Author's response to reviews

Title: Maternal fish and shellfish intake and pregnancy outcomes. A prospective cohort study in Brittany, France.

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Sir,

Please find the revised version of the manuscript referenced above.

Yours sincerely,

Laurence Guldner

Changes made in the revised version, in light of the reviewers’ comments

REVIEWER 1

Minor issues

Abstract: For a reader who only sees the abstract, it is difficult to interpret the measures of association given in there; e.g. specify what does highest and lowest shellfish consumption mean, and what does a fish meal refer to.

Highest and lowest categories of fish and shellfish consumption were specified (p2 lines 15-19). The term meal simply means a meal including fish or shellfish (as hot meal, sandwich, etc).

And the sentence “No other association …” is not understandable as written in the abstract.

This sentence has been revised and clarified (p2 line 21).

Page 4, dietary assessment: The authors should state precisely when during pregnancy the women completed the questionnaire.

This information has been added (p4 line 17).

The description of the classifications employed is not entirely clear to me and should be checked carefully again by the authors. E.g., for fish consumption the middle category (out of three categories) was “1-4 a month”, whereas for shellfish it was “2-4 times a month”; is it correct that there was a difference here?
Global shellfish intake was computed from the answers to the 3 questions about mollusks, large and small crustaceans, ranging from “never or less than once per month” (coded as 0.5 per month), “1 to 4 a month” (coded as 3 per month), “2 or 3 times a week” (coded as 10 per month), “4 to 6 times a week” (coded as 20 per month) to “Every day” (coded as 30 per month). Initially, the sum for the lowest consumption (corresponding to answer “never or less than once per month” for the 3 categories) was then 0.5+0.5+0.5=1.5 per month, which was not included in the same lowest category as fish intake, i.e. “never or less than once per month”. We can assume, however, that if the person reports eating mollusks and crustaceans, each at a frequency of less than once a month, the real total frequency is more likely to average never or less than once per month. We therefore changed the labeling for the shellfish categories for more homogeneity with those of fish intake.

*Page 10, line 6. “The trend towards …” I may be wrong but I do not fully understand the meaning of this sentence. Can it be the authors have erroneously switched between the words “positive” and negative” here?*

There was effectively a switch between both terms that has been corrected *(p10 line 6-8).*

*Table 5. It makes little sense to me to give a common estimate for the EPA and DHA (LC-PUFA n-3 FA) content in fish with such a variable content such as cod and salmon.*

We have deleted Table 5. We gave details in the text about the calculation and comparison of mean contents (n-3 PUFAs without distinction) and contaminations (organochlorines and metals) of fish and shellfish, based on data from the CALIPSO study *(p10 lines 13-20).*
Major Compulsory Revisions

1. The discussion of prior lit has some notable gaps. For example, the authors might want to reference the paper by Smuts et al. (OB GYN 2003), a RCT of DHA enriched eggs which found an increase in gestation length, and the study by Knudsen et al (BJOG 2006), an RCT of fish oil which found no effect on gestation length. Please also see the recent publication by Halldorsson et al. (IJE 2007), which includes a discussion of previous trials as well as new data suggesting that intake of fatty fish, which are likely to contain higher levels of persistent organic pollutants, is associated with lower fetal growth. References to Knudsen et al, BJOG 2006 (p3 line 10, ref 14) and Halldorsson et al, Am J Epid 2007 (p3 line 16, ref 20) were included. We also included references to 2 meta-analyses of n-3 PUFAs supplementation trials (p3 line5): Szajewska et al, Am J Clin Nut 2006 (ref 6) and Horvath et al, Br J Nutr 2007 (ref 7), the first one including the study of Smuts et al.

It would be helpful if the authors could specify which of the prior studies examined fish only, which fish and shellfish. This information has been added (p3 lines 21 and 22).

Background, line 10 – was the ‘negative effect on birthweight’ from an influence on fetal growth or on gestation length?

These studies showed a negative association between seafood intake (Oken 2004), marine n-3 PUFA intake (Oken 2004) or EPA cord serum concentration (Grandjean 2001) on the one hand, and birthweight (Oken 2004), fetal growth z value (Oken 2004) and birthweight adjusted for gestational age (Grandjean 2001).

We then changed the term birthweight to fetal growth (background p3 line 10), since the effect on BW in the first study was attributed by the authors to the effect on fetal growth. In these 2 studies, length of gestation was not related (Oken 2004) or positively related (Grandjean 2001) to seafood /n-3 PUFAs intake.

2. Methods – dietary assessment. Have the food frequency questions been validated or used previously?

Food frequency questions about seafood intake were derived from a previous survey. They were built using standard methods for food frequency questionnaires, based on the categories used for the study conducted by French Food Safety Agency (AFSSA) about dietary dioxin intake in the French population. In this study, dietary dioxin exposure was estimated by crossing contamination data and other information about nutritional habits (extracted from the results of 2 surveys using food diaries). Categories used in our frequency questionnaire correspond to those for which contamination data were available in the AFSSA study.

Please provide more detail about the fish question.

Were fresh water fish not included at all?

Which fish types were named?

Were portion sizes included for fish and for shellfish?

Information about fresh water fish intake was not included in our study. However, given Brittany’s location by the sea, we assume seafood consumption was much higher than fresh water fish intake. No fish species were mentioned in the saltwater-fish item (except that it was specified that salmon is included in this category). For more precision, the exact formulation of the seafood items has been added to the text (p4, line 21-22).

We had no information about portion sizes. A sentence about the lack of information about fish species, fresh water fish intake and portion sizes has been added to the text (p4, line 23-24).
3. Methods – outcome assessment. How was gestation length determined? i.e. based on LMP, ultrasound, or clinical assessment? Was it validated? This question is important as preterm birth was a primary outcome and SGA was of course based on the gestational age assessment. More explanation about estimation of length of gestation has been added to the text (p5 lines 4-9).

4. Table 2. Please include a column for all shellfish combined, which seems to be the primary exposure used in analyses. A column for all shellfish combined was added to table 2 (p15).

Also I don’t understand why the authors have different collapsed categories for fish vs. shellfish. Why not keep the same categories for both? Surely not b/c of N’s in each categories.
We have changed the labelling of categories of shellfish frequency to the same ones as for fish. (see answer to the same comment of the first reviewer).

5. Discussion. Please change the first line – the study was of seafood intake prior to pregnancy, not during pregnancy. “During pregnancy” was changed in “prior to pregnancy” (p7 line 19).

Minor Essential Revisions

6. Abstract – methods. Please clarify that fish and shellfish intake was pre-pregnancy, not during pregnancy as it would be reasonable to assume. The term “during pregnancy” was replaced by “prior to pregnancy” (p2 line 12).

7. Abstract – results. Please include the effect estimate and CI for fish intake with SGA and confidence limits for the effect on gestation length. Effect estimates and confidence interval were included in the result section of the abstract, for association between fish intake and SGA (p2 line 16) and length of gestation (p2 line 21).

8. Methods page 6. Did the authors include gestation length as a covariate for the SGA analyses? If not I suggest they do so, or report that such adjustment did not make any difference to their results. Regression models for SGA risk estimates were re-ran adding length of gestation as a covariate, but this didn’t change our results. We added information about this in the results section (p7 line 10-12).

9. Methods – statistical analysis. For the tests of trend, it would be preferable to model the categories using the mean intake within each category, rather than assigning ordinal values to each category. Trend tests used the method described by Olsen and colleagues in their paper (2006). We re-ran trend tests using mean intake in each category and found no change in the significance of our estimates:

<table>
<thead>
<tr>
<th>Categories using ordinal values</th>
<th>Categories using mean intake in each category</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB</td>
<td>LBW</td>
</tr>
<tr>
<td>fish</td>
<td>0.3</td>
</tr>
<tr>
<td>shellfish</td>
<td>0.3</td>
</tr>
</tbody>
</table>

The first paragraph of the results is not well organized, as the authors jump around among outcomes. Perhaps they could group the predictors for each outcome.
The first paragraph has been divided in 3 sections
1 : Description of socio-demographic characteristics of the women
2 : Description of birth outcomes
3: Crossed analysis and description of predictors for birth outcomes (p6 line 13-17)
This last paragraph was reorganised as following:
Predictors for PB/ Predictors for LBW / Predictors for SGA

10. Results. Please reference table 1 in the text.
Table 1 was already referenced in the text, in the results section and is now referenced as an additional file (p6 lines 7).

11. Table 1. The organization of the table is confusing. It seems the % under the header ‘overall’ is a column prevalence and the %’s under the outcome headers are row prevalences. Please label more clearly. For the dichotomous outcomes, please include the alternative category, e.g. married, >= baccalaureate, parous. It is difficult to interpret the reported p values in isolation (was the rate of preterm birth higher or lower among parous women??).
The percentages in the overall column of table 1 were added in the n’s column with brackets. Percentages in other columns are effectively row prevalences of adverse outcomes depending on covariate category, as mentioned in the title of the table. We changed the headers to PB rates, LBW rates, SGA rates.

12. Results. Please include effect estimates and confidence limits, not just p values.
ORs and 95%CIs have been added for the relation between shellfish intake and SGA (p7 line 7-8). Similarly the 95%CI for the linear coefficients in the analysis of birthweight and length of gestation have been added to table 4 and to the text for the significant association between frequency of fish intake and length of gestation (p7 line 15).

13. Discussion, page 8. Isn’t it also possible that enrollment bias may explain the low rate of adverse outcomes? (i.e. as seen elsewhere, women who enroll in research studies are likely to be healthier).
This is also a possible explanation that we cannot exclude.
A sentence about the enrollment bias was included in the text (p8 line 6-7).

14. Discussion, page 9. Where do these new results come from regarding the mean g/dy of fish and shellfish intake? How calculated? How related to the monthly servings used elsewhere? These results should be explained and presented in the Results section, not the Discussion.
The information on which we based portion sizes, which enabled us to calculate mean grams per day, comes from the SUVIMAX study, and we now reference it in the results section. (p6 lines 19-21).

15. Discussion, page 10. It would be helpful if the authors could clarify which studies examined birthweight (which reflects fetal growth as well as gestation length) and which isolated fetal growth as an outcome.
These details have been added to the text (p9 lines 19-24).

16. Discussion, page 11. From where do they get the data about which are the most commonly consumed types of fish and crustaceans?
Information about the most commonly consumed types of fish was abstracted from the same CALIPSO study from which we extracted seafood PUFA contents and pollutant concentrations (AFSSA 2006). This point was added in the text (p10 line 14).

17. Discussion – conclusions. The statement that their findings ‘confirm’ an association between fish consumption and length of gestation is too strong, since prior evidence is conflicting, including a recent large RCT that was null.
The term “confirms” has been changed to “suggests” (p10 line 24).
**Discretionary Revisions**

18. Discussion page 11. Are there any evidence that lead, arsenic, and cadmium are likely to be related to fetal growth or gestation length? If not the authors might want to limit their discussion to the persistent organic pollutants.