Author's response to reviews

Title: Metals and trace element concentrations in breast milk of first time healthy mothers

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Author's response to reviews: see over
Dear Editor,

Please find enclosed our revised manuscript “Metals and trace element concentrations in breast milk of first time healthy mothers”.

We would like to thank the reviewers for very good comments. The comments have been addressed and are described together with the authors’ answers below. Changes that have been made in the manuscript according to the reviewers’ comments are highlighted with “tracked changes”.

Kind regards,

Marika Berglund

**Reviewer: Birger Heinzow**

**Minor Essential Revisions**

1. Differences in mineral content between the two surveys are very interesting and some observations in the study deserve more discussion.

   “Large interindividual and over time differences were detected for Na concentrations...”

   It is quite remarkable, the difference in composition of milk and blood plasma, both have similar osmolarity but striking differences: lower sodium and higher Potassium concentrations in milk.

   **Response:** Indeed sodium concentrations were much higher in the present milk samples compared to 1989, however, they were comparable to Na concentrations measured in milk samples collected during early lactation (21 days pp) in the USA (mean 212 mg/L; Allen et al, 1991) and Gambia (30 days pp; 120 mg/L; Richards et al, 2010). Even though Na concentrations decrease over time of lactation, we believe that the relatively high concentrations found in our sample needs further investigations to elucidate the influence of dietary Na on BM concentrations. We have added information on this in the Discussion section.

2. “Arsenic and B were positively correlated with fish consumption...”

   Discussion if fish is a known source for boron, a comment on selenium, where seafood is a possible source would be welcomed.

   **Response:** Indeed Se and As in milk was statistical significantly correlated (rs=0.36; p<0.01), but Se and fish consumption was not (rs=0.22; p=0.10). It has been estimated that one-fourth of the Se
intake in the Swedish population comes from fish and seafood (Becker, 2000), i.e., fish is an important source of Se. However, we have previously analyzed Se in serum of Swedish non-fish eating women. The median concentration was 76 µg/L (range, 53-103 µg/L), which is in agreement with previous studies of the Swedish general population (75 µg/L; ÄK-LIVS, 1996), indicating that the Se status of non-fish-eating individuals is not substantially lower than that of people who include fish in their diet (Lindberg et al 2004).

The Se intake in Swedish women is relatively low, on average 40 µg/day (Riksmaten 2012), which is below the recommended intake for lactating women of 60-70 µg/day (NNR2012; US Institute of Medicine, 2000). A low intake of Se in Sweden has been attributed to the poor content of Se in Swedish soil (Bruce 1986). We have added information on this in the Discussion section.

3. Zinc is actively transported, and essential for growth, a reduced growth rate might be related to depletion and low zinc levels in milk, maternal supplements will then be required.

Response: Zinc deficiency is uncommon in Swedish women, and the Zn level in milk (3.5 mg/Kg) is adequate to meet the need of breastfed infants for the first several months of life (Krebs and Westcott, 2002). Human milk provides an excellent source of highly bioavailable zinc and generally meets the needs of the healthy young exclusively breastfed infants for the first several months of life. Adequate intake for Zinc in infants 0-6 months is 2 mg/day, set by Institute of Medicine (2001).

We have added information on this in the Discussion section.

4. Calcium and other minerals (Na, K) are comparably low in human milk compared to bovine milk, except copper which also shows great variation between mothers.

Response: We did not find great variation in Cu concentrations between mothers (CV: 0.16). Also, maternal intake does not seem to influence Cu concentrations in breast milk (Lonnerdal, 2000). Thus we believe that Cu is tightly regulated in human milk.

5. If available information on osmolarity of the samples and fat and casein content would be valuable information concerning homogeneity of the samples collected.

Response: Unfortunately, we do not have this information for the particular breast milk samples. In general, the fat content of milk collected in lactation week 2-3 is 3%.

6. Page 9: are the values normal or log-normal distributed?

Response: The macroelements were normally distributed (as evaluated by the Shapiro-Wilk test), except for Mg and Na. Also, Cu, Zn and Rb were normally distributed, as reported on page 9.
7. Page 11: “or is affected by” delete: is

Response: We have made the change accordingly.

8. Page 12: “Although, breast milk Na concentration is reported to decrease during the lactation period [10, 21], the observed difference may reflect a true increase in Na intake over time, since there has been a substantial increase in the intake of salt through food among Swedes since the 1980’s”

This is a very interesting because unexpected observation is there information on variation of Na because of salt intake? What is known: Sodium might be elevated during weaning! and impaired lactation ( inter alia: mastitis).

Response: We have not found any information on the influence of salt intake on BM Na concentrations.

9. Page 13

The strikingly low Selenium status of Swedish women should be discussed by means of recent information on serum Se in Sweden rather than a 20 year old view, was selenium related to fish and correlated with arsenic? If not, this might be worth mentioning. Selenium in human milk declines during the course of breastfeeding, depleting the mother within a couple of months: Jochum F, Fuchs A, Menzel H, Lombeck I (1995) Acta Paediatr 84: 859-862. Thus a low start at 10 ìg might have some health implication on selenium-status of mother and child and this information should be pointed out and might even require supplementation. Discussion of these aspects is essential.

Response: We fully agree with the reviewer and the text has been revised accordingly (see response to question number 2 above).

10. Page 15 “Based on the fact that breast milk B concentrations were 60-100% of plasma B concentrations...”.

Response: Unfortunately, we do not have that information for all those elements.

11. Page 16:

“Also, little is known about Ag and U concentrations in breast milk, which varied largely in our samples, indicating some influence of maternal exposure to these elements.”

Uranium is very important and shows high CV, drinking water might be a source, if the water supply comes from different wells.
**Response**: The U concentration in BM was low (median 0.3 µg/L), and well below the provisional DWG of 30 µg/L for U (WHO 2012). It is not known, however likely, that some of the women in this study had a private well with U concentrations above what is common in surface water, but in this area, below the DWG.

12. For some elements the correlations are mentioned in the text I encourage the authors to include a correlation table of all elements, this will be useful for studies in the future and in other countries.

**Response**: We have submitted a correlation table as supplemental material.

13. Finally a toxicological assessment of the values of the toxic elements, i.e. lead, uranium et al with a comment that the concentration of these elements is low compared to drinking water and that they pose no risk would be appropriate.

**Response**: We have added that information in the Discussion.

**Reviewer: Milena Horvat**

Reviewer’s report:

The following information should be provided:

1. LOD is useful, however LOQ is much more relevant in these studies. Uncertainty estimates are not given, as well as the calibration details not provided. This makes the data provided untraceable and consequently difficult to compare. The authors are advised to read the basic principles to describe essential measurement characteristics. Useful reading is provided in IUPAC Technical Report (de Bievre et al. Pure Appl. Chem., 2012).

**Response**: We thank the reviewer for the advice to read IUPAC Technical Report. We have added information in the Method section concerning the analytical procedures. We usually report LOD instead of LOQ, and the reader can easily recalculate LOD to LOQ by multiply LOD with 3.

Concerning the uncertainty of the estimates, the mean± SD includes the measurement error and other uncertainties as well as the variability related to the determinations of elements in breast milk. We have elaborated on the uncertainty in the sections Methods, Results and Discussion.

2. The matrix reference materials are certified only for a small number of elements, and moreover these are not either concentration neither matrix matched. As such insufficient data are provided for accuracy assessment. The uncertainty range for a number of elements is also rather large for the study implemented. The authors should explain their strategies for accuracy assessment more precisely.
Response: At the time of our analyses there were no appropriate reference materials for breast milk, and thus we used infant formula and blood reference materials. This is something that will add to uncertainty. We have included more information in the analytical quality control section.

3. It is unclear how the uncertainty of data was taken into account in statistical analysis. This should have been included.

Response: Uncertainty is always part of the analytical results, including sampling, sample handling, measurement errors, and thus the mean±SD includes all these errors as well as the variability. We have not separated the uncertainty from the variability in the statistical analyses, but we have tried to clarify the uncertainty associated with the analytical results in the manuscript.

4. Among the elements analyzed by ICPMS more precise information is needed for potential interferences for particular elements. This is insufficiently described.

Response: We use an ICPMS with collision and reaction cell systems, and analyze various elements in different modes (helium, hydrogen or standard mode, as described in the Method section, Analysis) in order to minimize potential interferences. This has been further described in the Analysis section.

5. The selection of elements is ultimately linked to the ICPMS analysis. Among the trace toxic metals mercury has not been assessed. Is there any particular reason for not providing the data for this high priority toxic metal? It would be useful to cover as large spectrum of elements as possible for the wider interest.

Response: We think we cover a large spectrum of elements (N=32), but this time we did not include Hg. However, we have previously analyzed total Hg in breast milk of Swedish women (Bjornberg et al, 2005). The median concentration was 0.14 μg/L (range, 0.07–0.37 μg/L), and concentrations were correlated with number of amalgam fillings rather than fish consumption of the mothers. Furthermore, we have analyzed THg in hair of these breastfeeding mothers, and the concentration was 0.4 mg/kg, which is rather low. We have added some of this information in the Discussion section.

6. The data are provided as mass unit per L of the milk. It is stated that about 1 g of the sample was taken for analysis (section Analysis). How the data were transferred from a mass unit to the volume?

Response: We assumed mass=volume. Density of milk has been reported to be 1.03 (Pao et al, 1980) and our own measurements indicate a density of 1.01 Kg/L. We have added this information in the Methods section.
7. Homogeneity of the samples was assured by the thorough mixing (Page 6) – more information is needed for how the samples were homogenized and checked for the homogeneity. This may affect the overall result and significantly contribute to its uncertainty.

**Response:** The samples were thoroughly homogenized by vigorous shaking prior to digestion. Digested samples (completely clear, colorless, and homogenous solutions) were analyzed by ICPMS. We have added the information in the Analysis section.

8. In reference to the above it is essential that this manuscript is seen by an expert statistician to accurately account for the uncertainties introduced by sampling preparation as well as analytical errors.

**Response:** We believe that the statistical methods used in this paper (Mann-Whitney U test, Shapiro-Wilk test, coefficient of variation and Spearman correlation test) are standard statistical tests that do not demand special expertise in statistics. For clarifications regarding uncertainties, we have stressed the uncertainty in the results throughout the manuscript.

**References**


