2. The manuscript in its current state presents large amounts of data. Seven outcomes (4 pregnancy outcomes and 3 pregnancy complications) were linked to 3 exposure variables. The manuscript would benefit from focusing on a limited number of outcomes and exposures. My suggestion is to focus on two pregnancy outcomes describing fetal growth and pregnancy duration (for instance preterm birth and term birth weight). Cumulative traffic density and distance weighted traffic density within a 150 m radius around the participants’ homes were highly correlated (r=0.93). Therefore, one of these two variables can be left omitted without losing information.

   We followed the reviewer’s suggestion, and have omitted the results focused on low birth weight as outcome. Furthermore, we have decided to focus on distance-weighted traffic density and distance to a major road as exposures, and to exclude cumulative traffic density. As a result, we have adjusted the sections of the manuscript in which either cumulative traffic density or low birth weight were discussed. For the same reason, we have shortened and restructured the paragraph in the beginning of the Discussion in which comparisons with other relevant studies are made.

3. Reducing the number of exposures and outcomes, would give the authors the possibility to present the results of the large number of sensitivity analyses they have done, but are not shown in the current version of the manuscript, e.g. the analyses in Dutch children and non-movers, and the analyses on the potential effect modification by socio-economic status. The paper would benefit a lot from showing the results of these analyses.

   We have included the results of the mentioned sensitivity analyses as Additional files. The analyses in Dutch children are shown in Additional file 4. The results of the stratified analyses by maternal educational level are presented in Additional files 5, 6 and 7 for selected outcomes. Additional file 8 and 9 show the results of the analyses in non-movers. These additional tables are added as supplementary tables to the paper.

4. The number of women with pregnancy complications is small in this population, which raises the question of statistical power. Did the authors perform any power calculations? A discussion of statistical power needs to be added.

   See point 1 of the first reviewer. We have elaborated on this topic in the Statistical Analysis paragraph and in the Discussion.

Major compulsory comments
Introduction
5. The authors review the types of exposure assessment that have been used in studies on air pollution and pregnancy outcomes and underline the importance of taking into account the intra-urban variability of air pollution levels. Using indicator variables like (distance
weighted) traffic densities is one way to approach this problem. However, this is only one possible approach, which has some limitations, which should be discussed in the discussion e.g. the fact that temporal variability of exposure is not accounted for. Recently, other approaches that take into account both the temporal and spatial variability of air pollution levels have been introduced in studies on air pollution and pregnancy outcomes (Slama et al. Environmental Health Perspectives 2007; Brauer et al. Environmental Health Perspectives 2008; Fanshawe et al. Environmetrics 2007; Aguilera et al. Environmental Health perspectives), which should be mentioned here.

See also point 2 of the first reviewer. We have included a discussion of the studies mentioned by the reviewer (see below). We also describe the limitations of using traffic indicators. The paragraph has been changed into:

“Moreover, traffic measures may be viewed as crude estimates of air pollution. They do not take into account influencing factors such as type of traffic, emission factors, meteorology (wind direction, wind speed and temperature), and land cover data. Furthermore, they are based on annual averages and do not reflect seasonal, monthly or daily differences in air pollution levels. Ideally, these temporal variations would be taken into account in the exposure assessment, next to the spatial variability. This has been done by a few earlier pregnancy studies, in which air pollution concentrations were modeled and subsequently adjusted for temporal variation [33, 34, 51, 57]. Unfortunately, we were not able to take into account temporal variations in our air pollution exposure assessment, but we are planning to do this for future analyses.”

Materials and methods
6. Page 7, line 1. It is not clear whether the complete address history of the mothers was included in the present analysis or whether exposure indicators were assessed for one specific address (e.g. birth address or address at recruitment). This needs clarification.

We have now clarified that the exposure indicators were assessed at one specific address. We have specified this further on in the mentioned paragraph, with the following sentence: “We geocoded the mothers’ home addresses at time of delivery using ArcGIS (v9, ESRI).”

7. Page 7 f. calculation of distance-weighted traffic densities. This is difficult to follow. The authors refer to papers by Pearson et al. and Wilhelm and Ritz, but if I understand it correctly, the authors did neither exactly what Pearson did nor what Wilhelm and Ritz did in their publications. My suggestion is to skip the references, and to describe what was actually done.

We have deleted the references of Pearson et al. and Wilhelm & Ritz in the first part of the section to improve the comprehensibility. At the end of the section, a comparison with the method used by Wilhelm & Ritz is made, also to illustrate that this method has been used in the literature before. We have changed the section into:

“Distance-weighted traffic density was calculated using MapInfo Professional (v9.0, Pitney Bowes). To estimate the dispersion of motor vehicle exhaust, we employed a model that was based on a Gaussian distribution that assumes that 96% of the emitted pollutants disperse up to 150m from the road:
\[ Y_i = \frac{1}{0.4\sqrt{2\pi}} \exp \left( -\frac{1}{2} \frac{D_i}{150} \right) \]

where \( D_i \) is the distance from the road segment \( i \). This curve was used to weigh the products of the length (in m) and the traffic intensities (in vehicles/24h) of all road segments within the buffer. The weighted values were summed for each subject to obtain the distance-weighted traffic density. As vehicles may use multiple segments in the buffer, the DWTD values can be relatively high (up to millions of vehicles/24h*m). Various definitions of DWTD are being used in the literature. We remark that our method to define DWTD is derived from the method used by Wilhelm & Ritz [30], with the difference that we take into account the length of the roads within the buffer.

8. Page 8f, birth and pregnancy outcomes. I agree that using the date of the last menstrual period for exposure assessment has limitations. Given the fact that ultrasound measurements were available for all children apart from the 3% of the population that was enrolled in late pregnancy, one option could be to limit the analyses to the 97% of the population for which gestational age was (consistently) determined by ultrasound measurements.

The reviewer is right that it should be verified whether the method for determining gestational age influenced the results. We have conducted sensitivity analyses in the 97% of the participants who were enrolled in early and mid pregnancy and for whom gestational age was determined by ultrasounds. These analyses did not show different results. Therefore, we decided to present the results for the total population in the manuscript. We included this explanation in the relevant sections in the manuscript. In the Sensitivity Analysis paragraph in the Methods section, we added the following sentences: “Furthermore, to evaluate whether the results were sensitive to the method of determining gestational age (ultrasound versus LMP), we repeated the analyses after excluding women who were enrolled in late pregnancy, since only mothers who were enrolled in mid- and late pregnancy were dated on ultrasound.”

In the Results section, we added: “Furthermore, results of the analyses did not change after excluding women who were enrolled in late pregnancy and for whom gestational age was determined based on LMP.”

And in the Discussion, we added the following sentences to the description of potential limitations: “Furthermore, gestational age could not be determined based on ultrasound examinations in 3% of the participants, because they were enrolled in late pregnancy. Nevertheless, results were comparable when these women were included or excluded.”

9. Page 9, birth and pregnancy outcomes. Swedish reference standards were used to define “small size for gestational age” although Dutch standards are available (www.perinatreg.nl/). Why did the authors decide to use the Swedish reference standards instead of the Dutch reference standards?
The reason that Swedish reference standards were used is that these are based on a larger population. Consequently, they include the extremes of the birth weight distribution, which is not the case for the Dutch reference standards. We have added this explanation to the text and rephrased the sentence into:

“Gestational age-adjusted standard deviation birth weight scores were based on published reference charts from a North European birth cohort [33], which are based on a large population and include the extremes of the birth weight distribution.”

10. Page 10, covariates. Maternal BMI was defined as maternal BMI at intake, which was in mid or late pregnancy for one quart of the population. This means that for this part of the population, maternal BMI as defined here most likely is an overestimation of maternal pre-pregnancy BMI. The fact that the correlation between pre-pregnancy BMI and BMI at enrolment is high \((r=0.97)\) does not convince me that BMI at enrolment is a good estimate of pre-pregnancy BMI. A high correlation can occur despite significant differences between pre-pregnancy BMI and BMI at intake.

We agree that the high correlation between pre-pregnancy BMI and BMI at intake does not justify the decision to adjust for the latter. Therefore, we conducted sensitivity analyses using pre-pregnancy BMI instead of BMI at intake. This information was available in 5,899 women (out of 7,339). The results of these analyses were similar to the results presented in the paper. Therefore, we decided to show the complete data, adjusting for BMI at intake. We have also explained this in the manuscript.

In the Sensitivity Analysis paragraph in the Methods, we added the following sentences:

“In addition, we repeated analyses in a subsample of women with data available on body mass index before pregnancy, and adjusted these analyses for BMI before pregnancy rather than BMI at intake.”

And in the Results section, we added:

“Results were also comparable when analyses were adjusted for maternal BMI before pregnancy rather than maternal BMI at intake.”

Population for analysis

11. What was the reason that exposure indicators could not be calculated for 1% of the population?

The exposure indicators could not be calculated for a small part of the population because no complete address data was available for these participants. In response to the reviewer’s question, we have changed the relevant sentences into:

“We were able to calculate traffic exposure for 7,339 of these 7,431 women (99%) due to incomplete address data in 92 subjects. The associations between traffic indicators and pregnancy-related outcomes in mother and child were analyzed in the 7,339 remaining mothers.”

Statistical analysis

12. All exposures were categorized. Later a number of sensitivity analyses were performed to examine the robustness of the results against the choice of the cut-offs. No results of these sensitivity analyses are shown, most likely to limit the number of tables. Nonparametric smootheners could be used to overcome the problem of the large number of sensitivity analyses. Smoothed curves could for instance be presented in an online supplement and justify the use of categorical exposure variables and the choice of the cut-offs.
We have examined the data in various ways. We did not observe clear associations in the main analyses, and neither did we see effects in the sensitivity analyses using different categorizations or buffers for the traffic indicators. As the results of these different analyses do not change the conclusions, we have decided to summarize the findings in the text, indeed to limit the number of tables. Instead, we have decided to present the results of several other sensitivity analyses as supplementary materials (see also reviewer’s point 3 and 16).

13. How exactly was gestational age used in regression models? Linear term only, linear and quadratic term, categorical? How were maternal age and maternal BMI defined, continuous or categorical?

We have clarified this in the Statistical analysis section with the following sentences:
“Models were adjusted for known determinants of birth and pregnancy outcomes (maternal age, maternal ethnicity, maternal education, maternal BMI, parity, maternal smoking, and maternal alcohol consumption). **Maternal age and BMI were included in the models as continuous variables.** Models with birth weight as outcome were additionally adjusted for gestational age **(with a linear term)** and fetal sex.”

14. The associations between traffic-exposures and pregnancy outcomes are highly non-linear. This is evident from the tables. In my opinion, no trend-test is necessary to prove that. I advice to skip the trend-tests.

We have deleted the trend-tests from the tables in accordance with the reviewer’s suggestion.

Results
15. Please add a table with the distribution of the main exposure variables.

A table with characteristics of the distributions of distance-weighted traffic density and distance to a major road is enclosed as an Additional file 1. We have added the following sentence to the Results section: “**Characteristics of the distribution of distance-weighted traffic density and distance to a major road are shown in Additional file 1.**”

16. Results of the sensitivity analyses need to be presented – either in an additional table or as part of the online supplement. I have concerns about the statistical power of the stratified analyses by maternal education. In particular the group of parents with low education is rather small (10%). This needs to be discussed.

As noted above, we have included the results of several sensitivity analyses as Additional files. Furthermore, we have addressed the reviewer’s concern by discussing the limited statistical power for the mentioned analysis. We have changed the text as follows: “Stratified analyses by educational level did not show different results (see Additional files 5, 6 and 7 for associations between proximity to traffic and selected outcomes). However, it must be noted that the statistical power for some of these analyses was limited due to small numbers of participants in the subgroups, especially in the lowest educational group.”

17. Page 15, maternal socio-demographic risk factors for traffic exposure. This paragraph following the main and sensitivity analyses seems to be a bit misplaced and the objectives of
these analyses are not entirely clear. It could be either skipped or extended to an analysis of the associations of all potential confounding variables and traffic exposures that could be used to explore the potential of these variables for being a confounder. The latter should be presented before the main results.

We agree that this objective may lead to confusion. In the revised version we have omitted the sections in which these analyses were discussed. This has also shortened the Discussion considerably.

Discussion
18. With regard to pregnancy complications and the interaction between SES and traffic exposure a discussion of the (limited) statistical power needs to be added.

We have addressed the limited statistical power for these analyses. See also point 16 above, and point 1 of the first reviewer.

19. Page 19, 1st paragraph. It is correct that there are no previous studies on the association between traffic intensities and pregnancy outcomes in Europe. However, there are other European studies on air pollution and pregnancy outcomes using time-space exposure models (e.g. Slama et al. Environmental Health Perspectives 2007; Aguilera et al. Environmental Health Perspectives 2009) from areas with similar air pollution levels and mixtures, which should be discussed here.

The studies mentioned by the reviewer are very interesting studies that examine the relation between air pollution and pregnancy outcomes. However, there are several studies that evaluated this relationship, using various exposure assessment methods. We specifically focused on studies using traffic indicators. In response to point 5 of the reviewer, on including a discussion of modeling temporal variation in air pollutant levels, we included references to these studies and other studies mentioned above. We also remark in the manuscript that some of these studies were conducted in Europe. The text has now been changed as follows:

“Ideally, these temporal variations would be taken into account in the exposure assessment, next to the spatial variability. This has been done by a few earlier pregnancy studies, some of them conducted in Europe, in which air pollution concentrations were modeled and subsequently adjusted for temporal variation [33, 34, 51, 57].”

20. Page 19, residential mobility. “Non-movers” were defined as women who did not move between 7 months before conception and five months of pregnancy. I personally would consider it more logical to define “non-movers” as those who did not move home during the entire pregnancy. Is that possible and if yes, what are the results? Please include a table or with the results.

Unfortunately, we did not have data on residential mobility during the whole pregnancy period. We are planning to make this data available for future analyses. However, for this manuscript, we conducted stratified analyses for movers and non-movers in the period indicated above. The results of the analyses in non-movers are presented in Additional file 8 and 9. We have changed the relevant sentences in the Methods section into:

“Finally, we conducted stratified analyses for residential mobility. We had information available on change of residence (yes/no/missing) in the period between
seven months before conception and five months of pregnancy, and repeated the analyses for the different strata.”
In the Results section, we have added:
“Finally, stratified analyses by residential mobility showed that results were not different across strata (see Additional files 8 and 9).”
And in the description of potential limitations in the Discussion, in order to avoid repetition, we have changed the text into:
“In stratified analyses, we observed that results were not different across those who did/did not change residence in the period between seven months before conception and five months of pregnancy. This indicates that residential mobility did not have a large effect on our effect estimates.”

References
21. The list of references currently includes more than 50 papers, which is rather long for an original paper and should be restricted to the most relevant references.

We have shortened our reference list. It now includes 45 papers instead of 58 papers.

Minor comments
• Abstract, conclusion. Mothers exposed to … please add “in this study”.
• Page 6, line 6. “briefly” should read “in brief”

Both sentences have been modified according to the reviewer’s suggestion.