Author's response to reviews

Title: Decline in blood concentrations of mercury and lead in the Inuit population of Nunavik (Quebec)

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Author's response to reviews: see over

We have considered combining the two manuscripts. However, since we have added new results related to food consumption, we thought that a single paper would have too many pages. Also, sources of cadmium are not related the food consumption describes in the Hg and Pb article, and the importance of smoking is not of interest for those two metals. We resubmit the articles separately, but if it is a major concerned for the journal, we still can combine the two articles in one.

Reviewer 1: Pál Weihe

Reviewer's comment 1.1
In order to ensure comparability I will recommend a table showing the number of samples taken in each of the 14 communities in Nunavik in 1992 and 2004.Furthermore a more detailed explanation about the randomization of the sampling should be given: How were the individual households selected and extracted from a public register, by random selection on a map or simply by including all houses in a community? Were all persons in the household invited or only the individuals, who were at home at the time of the visit? Was the randomization procedure the same in 1992 and 2004?

***Response 1.1*:** In order to give more information on the method of sampling in 2004, theses paragraphs were added to the population and sampling paragraph of the methodology (pages 6-7)::

The Institut de la statistique du Québec (ISQ) was given the mandate to develop the survey frame. Many sources of information were used by the ISQ to count all private Inuit households in Nunavik. Priority was given to municipal rolls as the most comprehensive source of information. When information was lacking, other lists were used such as those from employers who provide lodging to employees, (Ungava's Tulattavik Health Centre, Inuulitsivik Health Centre, Kativik School Board, etc.), the Quebec electoral roll, the Kativik Housing Bureau and the telephone directory.

The survey plan was for a complex two-stage stratified random sampling. The first stage was to select a stratified random sample of private Inuit households with proportional allocation. The community was the only stratification variable used. Since home addresses (civic numbers) in some municipalities are consecutive, the survey frame was sorted first by home addresses, followed by a systematic draw of a predetermined number of households to avoid selection of two immediate neighbours. Since many Inuit regularly move from one house to another, it was decided to sample households instead of individuals. To obtain a good representation of each community, a proportional allocation of sample units corresponding to the size of each village was chosen. In the second stage, all eligible people were asked to participate according to the survey steps or instruments.

More details on the method sampling of the Santé Québec Survey in 1992 were also added in the following paragraph (page 8).

To assess temporal variation of blood Hg and Pb concentration, we compared our results with data from a survey conducted with a similar protocol by Santé Québec in 1992. A systematic sampling was achieved after sorting the survey base by household address to favour a more complete coverage of the territory and to avoid the selection of next-door neighbours. Furthermore, so that each village would be represented, we stratified the sample by village, with quasi-proportional representation of the number of households in each stratum. A total of 400 household were randomly selected and visited by interviewers between September and December 1992, and a total of 493 people were recruited. Protocols for face-to-face interviews and blood sampling were similar to the ones used in 2004. However, the food frequency questionnaire was only administered to 226 women, and the list of country foods was les exhaustive than that used in 2004, hence making comparisons between the surveys difficult.

Regarding the breakdown by municipality, because some villages are very small, the Ethical Committees do not allow us to present data stratified according to municipality.

Reviewer's comment 1.2.
The authors find it likely that the decrease in blood mercury could be attributed by the changes of dietary habits. The reported reduction in daily intake of marine mammal meat of 40 % (reported in an other publication) is a sufficient explanation of the findings. However, since the consumption of marine mammals is so important when describing changes in arctic exposure to mercury, including dietary data from both 1992 and 2004 would increase the value of this paper.

Response 1.2: Many comments of the 3 reviewers are related to the dietary intake. In this response, we will consider all those concerns. First, we have added 2 tables in the results section to present the country food intake in 2004 for our sample, limited to the food items of interest.

(See table 2 and 3)

The mean daily consumption of the country food items of consumed by most of the participants is presented in table 2. These variables satisfied log-normality criteria and geometric mean are therefore presented. Only cconsumption of fish significantly increased with age ($p = 0.0001$). Consumption of marine mammal meat ($p < 0.0001$), fish ($p = 0.01$) and game birds meat ($p = 0.01$) was significantly higher in men than women. In Hudson's bay region, mean consumption of marine mammal meat ($p < 0.0001$), fish ($p = 0.04$), and game birds meat ($p = 0.01$) was significantly higher than in Ungava's bay. Other country food items were consumed by less then 50% of the sample, therefore these variables were treated dichotomously, and proportions of consumers are presented in table 3. Proportion of consumers of marine mammal's kidneys and liver (p-trend = 0.003), salmon and trout (p-trend <0.0001) and duck meat (p-trend <0.0001) increased significantly with age. Proportion of consumers did not vary according to gender, except for consumption of salmon and trout ($\chi^2 = 12,6$; $p<0.001$). In Hudson's bay region, the proportion of consumer of marine mammal's kidneys and liver ($\chi^2 = 107.7$; $p<0,0001$) and duck meat ($\chi^2 = 40.7$; $p<0,0001$) were

higher than in Ungava's bay, while it was lower for consumption of salmon and trout (χ^2 = 41.7; p<0,0001) (results not shown).

Secondly, it was not part of our objectives to evaluate changes in dietary intake between the two surveys, because we didn't have enough comparable data between the 2 surveys. In 1992, the food frequency questionnaire was only administered to 226 women, and the list of country foods was les exhaustive than that used in 2004. Data on food consumption in 1992 has been published in Blanchet et al, 2000, Contribution of selected traditional and market foods to the diet of Nunavik Inuit women, Canadian Journal of Dietetic Practice and Research, 2000, 61 50-59.

To avoid confusion, the objective has been reformulated. :

The objective of this study was therefore to evaluate blood concentration of mercury and lead and its sources among the Inuit of Nunavik in 2004 and evaluate the evolution of theses concentrations since the Santé Québec Survey

We have also added these news lines in the methodology section:

Protocols for face-to-face interviews and blood sampling were similar to the ones used in 2004. However, the food frequency questionnaire was only administered to 226 women, and the list of country foods was les exhaustive than that used in 2004, hence making comparisons between the surveys difficult.

Reviewer's comment 1.3 :
Geometric mean of blood mercury in 2004 is stated to be 26, 6 nmol/l. This must be a mistake considering the other data in this table.
Response 1.3: This mistake was corrected. The correct values were: geometric mean 52.2 nmol/l; IC 95% (4.79-54.6) and range: (0.4-1200)

Reviewer 2: Staffan Skerfving

Reviewer's comment 2.1 Is it really logic to have both "decline" and "cross-sectional" in the title?
Response 2.1: The title was change.

Reviewer's comment 2.2:: Information on levels of mercury and lead should be given, not only the change.
Response 2.2: This information was added:

Mean blood concentrations of Hg was 51.2 nmol/L, which represent a 32 % decrease (p<0.001) between 1992 and 2004. […] *In 2004, mean blood concentration of Pb was 0.19 µmol/L and showed a 55 % decrease since 1992.*

Reviewer's comment 2.3 P 4, line 4: "Acute"? Heavy?
Response 2.3: The English quality of the manuscript was revised.

Reviewer's comment 2.4 Methods: The method for obtaining information on food intake must be described.
Response 2.4: This information was added to the population sampling section:

Face-to-face interviews were conducted on board the Amundsen to collect information on socio-demographic characteristics and lifestyle habits. A food frequency questionnaire was administered to collect information on food intakes and eating patterns. The questionnaire used in 2004 measured the consumption of 25 food items of country foods which refers to food items derived from fishing, hunting and gathering, recorded for each of the four seasons (of the year prior the interview). Specification of the usual serving size was included in the questions on frequency. Pre-defined serving sizes were included in the questionnaire and a corresponding food model was shown to the respondents

Reviewer's comment 2.5 Methods. Population and sampling: It is difficult to follow the recruitment procedure.
Response: The entire section on recruitment was reorganised:

The Nunavik Health Survey was conducted in the 14 communities of Nunavik during fall 2004 on the Canadian Coast Guard icebreaker and scientific research vessel CCGS Amundsen (Fig.1), in collaboration with the Institut National de Santé Publique du Québec, the Nunavik Regional Board of Health and Social Services and the Institut de la Statistique du Québe (ISQ). The ISQ was given the mandate to develop the survey frame. Many sources of information were used by the ISQ to count all private Inuit households in Nunavik. Priority was given to municipal rolls as the most comprehensive source of information. When information was lacking, other lists were used such as those from employers who provide lodging to employees, (Ungava's Tulattavik Health Centre, Inuulitsivik Health Centre, Kativik School Board, etc.), the Quebec electoral roll, the Kativik Housing Bureau and the telephone directory.

A stratified random sample of private Inuit households was selected according to the municipalities. Since home addresses in some municipalities are consecutive, the survey frame was sorted first by home addresses, followed by a systematic draw of a predetermined number of households to avoid selection of two immediate neighbours. Since many Inuit regularly move from one house to another, it was decided to sample households instead of individuals. To obtain a good representation of each community, a proportional allocation of sample units corresponding to the size of each village was chosen. All eligible people of the household were asked to participate according to the survey steps or instruments. A total of 1058 people were recruited for the study and informed consent was obtained from all participants. Blood samples were obtained from

917 participants during the clinical session to evaluate levels of exposure to environmental contaminants. Face-to-face interviews were conducted on board the Amundsen to collect information on socio-demographic characteristics and lifestyle habits. A food frequency questionnaire was administered to collect information on food intakes and eating patterns. The questionnaire used in 2004 measured the consumption of 25 food items of country foods which refers to food items derived from fishing, hunting and gathering, recorded for each of the four seasons (of the year prior the interview). Specification of the usual serving size was included in the questions on frequency. Pre-defined serving sizes were included in the questionnaire and a corresponding food model was shown to the respondents

Reviewer's comment 2.6 P 7-8, Biological samples processing: What kind of tubes were used? Venous blood? Is relative bias the deviation from the average of participating laboratories? In what direction? Were the QC samples analysed parallel to the study samples?

Response 2.6: This information was added:

The blood sample for metal analysis was collected from a cubital vein in a 6-mL plastic vacutainer containing potassium EDTA as the anticoagulant (BD Medical).

[…]

Accuracy and precision were measured using reference material from the Interlaboratory Comparison Program of the Centre de Toxicologie (INSPQ). The coefficient of variation was 2.8%, and the relative bias was +3.1 % for the Pb reference specimen analysed on 10 different days (consensus median value from participating laboratories = 0.6 µmol/L). The coefficient of variation was 2.1 %, and the relative bias was +1.6 % for the total Hg reference specimen analysed on 10 different days (consensus median value from participating laboratories = 66 nmol/L).

Reviewer's comment 2.7 P 8, Statistical analysis: How was the confounding analysis made? Correlation between risk determinants (food intakes, etc., which is crucial for the possibility to separate the different issues)? What potential confounding factors were tried?

Response 2.7: The statistical analyses were not clearly explained, so we add theses lines to clarify what we did. We have build a predictive model with all the potentials risk determinants, therefore confounding is not relevant.

We performed analyses of variance to assess multivariate associations between metal blood concentrations and various variables. All personal characteristics (gender, age, region of residency) and consumption habits that were associated ($p \leq 0.10$) with metal

concentrations in blood were considered in the predictive model. To be retained in the final model, a variable had to show significant association (i.e., $p \leq 0.05$) with metal concentrations in blood.

We have also corrected the results part:

[…] *Analyses of variance (results not shown) also showed that Hg concentrations were significantly different according smoking habits ($p < 0.001$), and that Pb concentrations were associated with hunting frequency, but not with alcohol consumption ($p = 0.12$) and tobacco consumption ($p = 0.36$).* […]

A positive association (Table 5) was observed between blood Hg concentrations and consumption of marine mammal meat, marine mammal kidney and liver and salmon and trout. A model including consumption of marine mammals' meat, kidney and liver, age, gender and region of residence as independent variables explained 27% of the variation of Hg concentration ($p<0.0001$).Of all theses variables, age was the most highly associated to Hg blood concentrations (partial R^2 of 0.20), followed by marine mammal meat consumption (partial R^2 of 0.04)

As shown in Table 5, all the traditional food consumption variables selected were correlated with Pb concentrations. A model including consumption of game birds and marine mammal kidneys and liver, age, gender and tobacco consumption as independent variables explained 25% of the variation of Pb concentration ($p<0.0001$). However, associations with dietary variables were very low (partial R^2 of 0.01) and as for Hg, age was the most highly associated variable (partial R^2 of 0.20).

Reviewer's comment 2.8 P 9, para 1: CI=confidence interval?
Response 2.8: This information was added. when CI were first introduced in the results (page..) :

The mean age for men in 2004 was 36.4-yr old (95% confidence interval (CI) = 35.9-37.0) and 36.9-yr old for women (95%CI = 36.2-37.2).

Reviewer's comment 2.9 P 10, para 1: This is repeated in Discussion
Response 2.9 : This paragraph was cut is the results section to avoid repetition.

Reviewer's comment 2.10 P 10, last para, line 2: "¦confusionâ"? Confounding?
Response 2.10: The English quality of the manuscript was revised.

Reviewer's comment 2.11 Discussion: Information on the levels of Hg and Pb in different foods? Is the difference between regions in the final Hg model (Table 4) due to differences in Hg levels? Some information on hunting with Pb bullets should be given, tounderstand the risk of being exposed. Also, the possibility of decreasing long-range air-transport of lead (as a result of reduced use of petrol Pb) should be considered.

Response 2.11:
This answer will also take in consideration reviewer's comment 3.6 to give a single and complete answer. More information about the levels of Hg and Pb in different country food has been added in the introduction section (p.4)

Contaminant exposure from traditional food consumption among Inuit has been estimate during an extensive dietary survey covering 18 Arctic communities in the late 1990s [9]. Data showed that the mean intake levels of Hg were below the tolerable daily intake levels used by Health Canada. Ringed seal kidney had the highest concentration of mercury (2844 ng/g) but only contributed 2.5% of total intake during late winter, while ringed seal meat had much lower concentration (400 ng/g) but was an more important contributor (15.3%). Caribou meat had low Hg concentrations (57 ng/g) but due to its frequent consumption, it was the most important contributor (30.1 %) to Hg intake. A recent update in on dietary contaminant exposure done by the same team showed that the contaminants concentrations in 2007 appeared to be similar, with the exception of Hg in ringed seal liver, which was higher, and walrus blubber, which was lower [10].

[…]

In a previous dietary survey in Inuit communities of the Canadian Arctic, the major contributors to Pb dietary exposure were caribou meat (57.0 %; 783 ng/g) and Arctic char (26.4 %; 1009 ng/g) [9]..

Moreover regarding the temporal trends of Hg we have also pointed out in the discussion that :

[…], evidence for increasing levels of Hg in the Canadian Arctic is observed in a number of marine birds and mammals (35-38).

Regarding the difference between region in the final Hg model, this difference could be partially explain by the higher consumption of marine mammals meat in Hudson Bay compare to Ungava Bay, as explained in the following lines (page 15) :

As in 1992, blood concentrations of Hg were higher in Hudson's Bay residents compared to Ungava's Bay residents. A possible explanation for this difference is that Hudson's Bay residents consume significantly more marine mammal meat, kidney and liver, which are known sources of Hg intake.

More information on the risk to be exposed to Pb when hunting with Pb bullets was added in the introduction (page 5):

Hunter can be exposed to Pb by inhalation or ingestion of lead dust released by the friction of the shot against the barrel and by the combustion of high-explosive primers that contain lead styphnate. Moreover, consumer of wild game can be exposed to Pb by ingestion of whole pellets or fragments embedded in meat or by ingestion of game with biologically incorporated lead (mostly exposed through ingestion of spent shot and fishing sinkers).

Regarding the decreasing long-range air-transport of lead (as a result of reduced use of petrol Pb), we reformulated the discussion paragraphs to include this possibility (that was already underline in the introduction) (page 17):

This suggest that the strong decrease in Pb concentrations in adults and newborns could be not only attributed to the decreasing environmental levels (since the ban of leaded gasoline), but could also be a beneficial consequence of the ban on lead shot use for hunting wild game and birds, a policy implemented by the Public Health Directorate in 1998.

Reviewer's comment 2.12 11, last para, line 7: "¦bioaccumulation." Is this really reasonable? The biological half-time is about two months. May it be a difference of Hg levels in different foods?

Response 2.12: The biological half-time being around 60 days, this hypothesis was not possible. We have corrected this:

Hg blood concentrations were statistically higher in adults aged 45 to 74 compared to younger adults, as observed in other studies [1-4]. Mercury is not known to bioaccumulate in human tissue and this association could be explained by the tendency of young adults to eat less traditional food than previous generations. Indeed, in our population sample, we observed a significant lower intake of certain traditional food items (marine mammal kidneys and liver, fish, duck meat) in younger people. Moreover, the concentration of omega-3 fatty acid in plasma phospholipids, a biological marker of seafood consumption, was nearly two times lower ($p<0.0001$) in the 18-24 yr age group (5.0 % ; 95% CI = 4.8-5.3) than in the 45-75 yr age group (9.9 ; 95% CI = 4.8-5.3).

Reviewer's comment 2.13 P 13, last para: What about alcohol, which is a major source of Pb in some populations.

Response 2.13: Alcohol consumption was tested, but the correlation was not significative ($p=0.12$). This information was added in the results section (page 13):

[…] and that Pb concentrations were associated with hunting frequency, but not with alcohol consumption (p = 0.12) and tobacco consumption (p = 0.36).

Reviewer's comment 2.14 Reference list: 1. AMAP? 15. Complete! 17. Complete! 22. Cpomplete! 28.Author? 41. Font.
Response 2.14: There is a problem with the Reference Manager Style given by the journal, which keep a maximum of 10 authors. The reference style also put 'Reference list' as the title for the reference section instead of 'References'. Same thing for the font of reference 43 and 53 (both 'in press'). We have corrected all these references and the title by hand, but it is possible that formatting during transfer erased these corrections.

Reviewer's comment 2.15 Table 2: Mercury, All, Range: Why not 1.4-1200? Also, 1200 (and 820, 420) is more reasonable than 1200.0 (etc.). Lead (Âµmol/L, not nmol/L)!
Response 2.15: These mistakes were corrected.

Reviewer's comment 2.16 Table 3: Pearson´s coefficient suitable for for Gender, Costal region and Tobacco consumption?

Response 2.16: The reviewer is right; Pearson's coefficient is not suitable for dichotomous or categorical variables. The table and the title have been modified. We kept the correlation for dietary variables (continuous). To select the categorical variables to consider in the predictive model, we used the t-test and analyse of variance results presented in table 4.

Reviewer's comment 2.17 Table 4: Indicate that the right columns are for the total model.
Response 2.17: This title was added.

Reviewer's comment 2.18 Figures 2 and 3: It would be more informative to truncate Hg at 500 nmol/L andPb at 1.2 Âµmol/L, to allow more details at the lower ends (and indicate thenumber of higher levels in the legends).
Response 2.18: These figures were changed to take in consideration comments 2.18 and 3.8

Reviewer's comment 2.19 Figure 2: Where is the 1200 nmol/L sample?
Response: Figure 2 was modified and includes now all samples.

Reviewer 3: Greet Schoeters

Reviewer's comment 3.1 The manuscript gives interesting information on blood levels of two important pollutants in a high exposure population. However the data are explained by referring to changes in life style and dietary habits but the manuscript lacks concrete information in the results section on these topics. This should be further elaborated since ta data should be available based on the food frequency questionnaires.

Response 3.3: see response 1.2

Reviewer's comment 3.2. Are the data on lead and Hg measured in 1992 and in 2004 analytically comparable? Do the authors have evidence for this? More information is needed as to the analytical comparability of the data collected in 1992 and 2004.

Response 3.2: We have added this paragraph (page 9):

In 1992 blood Pb concentrations were determined by graphite furnace atomic absorption spectrometry (GFAAS) (Perkin Elmer, model ZL 4100). Samples were diluted and injected directly into the instrument. Blood Hg concentrations were determined by cold-vapor atomic absorption spectrometry (CVAAS) (Pharmacia Mercury monitor). Samples were microwave-digested with nitric acid, and an aliquot was used for the analysis. In order to compare the different laboratory procedures used in 1992 and 2004, the INSPQ performed a quality controle study within the framework of the Quebec Interlaboratory Comparison Program. The INSPQ used both methods to determine the Hg and Pb concentrations and obtained strong correlations. The biais observed was 8% for Hg ($y = 0.92x + 2.83$; $R^2=0.99$) and 6% for Pb ($y = 0.94x - 0.0025$; $R^2=0.93$). We can therefore assume that both methods (ICP-MS and furnace atomic absorption spectrometry) give comparable results.

Reviewer's comment 3.3 Methylmercury is the most toxicologically relevant form of mercury, the authors have reported the measurement of total mercury, do they have information on the proportion of methylmercury in the blood samples?

Response 3.3: These analyses were not conducted in 2004. In 1992, MeHg represented 82% of blood total mercury.

Reviewer's comment 3.4. Information on the dietary intake should be given in table 2 &3 since the decreases in human blood concentrations are discussed in terms of dietary intake
Response 34: see response 1.2

Reviewer's comment 3.5. Information on differences in dietary intake between the population sampled in1992 and in 2004 should be presented in the manuscript. Some data are included in the discussion, they should be part of the results section.

Response 3.5: see response 1.2

Reviewer's comment 3.6 On page 3 and 4 substantial information is given on eg acute toxicity of lead and methylmercury, this could be shortened since these are general findings from different epidemiological studies. Instead it would inform the reader to get more specific information on the levels of lead and methylmercury which have been measured over time in arctic food
Response 3.6: see response 2.11

Reviewer's comment 3.7. page 5 ; the decrease in lead concentrations has already be demonstrated in cord blood samples between 1994 and 2001(Dallaire et al, 2003). How does the new information collected in this study relate to those findings?

Response 3.7: (page 17)

Blood Pb concentrations showed a 55% decrease over the 12-year period. This decreasing trend has also been observed in cord blood obtained from Nunavik newborns, with markedly lower concentrations in 1999 [22]. This suggest that the strong decrease in Pb concentrations in adults and newborns could be not only attributed to the decreasing environmental levels (since the ban of leaded gasoline), but could also be a beneficial consequence of the ban on lead shot use for hunting wild game and birds, a policy implemented by the Public Health Directorate in 1998

Reviewer's comment 3.8. On page 5 the authors state that the objective of this study was therefore to examine the evolution of mercury and lead exposure and its sources among the Inuit of Nunavik and compare this information with data collected in 1992 in
the Santé QuÃ©bec Survey The time trend in blood levels has been investigated in the present study, the information on the sources should be elaborated if there are data on levels in different food items and if they have more detailed information on dietary intake and changes in dietary intake between1992 and 2004.
Response 3.8 : see response 1.2

Reviewer's comment 3.9. Page 9 figure 2 &3 do not add a lot, it would be more interesting to compare the distributions from the 2004 and 1991 samples. Is this possible?
Response 3.9: These figures were changed to take in consideration comments 2.18 and 3.8

Reviewer's comment 3.10.Table 3 & 4 : specify that the data analysed are from the 2004 study
Response 3.10: The title was corrected and year was added.

Reviewer's comment 3.11. Page 10: â##After controlling for confounding factors (Table 4), only marine mammalsâ## meat consumption remained significantly correlated with the blood concentrations of Hg, with a partial R2 of 0.12.â## Which confounders are used in the model, how did you identify the confounders?
Response 3.11 : see response 2.7

Reviewer's comment 3.12. â## when we included age and gender as confusion factorsâ## what areconfusion factors?
Response 3.12 : see response 2.7

Reference List

1. Bjerregaard P, Hansen JC: **Organochlorines and heavy metals in pregnant women from the Disko Bay area in Greenland.** *Sci Total Environ* 2000, **245:**195-202.

2. Dumont C, Girard M, Bellavance F, Noel F: **Mercury levels in the Cree population of James Bay, Quebec, from 1988 to 1993/94.** *Canadian Medical Association Journal* 1998, **158:**1439-45.

3. Milman N, Mathiassen B, Hansen JC, Bohm J: **Blood levels of lead, cadmium and mercury in a Greenlandic Inuit hunter population from Thule district.** *Trace Elements and Electrolytes* 1994, **11:**3-8.

4. Rhainds M, Levallois P, Dewailly E, Ayotte P: **Lead, mercury, and organochlorine compound levels in cord blood in Quebec, Canada.** *Arch Environ Health* 1999, **54:**40-7.