Author’s response to reviews


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Author’s response to reviews: see over
Dear professor Grandjean, Ozonoff,


We are grateful for the detailed comments of the editor and reviewers. We have made changes in our manuscript in response to these comments. Most importantly, we described our methods and discussed the strengths and limitations of our methodology in more detail. Also, we discussed and compared our results with previous studies more appropriate.

Please find attached our point-by-point responses to the questions and comments of the reviewers. We think that these changes have improved the quality of our manuscript and hope you will consider this revised manuscript acceptable for publication in Environmental Health.

Yours sincerely,

Agnes M.M. Sonnenschein-van der Voort, Msc
Liesbeth Duijts, MD, PhD
Reviewer 1
Using data from 4,634 children, the authors found an increased risk of wheezing during early-life associated with air pollution exposure, but only among children exposed to tobacco during both fetal life and infancy. This association was not observed in the cross-sectional yearly analyses. The hypothesis of this study is clearly stated, and it is novel in examining the effect of air pollutants at different exposure windows during early life. The detailed assessment of health outcomes and exposures at several time points is an important strength of this study. Weaknesses included the current description of the tobacco smoke variables, and the discussion of relevant results.

We thank the reviewer. Our specific responses to the comments are given below

Major Compulsory Revisions
1. How infant and fetal smoking was defined is poorly explained, and no descriptive statistics are given for these variables in Table 1. Was fetal smoking defined as yes if a mother answered yes to smoking during any of the three time points assessed, or all three? Was infant smoking related to both the mother and the partner smoking, or only one of the two? Providing descriptive statistics for these variables would also help the reader understand how many observations were included in each smoking category in the models. This information is currently lacking.

We agree that smoking variables should be explained in more detail. We have rewritten the sentences on tobacco smoke exposure in the methods section (page 6), and have rewritten the descriptives of these variables in table 1 more clearly.

Methods
"At enrolment, mothers received the first questionnaire containing data about their active smoking habits [1, 2]. Information about maternal smoking during other periods of pregnancy was obtained by postal questionnaires sent in second and third trimester of pregnancy. Based on the first questionnaire, we grouped mothers smoking habits during pregnancy into 'no' (never smoked during pregnancy or until first trimester only) and 'yes' (smoked continuously during pregnancy). Reported smoking habits in second and third trimester of pregnancy were used to reclassify the maternal smoking habits where appropriate [2]."

“…and sent at the age of 2 years on environmental tobacco smoke exposure by anyone in the house ("Do people smoke occasionally in your house?" no; yes). The correlation between fetal and infant smoke exposure was poor (kappa 0.409)."
2. Why did the authors also not examine the effects of PM2.5? This air pollutant is commonly monitored and may have different effects, (potentially more or less pronounced than those associated with PM10).

Unfortunately, information about PM2.5 levels was not available during these years (2002-2008). We added this as a limitation of our study in the discussion (page 13).

Discussion

“We had no information on smaller particle sizes than 10 µm. Smaller particles sizes such as PM2.5 might more adversely affect respiratory morbidity than PM10 due to deeper peripheral lung deposition. However, previous studies which measured both PM10 and PM2.5 observed strong correlations between exposure to PM_{10} and PM_{2.5} and similar effect sizes of these exposures on childhood asthma or wheezing [3, 4].”

3. Of the eligible children, only 61% participated. The potential for bias therein must be carefully explored and analyzed to the extent possible.

We agree that selection bias due to non-response, besides that of lost to follow-up, could have been present in our study and explored this in more detail (see discussion section page 12).

Discussion

“Non-response at enrolment and lost to follow-up would lead to biased effect estimates if the associations of air pollutants with wheezing would be different between those included and not included in the analyses. Selection bias due to non-participation at enrolment in the prenatal phase might have occurred because our study population of more affluent and healthy mothers [1] might have reported less wheezing symptoms, tobacco smoke exposure and might have been exposed to lower air pollutant levels [5]. If so, our current observed effect estimates would be underestimated. Mother and children lost to follow-up during the postnatal phase were lower educated (67% vs 47%) and smoked more frequently during pregnancy (21% vs 13%), and might be exposed to higher air pollutant levels. If children who were lost to follow up would have had more wheezing episodes, this could have led to an underestimation of the observed effect of air pollution and tobacco smoke exposure on wheezing as well.”

4. The authors do not clearly explain why a positive association is only found using the GEE modelling approach, but not in the cross-sectional yearly examinations. Some comment or suggestions as to why these results differ should be given.

Indeed, we observed positive associations of air pollution with wheezing among tobacco exposed children in our GEE modelling approach and not in our cross-sectional yearly examinations. A reason for this difference could be the increased power in the GEE modelling approach in the overall analyses. Lack of power in our annual analyses might have been occurred in our stratification by smoking analyses. To increase power, we now additionally assessed the interaction terms per year (instead of stratification) and observed in our annual
analyses that the associations between air pollution and wheezing among children exposed to fetal and infant tobacco smoke was significant at age 3 years only. A possible explanation is that from the age of 3 years onwards wheezing might represent another phenotype in which other factors in the origins of wheezing become more important such as genetic and atopic susceptibility instead of respiratory tract infections. Also, the age at which infant smoke exposure was assessed could have played a role. Infant smoke exposure was assessed at the age of 2 year after respiratory outcomes at age 1 year. This might be a reason for observing no associations between exposure to air pollutants, tobacco smoke and wheezing before the age of 3 years. We present our additional analyses and interpretation of the results on page 9 (results) and page 10 (discussion).

Results
“To increase power, we additionally assessed the modifying effect of tobacco smoke exposure by interaction terms which were all significant (P-values for interaction per year are PM$_{10}$ * smoking: p-value = 0.35 (age 1), p-value = 0.20 (age 2), and p-value <0.05 (age 3). P-values for interaction NO$_{2}$ * smoking are: p-value = 0.23 (age 1), p-value = 0.14 (age 2), and p-value <0.05 (age 3)).

Discussion
“We observed in our annual analyses that the associations between air pollution and wheezing among children exposed to fetal and infant tobacco smoke was significant at age 3 years only. This might be explained by the idea that from the age of 3 years onwards wheezing represents another phenotype than earlier wheezing in which other factors such as atopic susceptibility in the origins of wheezing become more important. Also, infant smoke exposure was assessed after respiratory outcomes at age 1 year. This might be a reason for observing no associations between exposure to air pollutants, tobacco smoke and wheezing before the age of 3 years.”

5. The authors should comment on how many children moved during each year. A major strength of this study is the detailed information on moving during early life, but no data are provided. It would also be useful if the authors provided summary information on the differences in air pollution levels across the years per child. Did most children have very similar air pollution exposures each year, or was the variable per child large? Moving behaviour may be especially interesting as the overall temporal variation in the air pollutants seems relatively low (Table S1)

In the first 3 years of life 39.9% of the children moved at least once. Levels of exposure to air pollutants were measured each day at each individual home address from which we calculated an average yearly exposure. Therefore, we think that it is an important strength of our study that we have taken this into account. We have added this information in the discussion part of our manuscript (page 13).
The differences in air pollution exposure across the years per child did not differ much for the total group. Furthermore, we observed no association between the per year change of exposure to air pollutants with wheezing. In this cover letter, we added a table on air pollution changes for reviewer purpose. If the reviewer prefers we can add this table to the supplementary files and describe the results in our manuscript.

Table R1. Air pollutant levels per year for total and smoking categories

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Never</th>
<th>Fetal</th>
<th>Infant</th>
<th>Fetal and infant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>90% range</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>NO\textsubscript{2} 1-2yr</td>
<td>-1.07 (2.01)</td>
<td>-4.5 – 1.08</td>
<td>-1.09 (2.03)</td>
<td>-1.11 (1.96)</td>
<td>-0.98 (1.81)\textsuperscript{a}</td>
</tr>
<tr>
<td>NO\textsubscript{2} 2-3yr</td>
<td>-1.15 (2.07)</td>
<td>-4.6 – 1.07</td>
<td>-1.18 (2.09)</td>
<td>-1.23 (2.39)</td>
<td>-1.14 (1.93)</td>
</tr>
<tr>
<td>PM\textsubscript{10} 1-2yr</td>
<td>-0.57 (2.56)</td>
<td>-6.6 – 3.03</td>
<td>-0.57 (2.53)</td>
<td>-0.61 (2.52)</td>
<td>-0.65 (2.63)\textsuperscript{a}</td>
</tr>
<tr>
<td>PM\textsubscript{10} 2-3yr</td>
<td>-0.34 (1.61)</td>
<td>-2.8 – 2.89</td>
<td>-0.38 (1.60)</td>
<td>-0.38 (1.61)</td>
<td>-0.21 (1.62)\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Values represent mean (SD) and 5-95% range for total group and mean (SD) for smoking categories and were calculated by subtracting the air pollutant levels of a specific year from the previous year.

\textsuperscript{a}p-value for difference of air pollution difference per year with never smoke exposed as reference group <0.05

Discussion

"A strength of our study is that we were able to consider detailed spatial and temporal contrasts in exposure, in which we were able to take home movements into account. In the first 3 years of life 39.9% of the children moved at least once."

6. A major concern is the authors’ apparent misunderstanding of the relevance of the Rabinovitch study and its comparison to the present manuscript. In Rabinovitch, albuterol usage and LTE4 were related to PM2.5 concentrations on days when urine cotinine levels were low (<10 ng/ml per mg creatinine); on these days, mean albuterol usage and LTE4 increased up to 5 or 6% per 10μg/m3 increase in mmPM2.5. As far as this reviewer can appreciate, this is in sharp distinct to the results of the present manuscript, in which ETS and traffic-related pollution seem to be reinforcing (or create ‘vulnerability’ as suggested by the authors). These divergent results need be reconciled and the authors need to demonstrate a reasonable interpretation of the existing literature or argue why this reviewer’s posited contradiction is not reasonable. This is critical to the success of this manuscript as it represents, in the mind of this reviewer, the most novel contribution that this manuscript has to offer to the literature. This reviewer appreciates that you studied ETS at a different timepoint relative to the health endpoint, and different health endpoint, than did Rabinvitch, but nonetheless to say
that “this effect modification has been previously assessed by Rabinovitch” (your page 1) is easily misleading.

We apologize for our misinterpretation and have rewritten the discussion section in which we more accurately discuss the study written by Rabinovitch (page 11).

Discussion

“Our results suggest that tobacco smoke exposure increases the vulnerability of the lungs to air pollutants. The interaction between particulate matter and tobacco smoke exposure was previously explored by Rabinovitch et al [6]. They observed that environmental tobacco smoke exposure modifies the acute effects of low-level ambient \( \text{PM}_{2.5} \) exposure on childhood asthma. Albuterol usage and leukotriene \( E_4 \) were only related to \( \text{PM}_{2.5} \) concentrations on days when urine cotinine levels were low, which suggest that only when children were not or only a bit exposed of tobacco smoke, exposure to air pollution was positively associated with asthma. Their results were in the opposite direction as we observed. This difference might be explained by differences in study design and methods. We assessed reported tobacco smoke exposure both in fetal and infant life, wheezing at younger ages, and assessed long term exposure to tobacco smoke and air pollution. Rabinovitch et al assessed biological markers of smoke exposure in childhood, used albutarol usage, a proxy for asthma, at an older age, and assessed the short term effects of air pollutants. Based on these two studies, we speculate that short exposure duration to air pollutants might be more important for developing respiratory symptoms, whereas in the presence of environmental smoke exposure, long exposure duration to air pollutants might be more important.

7. A further discussion as to why both fetal and infant smoke exposures are needed to increase the risk is required. This is interesting as the associations for the fetal smoking group are very close to null, and thus suggest that this exposure may not be as important as smoke exposure during infancy.

We have rewritten the discussion section on why both fetal and infant smoke exposure is required to have an increased risk of air pollution on wheezing (page 11).

Discussion

“Children who were exposed to tobacco smoke both during the fetal and infant period had increased risks of wheezing when exposed to higher air pollution levels. Previously, we have reported that children from mothers who smoked continuously during pregnancy and during the first years after pregnancy had increased risks of wheezing in the first years of life [2]. Fetal smoke exposure is suggested to have a different underlying mechanism in the pathway to wheezing than infant smoke exposure. For fetal smoke exposure this includes impaired lung development and immunological changes while for infant smoke exposure it includes bronchial hyperreactivity, immunological changes, and direct toxic and irritant effects [7-9]. Increased vulnerability of the airways and lungs to air pollutants might be caused by both fetal
and infant smoke exposure via their pathophysiological mechanisms. For infant smoke exposure we observed a more prominent (still not significant) interactive effect than for fetal smoke exposure. This might be due to the direct toxic effects of both infant smoke exposure and exposure to air pollutants which fetal smoke exposure only has not [10]."

8. If the results are the same between the imputed data and the complete cases, why not only show the data based on the complete cases, especially as the authors imputed for the outcome variable as well as the covariates (the former of which is less advisable). Multiple imputation is often used in large cohort studies for taking into account loss of information that may occur due to restriction to study participants with complete data and to adjust for bias caused by missing data. A recent simulation study showed that multiple imputed GEE models results in less biased and more accurate effect estimates than weighted GEE models [11]. So, to increase power and reduce bias caused by missing data and to provide less biased and accurate effect estimates, we used MI-GEE models. We now explain this in more detail in the methods (page 7) and if the reviewer wishes we can additional show the main results of complete case analysis in the supplementary files.

Methods

“To handle missing values of the covariates and outcomes we performed multiple imputations, which are used to select the most likely value for a missing response, by generating 25 independent datasets [12]. We imputed both covariates and outcomes, as missing values may introduce bias in GEE models [11]. Imputations were based on the relationships between all covariates and outcomes included in this study plus paternal age, educational level, history of asthma or atopy and information about shortness of breath in the past year of the children at the age of 1, 2 and 3 years. All datasets were analyzed separately after which results were combined. No differences in results were observed between analyses with imputed missing data or complete cases only. We only present results based on imputed datasets.”

9. The results for the sensitivity analysis should likely be presented in the results section. Introducing a new Table and results in the discussion section is unusual. Also, Table S3 does not show that a larger variation in exposure levels of air pollutants were measured in the previous month at the age of 1 year, as is indicated in the text (odds ratios are presented in this table). In fact, this information does not appear to be presented at all.

We now present table S3 in the main manuscript as Table 3 and discuss the results, page 8. Furthermore we added the descriptive air pollutant levels measured in the previous month to Table S1.

Results
“At the age of 1 year only, we were able to obtain information about wheezing in the last month and the average exposure to air pollutants during that month. We observed a larger variation in exposure levels of air pollutants measured in the previous month at the age 1 year (Table S1). Furthermore, increased levels of air pollutants exposure during the previous 1 month were associated with increased risks of wheezing (odds ratios 1.25 (0.98, 1.58) and 1.32 (1.11, 1.55) per 10 μg/m³ increase PM₁₀ and NO₂, respectively)(Table 3).”

10. Where the GEE models adjusted for respiratory infections at any age, or only in the previous year as is indicated in the legend of Figure 2? Also, given the high rate of missing values for this covariate (46%), are the results similar when the models are not adjusted for respiratory infections?

We adjusted the GEE models for ever doctor diagnosed lower respiratory tract infections [no;yes] in the first 3 years of life, including pertussis, bronchitis, bronchiolitis and pneumonia. Using the same questionnaires as for wheezing, infections were annually reported over the last 12 months and we recorded them into respiratory tract infections (no, yes) in the first 3 years as follows: ‘no’ when no infections at any age occurred; or ‘yes’, when at least one infection had occurred; the remainders were set to ‘missing’. Because of this recoding of 3 variables into 1 variable we ended up with many missing values before the imputations. Based on the comment from the reviewer, we now use doctor diagnosed lower respiratory tract infections at the corresponding ages in our GEE models, present respiratory tract infections per year in table 1 and changed the legend of figure 2. The number of missing is lower (range 14-21%) (Table 1). Furthermore, the effect estimates did not change when the models were not adjusted for respiratory tract infections (results not shown).

Legend figure 2:
“…Models are adjusted for maternal age, education, parity, history of atopy or asthma and children’s ethnicity, sex, gestational age, birth weight, breastfeeding, daycare attendance, pet keeping and doctor-diagnosed lower respiratory tract infections at 1, 2 and 3 years of age. P-values for interaction: tobacco smoke exposure * average level PM₁₀, p-value <0.05; tobacco smoke exposure * average level NO₂, p-value <0.01. “

11. Page 10 says “confirms the earlier results that air pollution is associated with doctor diagnosed asthma and not with wheezing due to infectious mechanisms”. This statement is problematic for 2 reasons: a) Morgenstern did not look for infection, and since the 2 mechanisms are not mutually exclusive both could have been at play; b) adjusting for such infections is not the same as a design/analysis focused on whether or not such infections are caused by air pollution and then lead to wheezing; this is especially true when looking at complex interaction dynamics such as you posit (ETS and ambient pollution #wheeze)
We agree with the points of the reviewer. Morgenstern did analyze the associations between air pollution and respiratory infections (no effect) but, indeed, did not analyze the possible modifying effect of respiratory infections on the association of air pollution with wheezing. In the revised manuscript, we explored whether there is a statistical interaction between respiratory infections and air pollutants. We observed that all p-values were >0.05, indicating no interaction. We did also not observe an association of air pollutants with lower respiratory tract infections in the first 3 years. We now present this in the results, page 8, and discuss this in the discussion section as follows, page 12.

**Results**

We explored the confounding and modifying effect of lower respiratory tract infections and did not observe changes in our effect estimates after adjusting the analyses for lower respiratory tract infections and testing for interaction (all p-values for interaction: >0.05). Furthermore, we observed no associations between air pollutants and lower respiratory tract infections (data not shown).

**Discussion**

“The mechanisms underlying the association of air pollution exposure with wheezing or asthma might also include the induction of airway inflammation and oxidative stress, modification of enzyme functions, disruption of immune responses and increased reactivity to allergens [10, 13-15]. Also, respiratory infectious diseases might play a role. However, we did not observe a confounding or effect modifying effect of respiratory tract infections nor associations between air pollutants and respiratory tract infections. Therefore, the associations of air pollution with wheezing in this study are probably not explained by infectious mechanisms. Further studies exploring potential underlying causal mechanisms are needed.”

12. Why do the authors believe that exposure misclassification (eg. indoor air pollutants or commuting) would lead to an underestimation of the associations?

We suggest that misclassification of the exposure will be non-differential and this usually leads to an underestimation of the effect estimates [16]. We added this in the discussion (page 13).

**Discussion**

“Furthermore, other types of exposure (indoor or commuting) were not taken into account. If any, we expect that this misclassification is non-differential and may have led to an underestimation of the associations [16].”

13. The third sentence of the introduction is confusing. Do the authors want to suggest that children are more exposed to air pollutants than adults (which is what I suspect) or less exposed. The current sentence suggests the latter.
We agree that the word choice or statement confusing. Our suggestion is that children are more exposed to air pollutants than adults. We have written this more clearly (page 4).

**Introduction**

“The effects of air pollutants on airway symptoms may differ between children and adults. Children > 6 months of age may breathe more through the mouth than adults, and benefit less from the filtering, humidifying and temperature raising effect of the nose and might therefore inhale higher air pollutants levels [17].”

14. Not all environmental exposures negatively influence the risk of asthma and symptoms, as is suggested by the authors in the introduction. For example, farming exposures are believed to be protective. Possibly adding “some environmental exposures” would help clarify this point.

We agree and changed it as suggested (page 4):

**Introduction**

“Like some other environmental exposures, fetal and infant tobacco smoke exposure negatively influence the risk of asthma symptoms in early childhood, and might increase the susceptibility for the adverse effects of air pollution [2].”

15. There appears to be a significant interaction between air pollution and tobacco smoke exposure at age 3 for both PM10 and NO2. This is mentioned in the legend of Table S2, but may be of some importance and could be discussed in the results.

We agree and now show the p for interactions in the results. See also point 4 above.

16. Page 7 say “. . .(95% range 37.0-42.1)”. What does that mean? I suspect this is either 95% CI or range, but this is unclear and perhaps sloppy.

We mean the range between 2.5% and 97.5%. We now write it as (2.5-97.5% range).

**Minor Essential Revisions**

17. There is a mistake in abstract: “per 10 mg/m3 PM10” is written twice in the last sentence of the results section.

We deleted the first per 10 mg/m3 PM10.

18. First sentence of results: the authors should indicate what (556) is (the standard deviation?).

Indeed, we meant standard deviation and changed it.

19. In the list of abbreviations, “Matter” should not be capitalized.

Matter is now written “matter”.

.
20. Reference 14 is not formatted like the others (capitals used in title)
   We changed reference 14 accordingly.

21. In Table S2: * and ** do not appear in the table, and thus should be deleted from the legend.
   We removed * and ** from the legend.
Reviewer 2

We thank the reviewer. Our specific responses to the comments are given below

Minor essential revisions

1. You cited Figure 2a and 2b into the text, whereas the 2 figures are named figure 2 and figure 3.
   We apologize for this typo. We uploaded A and B as two different figures. We now combined those.

Discretionary revisions

2. Pag. 7: the very low % of preterm (if present – 5th perc.=37.0) indicates a healthy population, as you indicated in your previous article (ref. 16). A comment on this possible selection bias and its consequence on results could be useful.
   In the Netherlands, the average prevalence of preterm birth (<37 weeks) is 7.7%. In our population for analyses this was 4.7%. So, indeed our population might reflect a selection towards a more healthy population. A more homogeneous population as ours does not affect the true association between air pollution and wheezing among children exposed and not exposed to tobacco smoke. However, it does affect the generalizability. The observed effects might be different in a population with more preterm born children. Preterm birth could also modify the effect between air pollution, tobacco smoke exposure and wheezing, because fetal smoke exposure could lead to preterm birth and therefore airways and lungs might be even more vulnerable to air pollution. We added this information in the discussion section (page 12)

   Discussion
   “One of the limitations of our study is that we might reflect a selection towards a more healthy population as the prevalence of preterm birth is lower than average in The Netherlands, 4.7% versus 7.7%. A more homogeneous population as ours would not affect the association between air pollution and wheezing among children exposed and not exposed to tobacco smoke. However it might affect the generalizability. The observed effects might be different in a population with more preterm born children. Also, preterm birth could modify the effect between air pollution and wheezing, because airways and lungs of preterm born children might be less developed and therefore might be even more vulnerable to air pollution.”

3. Pag. 7: The tobacco smoke variables were combined into a new variable with 4 categories: never; only fetal; only infant; fetal and infant smoke exposure. Is this variable a priori constructed based on a hypothesis of growing risk for respiratory outcomes? It appears strange the lower risk for fetal exposure compared with infant exposure (even if not both not significant). A discussion about this is recommended,
with possible explanation in healthy population (see note above) or bias in pregnancy smoke reporting or other.

Indeed, the combined tobacco smoke variables were a priori constructed based on results from our earlier study to the association of smoking and wheezing [2]. We suggest that infant tobacco smoke exposure and air pollutants might affect the lungs in different ways. As a result of this increased exposure the effect of air pollution might be larger in children which are also exposed to tobacco smoke in infancy, although not significant. We discuss this issue now in the discussion section (page 11).

Discussion

“Children who were exposed to tobacco smoke both during the fetal and infant period had increased risks of wheezing when exposed to higher air pollution levels. Previously, we have reported that children from mothers who smoked continuously during pregnancy and during the first years after pregnancy had increased risks of wheezing in the first years of life [2].

Fetal smoke exposure is suggested to have a different underlying mechanism in the pathway to wheezing than infant smoke exposure. For fetal smoke exposure this includes impaired lung development and immunological changes while for infant smoke exposure it includes bronchial hyperreactivity, immunological changes, and direct toxic and irritant effects [7-9].

Increased vulnerability of the airways and lungs to air pollutants might be caused by both fetal and infant smoke exposure via their pathophysiological mechanisms. For infant smoke exposure we observed a more prominent (still not significant) interactive effect than for fetal smoke exposure. This might be due to the direct toxic effects of both infant smoke exposure and exposure to air pollutants which fetal smoke exposure only has not [10].”

4. You clearly reported various exclusions in your population (pages 4-5 and figure 1). Do you think these selections could have biased your sample? Did you make analysis in this sense, where possible?

We agree that selection bias due to non-response, besides that of lost to follow-up, could have been present in our study and explored this in more detail (see methods page 7 and discussion section page 12).

Methods

“To handle missing values of the covariates and outcomes we performed multiple imputations, which are used to select the most likely value for a missing response, by generating 25 independent datasets [12]. We imputed both covariates and outcomes, as missing values may introduce bias in GEE models [11]. Imputations were based on the relationships between all covariates and outcomes included in this study plus paternal age, educational level, history of asthma or atopy and information about shortness of breath in the past year of the children at the age of 1, 2 and 3 years. All datasets were analyzed separately after which results were
combined. No differences in results were observed between analyses with imputed missing data or complete cases only. We only present results based on imputed datasets."

"Non-response at enrolment and lost to follow-up would lead to biased effect estimates if the associations of air pollutants with wheezing would be different between those included and not included in the analyses. Selection bias due to non-participation at enrolment in the prenatal phase might have occurred because our study population of more affluent and healthy mothers [1] might have reported less wheezing symptoms, tobacco smoke exposure and might have been exposed to lower air pollutant levels [5]. If so, our current observed effect estimates would be underestimated. Mother and children lost to follow-up during the postnatal phase were lower educated (67% vs 47%) and smoked more frequently during pregnancy (21% vs 13%), and might be exposed to higher air pollutant levels. If children who were lost to follow up would have had more wheezing episodes, this could have led to an underestimation of the observed effect of air pollution and tobacco smoke exposure on wheezing as well.

5. Pag. 9: you discussed about long term averages, and their smoothing of high level of exposure. Do you assert that average is not the more suitable exposure index? Actually the log-normality of distribution of pollutants increase the distance between the average and high values. So, a suggestion could be to use, for example, the 75th percentile of the hourly distribution of pollution values for each subject, or an index more suitable to take into account high values. Or your suggestion is that it could be better to consider a lag of 1 month (with average) to relate pollution and wheezing?
Indeed our suggestion was that it might be important to consider a lag of 1 month (with average) to relate pollution and wheezing. However, we did not have data on wheezing in the last month at the ages older than 1 year. We discuss this issue as one of the limitations of our study. Secondly, apart from taking the air pollutants as continuous variables, we also explored the effect in quartiles. The highest 25% was not associated with wheezing in the first 3 years (results not shown).

Results
“We observed no associations of average PM$_{10}$ and NO$_2$ concentrations during the previous year with the risks of wheezing at the ages of 1, 2 or 3 years nor an overall effect (Table 2). The highest 25% was also not associated with wheezing in the first 3 years (results not shown).”

“At the age of 1 year only, we were able to obtain information about wheezing in the last month and the average exposure to air pollutants during that month. We observed a larger
variation in exposure levels of air pollutants measured in the previous month at the age 1 year (Table S1). Furthermore, increased levels of air pollutants exposure during the previous 1 month were associated with increased risks of wheezing (odds ratios 1.25 (0.98, 1.58) and 1.32 (1.11, 1.55) per 10 μg/m³ increase PM₁₀ and NO₂, respectively)(Table 3)."

Discussion

“Unfortunately, we were not able to asses this short time interval at older ages.”

6. Pag. 6: “We used a compound symmetry correlation matrix”. Due to the small number of time points, why to use this type of matrix (instead of unstructured matrix, for example)?

We explored the unstructured matrix, toeplitz (3) and compound symmetry correlation matrix. The best method to use seems an unstructured correlation matrix, allowing for all sorts of correlations, though this matrix costs many degrees of freedom and thereby makes the model more unstable. Because results of the various used matrices were comparable, we decided to use the most stable model, the exchangeable correlation matrix. If the reviewer prefers we can add this information in the manuscript.

7. The range of pollutant values is effectively small. This could be due to model used (dispersion model). The article you referred as comparison of pollutant ranges made use of Land Use Regression (LUR) model for exposure assessment of birth cohort. Do you know Dutch cohort studies that used dispersion models to assign exposure, to compare with your values?

As far as we are aware of, there are no other Dutch cohort studies using the dispersion model. The performance of this model in the same study area has been evaluated and compared with the LUR model [18, 19]. We now refer to these studies in the methods section in the manuscript (page 5)

“The performance of this model has been evaluated by two studies in the same study area and show a good agreement between predicted annual average PM₁₀ and NO₂ concentrations and concentrations measured at monitoring stations [18, 19]”

8. Did you check the possible effect modification due to different study periods within the GEE model, considering an interaction factor between the period of follow-up and exposure of interest?

We tested in the GEE models whether the interaction between time in years*air pollutant was significant. We observed p-values for interaction of >0.05 for both PM₁₀ and NO₂ and described these results in the results section, as follows, page 8.

Results
“We observed no time-dependent effect of air pollutants on wheezing in the first 3 years (p-values for interaction time*air pollutant: >0.05).”

References


