PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Cohort profile: The Caribbean Consortium for Research in Environmental and Occupational Health (CCREOH) Cohort Study: Influences of complex environmental exposures on maternal and child health in Suriname
AUTHORS	Zijlmans, Wilco; Wickliffe, Jeffrey; Hindori-Mohangoo, Ashna; MacDonald-Ottevanger, Sigrid; Ouboter, Paul; Landburg, Gwendolyn; Codrington, John; Roosblad, Jimmy; Baldewsingh, Gaitree; Ramjatan, Radha; Gokoel, Anisma; Abdoel Wahid, Firoz; Fortes Soares, Lissa; Alcala, Cecilia; Boedhoe, Esther; Grünberg, Antoon; Hawkins, William; Shankar, Arti; Harville, Emily; Drury, SS; Covert, Hannah; Lichtveld, Maureen

VERSION 1 – REVIEW

REVIEWER	Caroline Taylor University of Bristol, UK
REVIEW RETURNED	25-Oct-2019

GENERAL COMMENTS	The cohort seeks to address an important issue in environmental exposures: that of the effects of complex multiple exposures to toxic metals and pesticides in high risk areas in a developing country. The evaluation of biomarkers, dietary assessment and food analyses provides a multi-faceted approach. However, I found the description of the study procedures confusing. The paper could be greatly re-organised with a more logical flow for clarity. In addition, the writing switches between past and present tense in a way that does not help the reader grasp the timelime readily, and where we are presently in the timespan of the study. In the aims you mention evaluation of mobile health technology in pregnancy but this is never referred again. Biomarker analysis: there is no reporting of any quality control measures for the analyses. Again, the timeline is confusing because you describe the analyses and then go on to further describe sample collection of other tissues. What was the protocol for the hair sampling? How were the pesticides analysed? There are quite a few details that it would be helpful to add. What determines whether the infant has a cord blood or a heel prick (I see on further reading there is an explanation at the end of the Strength and Limitations section)? Can you give some details of the questionnaires – are they standard questionnaires? Have they been validated? The data management description needs more detail. For example, will the data be placed in a repository for controlled access by researchers? Please consider placing this either at the

	beginning or end of the paper so that it encompasses all your data collection rather than in the middle of the paper. Details of sample collection and analysis should not be in the data management and stats section. It would be helpful to have more details in the stats plan – an important feature of this cohort is the array of environmental data available – what is the general approach to multiple exposures? How will you deal with the three sites – are they to be compared or will you do some cluster analysis? There is no explanation of how you modelled hair Hg and birth outcomes – if it's likely that this will be the topic of a full paper I would delete it here. Table 1: include 'cord blood' as 'cord or heel prick blood'? In summary this is a very interesting study and well worth publishing the cohort description. In my view It is essential to re-organise the content for clarity and include sufficient detail before publication.

VERSION 1 – AUTHOR RESPONSE

COMMENTS REVIEWER 1

The cohort seeks to address an important issue in environmental exposures: that of the effects of complex multiple exposures to toxic metals and pesticides in high risk areas in a developing country. The evaluation of biomarkers, dietary assessment and food analyses provides a multi-faceted approach.

However, I found the description of the study procedures confusing. The paper could be greatly re-organised with a more logical flow for clarity. In addition, the writing switches between past and present tense in a way that does not help the reader grasp the timelime readily, and where we are presently in the timespan of the study.

Thank you dr Taylor for your most valuable comments. We have made several adjustments in order to properly address your comments:

1) we re-organised the timeline and adjusted the topic order for more clarity.
2) we deleted the hair Hg and birth outcomes section since that indeed will be the topic of a separate full paper
3) for the same reason, we have deleted the urinary pesticide findings section including table 3 since this will be the topic of another full paper that is currently being drafted
4) we have addressed some inaccuracies or oversights from our first draft that we have just noticed.
5) Finally, for word count sake, we have rearranged the wording in certain sections

More specifically we have addressed your comments point by point:

In the aims you mention evaluation of mobile health technology in pregnancy but this is never referred again.

This has been added in the recruitment section, 2nd paragraph

Biomarker analysis: there is no reporting of any quality control measures for the analyses. Again, the timeline is confusing because you describe the analyses and then go on to further describe sample collection of other tissues.

Thank you for pointing this out. A description of quality control for the analyses has been added, biospecimen collection section, 2nd paragraph

What was the protocol for the hair sampling?

A description of the hairprotocol has been added, biospecimen collection section, 4th paragraph

How were the pesticides analysed? There are quite a few details that it would be helpful to add.

We have added a more detailed description of the pesticide analyses, Urine collection for pesticides analyses section, 1st paragraph.

What determines whether the infant has a cord blood or a heel prick (I see on further reading there is an explanation at the end of the Strength and Limitations section)?

Thank you for this comment. In certain cases, especially in the interior, it was not always feasible to obtain a cord blood sample at birth. In that case a heelprick sample was taken as soon as possible after birth. We have added this in the paragraph on data collection from birth through 48 months, 1st paragraph.

Can you give some details of the questionnaires – are they standard questionnaires? Have they been validated?

All questionnaires used were standard validated questionnaires. We have added this in the Data collection during pregnancy, Questionnaires section, 1st paragraph.

The data management description needs more detail. For example, will the data be placed in a repository for controlled access by researchers?

This has been addressed in the data management and statistical plan section

Please consider placing this either at the beginning or end of the paper so that it encompasses all your data collection rather than in the middle of the paper.

The data management and statistical plan has been moved to the beginning of the paper immediately after the recruitment section

Details of sample collection and analysis should not be in the data management and stats section. This has been moved to the recruitment section

It would be helpful to have more details in the stats plan – an important feature of this cohort is the array of environmental data available – what is the general approach to multiple exposures?

These have been added in the data management and statistical plan section

How will you deal with the three sites – are they to be compared or will you do some cluster analysis?

Comparison between study sites will primarily be analyzed by comparing participants, but can also be clustered depending on the research question. This has been added in the data management and statistical plan section

There is no explanation of how you modelled hair Hg and birth outcomes – if it's likely that this will be the topic of a full paper I would delete it here.

Indeed this will be the topic of a separate full paper, we therefore deleted this paragraph that included information on hair Hg results of the studypopulation.

Instead, in the section ''Findings to date'' subparagraph 'Biospecimens' we added the hair Hg results of the total number of 876 pregnant women and mention the percentage of women that had levels at or above the USEPA action level 1.1 ug/g (indicating that overall 39,1% of women had levels that would require further action). This was also adjusted in the abstract

Table 1: include 'cord blood' as 'cord or heel prick blood'?

This has been corrected in table 1

In summary this is a very interesting study and well worth publishing the cohort description. In my view It is essential to re-organise the content for clarity and include sufficient detail before publication.

Thank you very much for your compliments. Again we are very grateful for your comments, we hope to have sufficiently addressed these to help improve our paper.

VERSION 2 – REVIEW

REVIEWER	Dr Caroline Taylor University of Bristol, UK
REVIEW RETURNED	18-May-2020

GENERAL COMMENTS	Thank you for your revisions. In general the paper is much improved. In particular the timeline, and the description of what has been done so far, is clearer, with the exception of the abstract. Abstract In general, the abstract does not have the right level of details. For example: The statement that environmental assessments included fish etc is not very helpful. What was assessed in the fish and other foods? Or do you mean dietary assessment? You don't mention anything about biological assessments. The Participants section needs to make it clear exactly what has been done to date in the study – from further reading you have recruited and completed the data collection in pregnancy and started data collection in infants but this is not clear.

	The Future plans section opens with a statement that would be better placed in the Findings to date section. What were the blood mercury concentrations? Recruitment Are there differences in the literacy levels between the three sites? Do the mothers need to be literate to take part? If so, please expand on this in the limitations section. If not, please describe how you enable mothers to take part if they are unable to read/write. Do you need to involve any translators? What percentage of mothers deliver outside hospitals? Is this also a source of potential bias? Data collection from birth to 48 months Buccal swab – this section should include details of collection and analysis only rather than background to justify the analysis. Data management and statistics You mention cross-checking with medical records – please could outline what this involves and your methods of accessing medical records? Are you undertaking data linkage to electronic records? The description of the statistical methods is a little sketchy. Please could you add more details – for example how will you analyse associations with multiple exposures to toxic metals? What confounders might be considered (for example, have you collected data on smoking). How will you account for exposures during childhood? Do you have any power calculations? Findings to date How do the hair levels compare with values found in other studies? You do not mention how you derived the mercury exposure from fish from the dietary assessments – did you use a database of the mercury content of fish or direct analysis of local fish? Dietary exposures to mercury Here you include details of additional analyses on species of fish – this should be documented in your study description. You have not provided any citations to support the concept of the balance between positive benefits of nutrients in fish versus negative effects of mercury. Strengths and limitations You give a good account of the strengths, but the limitations are not described in any detail. Please could you expand on this part of this section (I mentioned some potential sources of bias above). I note also from Figure 2 that you were unable to attain your target recruitment number and have a substantial number of participants lost to follow up for various reasons. How will this affect your results as the study proceeds? (There are no power calculations provided to support the inclusion of 1200 women in the study.) Data sharing statement Will the data be placed in a data repository for controlled access? You state that the data are partially available. Is this the case? Will you not embargo data until they are in the repository?

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name: Dr Caroline Taylor
Institution and Country: University of Bristol, UK
Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below
Thank you for your revisions. In general the paper is much improved. In particular the timeline, and the description of what has been done so far, is clearer, with the exception of the abstract.

Response: thank you once again dr. Taylor for your insightful comments. We have tried to address these point by point to the extent possible and have revised the manuscript accordingly, including the abstract.
In addition:

1) at the request of the editor we have deleted figure 1 since this was seen as copyrighted material
2) we have updated the numbers of enrolled women and infants
3) we refrained from testing the children at 24 months for logistic reasons and have removed these assessments from the paper
4) for word count sake, we have rearranged the wording in certain sections

Abstract
In general, the abstract does not have the right level of details. For example:
The statement that environmental assessments included fish etc is not very helpful. What was assessed in the fish and other foods? Or do you mean dietary assessment? You don't mention anything about biological assessments.

Response: thank you for this comment, we have included additional references regarding information on environmental source characterization, dietary assessment, and biomarker evaluation in a sub-cohort available to date. Other analyses are ongoing.

The Participants section needs to make it clear exactly what has been done to date in the study – from further reading you have recruited and completed the data collection in pregnancy and started data collection in infants but this is not clear.

Response: thank you for pointing this out. This has been added in more detail to the Participants section of the abstract

The Future plans section opens with a statement that would be better placed in the Findings to date section. What were the blood mercury concentrations?

Response: Thank you for this suggestion. We have revised the statement on high mercury exposure in interior women and added mercury concentrations to the Findings to date section of the abstract

Recruitment
Are there differences in the literacy levels between the three sites?

Response: thank you for the comment. We are not aware of any previous data on (differences in) literacy from the three inclusion sites. To assess this, we have developed a pesticide literacy scale as mentioned earlier in the Future plans section.

However, based on our data on education, women in the interior had lower levels of education than women from Paramaribo and Nickerie. This could confound or impact literacy. We have added this to the limitation section

Do the mothers need to be literate to take part? If so, please expand on this in the limitations section. If not, please describe how you enable mothers to take part if they are unable to read/write. Do you need to involve any translators?

Response: thank you for the comment. Informed consent forms and questionnaires were translated in local languages Sranan Tongo, Sarnami, Saramaccan or Trio. In case the participant was unable to read the recruiter would read the questions in the local language. This has been added to the recruitment section.

What percentage of mothers deliver outside hospitals? Is this also a source of potential bias?

Response: thank you for the question. The annual births in Suriname are estimated at 10,000. 85% of all deliveries are hospital-based, with the remainder taking place in the primary health care under the supervision of a general practitioner or midwife at the Regional Health Service or a trained healthcare worker at the Medical Mission. A small proportion (1%) occurs at home. This implies that the vast majority (99%) of all deliveries take place in one of the recruitment sites used in this study. This has been added to the recruitment section.

Data collection from birth to 48 months
Buccal swab – this section should include details of collection and analysis only rather than background to justify the analysis.

Response: thank you for the suggestion. Detail of collection and analyses have been added to the Buccal swab collection for telomere assessments in the Data collection from birth to 48 months section

Data management and statistics
You mention cross-checking with medical records – please could outline what this involves and your methods of accessing medical records? Are you undertaking data linkage to electronic records?

Response: thank you for the comment. In case of missing data or incorrect data entries we consult the general practitioner, midwife, health assistant or hospital administration for permission to review the hard copy medical record of the participant.

This has been added to the Data management and statistics section

All medical records used were written hard copies, no linkage to electronic records.

The description of the statistical methods is a little sketchy. Please could you add more details – for example how will you analyse associations with multiple exposures to toxic metals? What confounders might be considered (for example, have you collected data on smoking). How will you account for exposures during childhood? Do you have any power calculations?

Response: thank you for the comment. We have added additional text in the Data management and statistics section to address these issues.

Findings to date
How do the hair levels compare with values found in other studies?

Response: thank you for the question. Mercury hair levels from women in the interior (median 3.48 ug/g) were well above international accepted health action levels (1.1ug/g hair). Mercury concentrations from women living in Paramaribo and Nickerie are similar to those found in other studies. This has been added in Findings to date section.

You do not mention how you derived the mercury exposure from fish from the dietary assessments – did you use a database of the mercury content of fish or direct analysis of local fish?

Response: thank you for the question. Local fish was previously analyzed for mercury content by co-author dr Ouboter, we sited his paper earlier and added the fish Hg concentrations, this has been added to the Cohort description section as a separate paragraph 'Data collection from fish'.

We have also completed dietary assessments that focus on fish consumption to support our hypothesis that fish consumption is consistent with the mercury exposures. In addition, we have speciated mercury in blood and hair and have found that the majority of the mercury in these human samples is methylmercury which again is consistent with fish consumption being the primary source of mercury exposure in our participants. This has been added to the Dietary exposure to Hg in fish section

Dietary exposures to mercury
Here you include details of additional analyses on species of fish – this should be documented in your study description.

Response: thank you for pointing this out. This has been added to the new paragraph 'Data collection from fish' section
You have not provided any citations to support the concept of the balance between positive benefits of nutrients in fish versus negative effects of mercury.

Response: thank you for the suggestion. These have also been added to the new paragraph 'Data collection from fish'

Strengths and limitations
You give a good account of the strengths, but the limitations are not described in any detail. Please could you expand on this part of this section (I mentioned some potential sources of bias above).

Response: thank you for this comment. We added the potential differences in literacy to the limitations section as well as some other limitations. Some participants from the interior sub-cohort were recruited in the second or early third trimester because of the distance to prenatal clinics. For these participants we miss biospecimen and questionnaire data for an earlier study timepoint. This may limit our ability to understand differential effects of exposure across gestation for this sub-cohort. This has been added to the Strengths and limitations section.

I note also from Figure 2 that you were unable to attain your target recruitment number and have a substantial number of participants lost to follow up for various reasons. How will this affect your results as the study proceeds? (There are no power calculations provided to support the inclusion of 1200 women in the study.)

Response: thank you for this question. Our initial study design was to recruit 1000 women. We asked permission from the Institutional Review Boards to consent 1200 pregnant women, because we

planned to over-recruit given the expected lost to follow up, as you rightfully pointed out. This has been added to the recruitment section.

Sample size was calculated based on a multiple linear regression model using a coefficient of determination of 0.10 and an R-square differential of 0.02. Using these parameters, an N of 495 was needed to have an 80% power at a 0.05 level of significance. We have added this text to the Data management & statistical plan section

Data sharing statement
Will the data be placed in a data repository for controlled access? You state that the data are partially available. Is this the case? Will you not embargo data until they are in the repository?

Response: thank you for the opportunity to clarify data access. We are currently collaborating with RTI, a global data management enterprise, to develop an integrated database of biospecimen and non-biospecimen data. Once that is fully developed, data can be made available based on a reasonable request to the PIs. Such requests will be discussed with the full investigator Committee, the Data Management Committee, and the Administrative Oversight Committee.

VERSION 3 – REVIEW

REVIEWER	Caroline Taylor University of Bristol, UK
REVIEW RETURNED	22-Jun-2020

GENERAL COMMENTS	Abstract: The Section 'Participants' contains information about what has been done with the participants (this should perhaps be in the 'What has been done to date' section) rather than a description of the characteristics and demographics of the participants. Please review the whole abstract with the headings in mind to ensure that information is in the correct section. in 'Future plans' please change 'fish consumption is the primary source' to 'fish consumption is likely to be the primary source' as you haven't shown it is the main source yet. Power calculation: if your power calculation indicates that 485 participants are sufficient to give 85% power, it could be considered unethical to recruit more than this number as you are collection data that is not strictly needed. What is the reason for the extra recruitment? I note, however, that biosamples are not taken from all participants - below 495 in most cases. The reason for this is not completely explained. Does it then limit the power for the data from these sample? Sections on data collection: Please review the tense that you use in these descriptions to make a clear distinction between what has already been done and what is planned for the future. This applies also to the dissemination section. Fish analysis: please give details of the fatty analysis that you mention. Dietary exposure: You give a value for the percentage of fish-consumers. Do you have data on the frequency of consumption among consumers? References: Please see also the work from ALSPAC and the Seychelles study. etc. on the conflicting roles of prenatal mercury exposure and fish consumption.

VERSION 3 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Caroline Taylor
Institution and Country: University of Bristol, UK
Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below

Response: thank you once more dr. Taylor for your insightful comments. We have addressed these to the extent possible and have revised the manuscript accordingly, including the abstract.

Abstract: The Section 'Participants' contains information about what has been done with the participants (this should perhaps be in the 'What has been done to date' section) rather than a description of the characteristics and demographics of the participants. Please review the whole abstract with the headings in mind to ensure that information is in the correct section.
Response: we have made suggested corrections to the Participants section. We have also revised the required sections of the abstract (Purpose, Participants, Findings to date, Future plans) as needed to ensure that information is in the correct section.

In 'Future plans' please change 'fish consumption is the primary source' to 'fish consumption is likely to be the primary source' as you haven't shown it is the main source yet.
Response: this has been revised.

Power calculation: if your power calculation indicates that 485 participants are sufficient to give 85% power, it could be considered unethical to recruit more than this number as you are collection data that is not strictly needed. What is the reason for the extra recruitment?
Response: thank you for pointing this out. Originally we planned to only compare the highest exposed children versus the lowest exposed children, therefore the sample size was calculated based on an N of 495. Since we decided to do neurodevelopmental testing in all children, the sample size calculation was adjusted. We have revised this in the ‘Datamanagement & statistical plan’ section, last paragraph. Text is highlighted.

I note, however, that biosamples are not taken from all participants - below 495 in most cases. The reason for this is not completely explained. Does it then limit the power for the data from these sample?
Response: apologies for this misunderstanding. Sofar we have collected: from 1143 pregnant women: 1994 whole blood samples for trace elements 1994 whole blood samples collected in K2EDTA anticoagulant, 1994 serum collected in serum separator tubes, 1994 plasma samples, 1980 urine samples, 941 buccal swabs, and 876 hair samples; From 992 infants either cord blood (N=441) or blood from heel prick (N=323) at birth, as well as 842 buccal swabs. Please see ‘Findings to date’section, second paragraph.

Sections on data collection: Please review the tense that you use in these descriptions to make a clear distinction between what has already been done and what is planned for the future. This applies also to the dissemination section.
Response: we have reviewed all of these sections and made changes throughout to make this clearer.

Fish analysis: please give details of the fatty analysis that you mention.

Response: these have been added to ‘Data collection from fish’ section. Text is highlighted.

Dietary exposure: You give a value for the percentage of fish-consumers. Do you have data on the frequency of consumption among consumers?
Response: we are presently analyzing these data and preparing for a separate publication. Data summarized so far represents certain species reported to be among the top three preference of the full cohort. Intake rates (based on reported meal frequency and portion sizes) for these three carnivorous species ranged between 0.01-2.5 kilograms per week. This has been added to ‘Dietary exposure to Hg in fish’ section. Text is highlighted.

References: Please see also the work from ALSPAC and the Seychelles study. etc. on the conflicting roles of prenatal mercury exposure and fish consumption.
Response: thank for pointing this out. We have added these references. Please see ‘Data collection from fish’ and references section. Text is highlighted.

VERSION 4 – REVIEW

REVIEWER	Dr Caroline Taylor University of Bristol, UK
REVIEW RETURNED	03-Aug-2020

GENERAL COMMENTS	Thank you for the patient work on your paper in response to my comments.