Author’s response to reviews

Title: Maternal occupational exposure and oral clefts in offspring

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Author’s response to reviews:

Reviewer #1: In this case-malformed study the authors investigated the relationship between maternal occupational exposure and oral cleft. Assuming that a lot of pregnant women work during the windows of susceptibility of palate and lip formation, there is a need to better understand the relationship between occupational exposure and the occurrence of oral cleft. I am not sure that the present study add a lot considering several limitations (exposure assessment and choice of the control group) to the existing published ones in particular concerning solvents and pesticides exposure and need at least major revision.

Comment 1:

Keywords: add occupational exposure.

Answer:

We added ‘occupational exposure’ as keyword as requested by the reviewer.

Abstract and Introduction:

Comment 2:
In the abstract and the introduction, the authors said that there is inconsistent results concerning occupational exposure to solvents and oral cleft. But in the literature review (line 68-71) they said that six studies reported an association between solvents exposure and oral cleft and two (including Lamon et al) did not. I not agree with the interpretation of Lamon et al study considered by the author as given 'negative' results. Please verify the interpretation of this study. Additional studies can be add in this literature review Holmberg 1982 in Finland, Cordier 1997 and Shaw 2003 (which is cited in the discussion).

In the lines with this comment (interpretation of Laumon et al study) it's seems for me that most of existing studies are concordant for the association between maternal exposure to solvent and oral cleft (at least 7 positive studies vs 1 negative studies or 2 including Shaw 2003).

Line 71-73: if there is some inconsistent results it can be also due to definitions of solvents (including oxygenated solvents or not), case assessment, study period (with changed in solvents uses) and study place.

Answer:

We thank the reviewer for this valuable comment to improve the Introduction. We added the studies of Holmberg, Cordier and Shaw to the literature discussed in the Introduction. Furthermore, we verified the study of Lamon et al. and revised the consideration as negative result. Now there is one negative result and 7 positive results in the solvent exposure literature discussed. This means that there are no major inconsistencies in the literature of occupational solvent exposure.

We changed a sentence in the background of the abstract as follows: ‘Previous studies suggest that periconceptional maternal occupational exposure to solvents and pesticides increase the risk of oral clefts in the offspring’. Page 2, lines 28-30.

We rewrote the introduction discussing inconsistencies as follows: ‘Large population based case-control studies suggest a relationship between exposure to organic solvents and oral clefts [1-8], whereas one other study did not find a higher risk of oral clefts in the offspring after maternal occupational exposure to solvents [9]’. Page 3, lines 67-69.

Comment 3:

Line 82-89 : It seems that the author put some methods information here, please remove this part to the corresponding method section.

Answer:

We apologise that there was some Methods information in the Introduction section. We moved the first sentence of this part from the Introduction to the first part of the Methods: ‘To examine
the possible association between maternal occupational exposure and oral clefts in the offspring. A case-malformed control study was performed. Cases and malformed controls were selected from the European Registration of Congenital Anomalies and Twins database of the Northern Netherlands (Eurocat NNL). The second sentence is moved to the Exposure assessment part: ‘A community-based JEM (ALOHA+ JEM) is applied to translate self-reported information about mothers’ occupation during the periconceptional period (three months before conception through the first trimester) into occupational exposures to solvents, pesticides, metals and more generic categories like mineral and organic dust, and gases and fumes’. Comment 4: Line 91-92: The objective of the study include in addition to exposure to solvents and pesticides, exposure to metals, dusts, gases and fumes but there is no information in the literature review on the existing (or not) studies concerning these exposure in relation the occurrence of malformations. Answer: We completely agree with the reviewer that literature about exposure to metals, dusts, gases and fumes is missing in the literature review. We added a sentence about occupational exposure to metals in relation to congenital anomalies. As far as we know, there is no literature concerning the relation between occupational exposure to dust/gases, and fumes and oral clefts. However, these exposures occur often in the same workplace as exposure to solvents/pesticides. For this reason we took these exposures into account as well. We added a sentence about that in the end of the Introduction: ‘There is one previous study that suggested an association between maternal occupational exposure to metals and oral clefts in the offspring [21]. As far as we know, there is no literature concerning occupational exposure to mineral and organic dust, and gases and fumes in relation to the occurrence of oral clefts. However, since these exposures often occur in the same workplace as exposure to solvents and pesticides, these exposures were also taken into account in this study’. Methods Section: Comment 5: Line 112: is it possible to add the percentage of informed consent. Answer:
In general, the informed consent rate is around 80% for all types of congenital anomalies for several years. We added this information at page 5, lines 114-115: ‘In general, the informed consent rate is around 80% for all types of congenital anomalies’.

Comment 6:

Line 122: smoking and alcohol consumption preconceptional, beginning of pregnancy, anytime during pregnancy? Please specify.

Answer:

We have specified this as follows: ‘For smoking habits, alcohol consumption, and the use of medication, information is gathered from three months before pregnancy until the end of pregnancy’. Page 5, lines 125-126.

Comment 7:

Line 128-145: Definition of cases and control: I think it would be more logical to present first the definition of the case (P.135) and after that the exclusion criteria unemployed housewives….I think it's important to have an idea of the percentage of non-responder among the eligible cases and control. The number of non-responder are cited in the same way as for exclusion criteria (unemployed, housewife…). For me it's not the same.

Answer:

We thank the reviewer for this suggestion. We rearranged this part of the method section (page 6, lines 131-155). First we defined the case group and show the number of non-responders with the respective percentages. We repeated this for the first and second control group. However, the ascertainment for cases and malformed controls is exactly the same, because we register all babies/foetuses with a congenital anomaly born in the Northern part of the Netherlands.

Comment 8:

In the case group, why have you include the Pierre Robin sequence?

Answer:

We included babies with a Pierre Robin sequence, because the etiology of the Pierre Robin sequence is not fully understood. Almost all cases with a Pierre Robin sequence were seen by a geneticist and therefore an underlying monogenic or chromosomal cause is unlikely.
Comment 9:

Control group : the author choose as control group chromosomal/monogenic defects. Because the age of the parents is associated at least with chromosomal defects and because the advance age at pregnancy is very often associated with educational level and by consequence occupational exposure, I think that the control group is highly selected. Did the author conduct sensibility analyses with all other malformation as a control group?

Answer:

Since both reviewers suggested this, we performed additional analyses with a second control group (non-chromosomal/non-monogenetic malformed infants and foetuses) as a sensitivity analysis. The baseline characteristics were more similar for the non-chromosomal control group (page 9, lines 217-219 and table 1).

Occupational exposure overall was lower in this new non-chromosomal control group compared to the chromosomal control group and the case group (page 9, lines 224-229, table 3). The multivariate regression analyses showed significantly increased aORs for infants with an oral cleft whose mothers were exposed to pesticides, especially for fungicides (aOR=2.0, 95% CI 1.1-3.7) and insecticides (aOR=1.8, 95% CI 1.0-3.2). Furthermore, a significantly increased aOR for organic dust was found (aOR=1.3, 95% CI 1.1-1.7). We added the results of these additional analyses in the paper on page 10, lines 236-240, and in table 3, page 24-25.

Comment 10:

Line 149 : How did you define periconceptional period did it include the windows of palate and lip formation ?

Answer:

The window of palate and lip formation is from week 4 till week 12 after conception. We ask women about their job during the beginning of the pregnancy (3 months before conception until the first trimester). The palate and lip formation period is within this period. This was added to the methods section (page 7, lines 161-162).

Comment 11

Line 152: 'other' include the oxygenated solvents family (eg alcohols, cetone or glycol ethers) ?

Answer:
Other solvents include higher alkanes, alcohols and esters. We added this information at page 7, lines 169-170, as follows: ‘The ALOHA+ JEM assigned occupational exposure to solvents (aromatic, chlorinated and other [e.g. alkanes, alcohols, and esters]).’

Comment 12

Line 177-180: it seems that the authors choose a restrictive criteria for selection of confounders included in the multivariate models (p=value of 5%). This criteria may be too restrictive and may lead to residual confounding but in light of the table 1 it include most of the potential confounder.

Answer:

The criteria might be too restrictive. However, we know from literature what are important potential confounders. Apart from statistics, we want to decide a priori which of these potential confounders, as biological plausible explanations for congenital anomalies, are needed to put in the model. Therefore, we do not want to do a backward/forward regression.

Comment 13:

Results: It's not clear for me, why the table 1 is in the text and why table 2 is after the references.

Answer:

We uploaded table 1 as additional file, because of landscape page. We moved table 2 and table 3 to after the references on reviewers’ request (page 22-25).

Comment 14:

Concerning the exposure description (line 235-242), it could be preferable to present the text in the same order that the table (any agents, and may be some information concerning solvents exposure which seems according to the literature review an important objective of this work, and then pesticides. …).

Answer:

We agree with the reviewer and changed the order in the text according to the order in the table, as follows: ‘Prevalence of maternal exposure to solvents was similar among cases and controls. The most frequent type of solvent exposure was exposure to ‘other solvents’. Mothers exposed to
‘other solvents’ were mainly working in healthcare. The prevalence of occupational exposure to pesticides was low, but was higher among cases than controls (3.6% versus 2.4% for chromosomal controls and 2.0% for non-chromosomal controls). Maternal occupational exposure to organic dust occurred most frequent, with case mothers being more often exposed to organic dust than chromosomal/non-chromosomal controls (36.7% versus 32.6%/29.6%). Mothers exposed to organic dust were working in e.g. healthcare or agriculture. Page 9, lines 224-233.

Comment 15:

Concerning solvents exposure, the most frequent exposure is the exposure to 'other' class of solvent. Is it possible to have some information about this class and the occupation mainly classified as exposed to this class in this study?

Answer:

We added information about the type of solvents in the class ‘other solvents’ in the method section, namely: ‘ALOHA+ JEM assigned occupational exposure to solvents (aromatic, chlorinated and other [e.g. alkanes, alcohols, and esters])’ (page 7, lines 169-170). Furthermore, we added a sentence about the occupations in the population which were assigned an exposure to “other solvents” at page 9, lines 227: ‘Mothers exposed to ‘other solvents’ were mainly working in healthcare’.

Discussion:

Comment 16:

Line 276-280: It's not all the same studies that those present in the introduction. There is the need to check the literature review.

Answer:

We checked the literature and made some changes to be more consistent between the introduction and discussion.

Comment 17:

Line 284-285: I Don't understand why expert assessment could have led to differential recall bias. It may be concerned the self-reported on which expert evaluated the exposure? Please clarify.
Answer:

It’s true that expert assessment itself does not lead to differential recall bias. We meant to say that the self-reported exposure of cases can be more extensive than those of controls, which can then lead to a biased expert assessment. We have removed this sentence and added as strength that the JEM avoids recall bias, namely: ‘The benefit of using a JEM is that it avoids recall bias since the mother is not directly asked about her occupational exposure during pregnancy’. Page 12, lines 309-310.

Comment 18:

I think that there is a prospective study in the literature cited, this kind of study is rare for studying malformation and may be some word on it could be important even if of course it have some limitations (the number of oral cleft) but this study gives significant results with direct and indirect assessment methods of exposure.

Answer:

We agree it is important to highlight this study because the strength of this study is the prospective design, and this study used a JEM as well. We added a sentence about this study at page 12, lines 288-291: ‘However, there is a prospective study, using self-reported exposure assessment as well as a JEM, that reports a significant increased risk of oral clefts in the offspring for mothers exposed to solvents [3]’.

Strengths and limitations:

Comment 19:

Line 300-305: number of cases what about Desrosiers et al? (>1000 I think). Shaw? (preferable to have a larger sample size with a JEM assessment than a smaller one with expert assessment?)

Answer:

We completely agree with the reviewer. With the new literature review in the Introduction and Discussion section we think the power of this study is not a major strength. Therefore we removed lines 300-305 in the Strengths and limitation section.

Comment 20:
Line 306: What about limitation of JEM exposure assessment comparing to expert assessment which is usually considered as the reference method in population based case-control study (Teschke et al OEM 2002).

Answer:

We thank the reviewer for suggesting this paper. We have read the paper of Teschke et al. and added a sentence about JEM exposure assessment compared with expert assessment in the limitations section, as follows: ‘Another limitation of using a JEM, compared to expert assessment, is that JEMs have often low sensitivity. Partly, this low sensitivity is due to the variability in exposure across time which is not taken into account by the JEM [29]’. Page 13, lines 317-319.

Comment 21:

What about the fact that low and high exposure categories were merge in a single class. What could be the impact on the results ? Is it possible for the more frequent exposure classes (any agents, solvents, other solvents, dusts, gases and fumes) to have the results by classes low and high ?

Answer:

We performed an additional analysis with low and high exposure for the categories ‘other solvents’, organic dust, and gases and fumes, because high exposure is more frequent in these subclasses.

There were 10, 11 and 4 oral cleft cases respectively, with high exposure for these categories.

When we performed a multivariate logistic regression for ‘other solvents’ exposure, the aOR for cases with low exposure was 1.1, 95% CI 0.8-1.5, and increased to 1.5, 95% CI 0.8-3.0, for cases with high exposure. However, both ORs are non-significant and the OR for low exposure is equal to the aOR for merged low and high exposure.

For occupational exposure to organic dust we observed the same trend. The aOR increased from 1.3, 95% CI 1.1-1.6, for low exposure, to 1.7, 95% CI 0.9-3.2, for high exposure. Because there were only few cases with high exposure to organic dust (n=11) and many cases with low exposure to organic dust (n=131), the aOR for low exposure is equal to the aOR for merged low and high exposure.

We did not observe this trend of increased OR for occupational exposure to gases and fumes.

Based the frequency of cases with high exposure and the logistic regression we do not think merging low and high exposure categories in a single class had impact on our results.
We described the results of these additional analyses on page 10, lines 251-255.

Comment 22:
Line 309-310: during pregnancy or during periconceptional period?
Answer:
We did mean during the periconceptional period. We have changed this on page 13, line 315 in the revised manuscript.

Comment 23:
Line 311: What is the windows of susceptibility of oral cleft? Do you have any information concerning work change during pregnancies to avoid potential Hazards in the NL?
Answer:
The window of susceptibility of oral clefts is in the first trimester. In the Netherlands, pregnant women are advised to avoid hazardous exposures in certain occupations. For example, women in agricultural sector are advised to avoid pesticides before and during pregnancy. This means that by using a JEM we might have overestimated the true exposure. We have added this as a limitation in the discussion (page 13, lines 314-317).

Comment 24:
Line 325-326: … and may explained why the authors did not found associations with solvents exposure. Is it feasible to conduct a sensibility analysis with another group of malformed birth as control group.
Answer:
We did additional analyses with a second (non-chromosomal malformed) control group as mentioned earlier. The results of these additional analyses are described in the answer to comment 9 and in the Results section of the revised manuscript on page 10, lines 236-240, and in table 3, page 24-25.

Comment 25:
Conclusion: line 343-345: in the text and in the table 2, the author reported a % of exposure to any agents >40% and for other solvents>20%, I think that it's not in accordance with the sentence in the conclusion (rare exposure)?

Answer:

We apologise this is not clear. The sentence on low number of individuals exposed was about pesticide exposure. We removed this sentence in the revised manuscript.

Reviewer #2: overall, this is an interesting paper that reads very well and adds useful information to the literature, but there are some issues in the adjusted analysis that I believe must be addressed. Although they are important, I consider them minor because I do not think these would be very time-consuming to correct.

Major issues in the adjusted analysis:

Comment 1:

Effect modification is completely ignored. Specifically: infant gender was different between the malformed control and case infants. Previous research on the association between herbicides and oral clefts has shown effect modification by infant gender, as the authors note in their background section. These are excellent reasons to examine results stratified by infant gender, not merely adjust for infant gender. Did the authors consider this?

Answer:

We thank the reviewer for this valuable comment. We did additional analyses where we stratified the results by gender using non-chromosomal controls.

We performed this additional analysis only for cases with a CL(P), because this is the group of oral clefts where significant gender differences were observed. We observed an increased aOR for occupational exposure to ‘other solvents’ in male infants (aOR=1.5, 95% CI 1.1-2.1).

Furthermore, the aOR for exposure to herbicides, and mineral dust was increased for female infants (aOR=3.8, 95% CI 1.1-13.4 and aOR=2.0, 95% CI 1.2-3.5, respectively). However, increased risk for herbicides was only based on three exposed cases.

In conclusion, we observed differences in risk factors based on infant gender.
We added this to the Methods (page 8, lines 200-201) and the Results section (page 10, lines 245-250).

Comment 2:

The authors defined family history as a positive history of a chromosomal or genetic disorder for controls, not a family history of orofacial clefts. This is unusual; family history is usually defined as family history of the outcome under study (i.e. oral clefts) for both cases and controls. Please explain the rationale and how this would impact interpretation of results.

Answer:

We do not agree on this point with the reviewer. Family history is used to correct for the genetic predisposition. Usually family history is defined as the same outcome under study if healthy controls are used. However, in our study malformed controls are used. We defined positive family history as a first degree family member having the same condition as the child under study. For instance, family history is positive when a child with a cleft has a sibling/mother/father with a cleft. But family history in controls is positive, when, for instance, a child with a congenital heart defect has a sibling with a congenital heart defect. There is a genetic component in the etiology of congenital heart defects, where you have to account for as well. Besides, in our opinion you cannot speak of a positive family history, when for example a mother of a child with a heart defect has an oral cleft, because these are different congenital anomalies, with different etiologies.

Comment 3:

There are more degrees of freedom than exposed cases in most comparisons in table 2. These results are overadjusted, and there will be positivity violations that can cause errors in estimates (even though models will converge in a logistic regression). Common tactics to prevent unnecessary adjustment are backwards selection and/or using a criteria of "change in the estimate of the main effect" criteria.

Answer:

We apologise this was not clear. First we compared the exposures of all oral cleft cases with those of the control group. In a first subgroup analysis we compared the exposures of CP cases with the control group. In a second subgroup analysis we compared CL(P) cases with the same control group. These last two analyses are subgroup analyses of the fist analysis. We rewrote a part of the Methods section to clarify this, namely: ‘Separate subgroup analyses were conducted for CP and CL(P) alone compared with both control groups’ (page 8, lines 197-199).
We did not consider forward/backward selection of potential confounders, because we know from literature what are the important potential confounders for oral clefts. Apart from statistics, we want to decide a priori which of these potential confounders, as biological plausible explanations for congenital anomalies, are needed to put in the model. Therefore, we do not want to do a backward/forward regression.

Comment 4:

I am not clear on how the adjusted models shown in table 2 were developed. Were the terms for fungicides, insecticides, and herbicides included simultaneously in the same model or separate models? At lines 246-249, it sounds like terms were simultaneously added to the model for pesticide and organic dust, but all cases were co-exposed to organic dust-- again, this can cause biased effect estimates due to positivity violations.

Answer:

The adjusted models shown in table 2 are developed as we explained on the reviewers’ 3rd comment. The terms fungicides, insecticides, and herbicides were not simultaneously included in the model. It were separate models.

In the original manuscript we performed an additional analysis were we simultaneously added pesticide and organic dust in the model. The reviewer is right that we caused biased effect estimates due to positivity violations with this additional analysis, because the correlation between pesticide exposure and organic dust exposure is high (all cases exposed to pesticides are exposed to organic dust). We decided to remove that part from our results.

Comment 5:

I suggest a more thorough evaluation of the potential bias that can be introduced by using malformed controls. One relatively quick and easy approach I've seen in registry-based studies is to use two control groups (one is infants with chromosomal/genetic disorders, the other is infants with non-chromosomal/genetic disorders other than the defect under study). This might be a useful approach; otherwise I suggest including some mention in the methods of steps the authors took to address these.

Answer:

Following the reviewer’s suggestion, we have now added a second control group consisting of infants with non-chromosomal/non-monogenetic disorders, with other defects than oral clefts. We performed all analyses with these non-chromosomal controls and added the baseline characteristics in table 1. Table 3 shows the prevalence of exposure and logistic regression
analyses with the non-chromosomal malformed controls. For this second control group we rewrote the Methods, Results and Discussion.

We described the results of these additional analyses at comment 9 of the first reviewer.

Minor suggestions:

Comment 6:

Please give a brief background of the ALOHA+ JEM. A few sentences should be sufficient.

Answer:

We gave some background information in the Method section, subheading Exposure assessment (page 7, lines 163-166).

Comment 7:

Line 133, typo: divided, not dived.

Answer:

We corrected the typo.