Author's response to reviews

Title: Exposure Assessment of Dietary Cadmium: Findings from Shanghainese over 40 years, China

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

Thank you for your letter of February 22, 2013 regarding our manuscript entitled “Exposure Assessment of Dietary Cadmium: Findings from Shanghainese over 35 years, China” (Ms. ID: 1843381576886802). We are very pleased to be asked for a revision and appreciated by four reviewers for the experimental design. We found the comments and suggestions of the reviewers to be extremely helpful.

Please find our uploaded revisions and responses on separate sheets. We would like to re-submit it for your consideration. The amendments were highlighted in red in the revised manuscript. Line numbers were also showed for reviewers’ convenience reading. Point by point responses to the reviewers’ comments are listed below.

Thanks again for your consideration of our manuscript for publication in your journal. We look forward to your favorable decision.

With best wishes,

Yours sincerely

Ping He
Comment from reviewer 1:

Major Compulsory Revisions
1) Methods for cadmium analysis are not well described or not described at all.
   Answers:
   Thanks for reviewer’s suggestion. I have rewritten the analysis method to make it more informative and quoted several references about the cadmium analysis method which were developed by our team. (Page 5, row 32)

2) Sample population for smoking women (4) seems to not to be enough for a statistical evaluation related to smoking exposure (e.g. Section Results: p 9 raw 22).
   Answers:
   Smoking men were 57 and smoking women were 4 in this survey. Over 15-years smoking rate of the total population was 35.8% according to the Chinese National Survey in 2002. The smoking rate of men and women were 66.0% and 3.1% respectively. The smoking rate (66.2% for men and 3.3% for women) in our study was close to the National Survey. So I just described the distribution of the total smoker and assessed the risk for them. Date for smoking women was not evaluated separately.

Minor Essential Revisions
1) Significant figures may be rounded in several cases (e.g. Results page 7 line 15 37.35%, line 19 38.81%, 36.65% ).
   Answers:
   Correction has been made in the revised version.

2) Results (P 7 row 25): Authors declare that the “level of water cadmium was lower than Limit of detection (LOD) as 0,00005 mg/l. Which is the authors’ estimation for censored data?
   Answers:
   Considering the low contribution of the water cadmium and the censored data in this study, I assumed 0.025µg/L as the average mean between 0 to 0.05µg/L (LOD). As the result, the mean water cadmium exposure was 0.03µg/day which accounted for 0.2% of the total environmental. (Page 8, row 20)

3) Interpretation of results is generally balanced and supported by the data. Authors should indicate NML for each food categories.
   Answers:
   Table 5 was added in the revised version.

Discretionary Revisions
1) Discussion (page 10 line 17): why didn’t the authors show data for other non-populated provinces in China?
Answers:
I have changed it in the revised version. (Page 11, row 27)

Comment from reviewer 2:

Is the question posed by the authors well defined?
1) The Authors consider the “dietary” cadmium exposure in citizens aged over 35 years in Shanghai, China (also including smoke; is this a dietary source? In the background, they indicate “non-occupational” exposure may be this definition is more appropriate. In the abstract-conclusion is reported “… as a result of recent total cadmium exposure”. In the discussion, they indicate, “This study provided a detailed assessment of the environmental cadmium exposure especially….” I think that the purpose is confuse!).

Answers:
Thanks for the reviewer’s good evaluation and kind suggestion. In this study, the main objective was to understand the contribution of the dietary cadmium exposure. Other environmental factors such as water and tobacco were considered to make our research more comprehensive. I have rewritten the aim as “assess the contribution of dietary cadmium exposure in the environmental cadmium exposure and its risk to adults in Shanghai, China”. Also the discussion part was rewritten in the revised version.

2) The main question the AA wish to give an answer is: are adults in Shanghai exposed to dietary/non occupational cadmium above the tolerable intake? As a general consideration, they give an answer to the question but criticism can be made for several details that are listed below. Are the methods appropriate and well described?

Answers:
Thanks for the reviewer’s kind suggestion. I revised the article carefully. Please read the revised manuscript and the answers below.

3) The methods are generally/probably adequate but the description is rather confuse and not exhaustive. Just for example:- they did not explicit formally the model trough with the probabilistic estimation was performed - the method used to determine the biomarker “creatinine” has not been described in the M&M section - concerning cadmium determination “cadmium (BCd) were tested using the national standard methods of WS/T 32-1996 and WS/T 32-1996” I don’t know this methods, I suppose are adequate but a wide literature is available
worldwide that should be used to validate the method. The authors did not provide both limit of detection and limit of quantification of the AAS methods they used for the Cd quantification in foodstuffs and urine - uncensored data treatment has not been described and it is not clear if it was performed (see comment 12 hereafter).

Answers:
As our team has published papers which described the method of urine and blood cadmium detection, I described the method briefly and quoted the references. (Page 5, row 32)

4) In more details the sample population is indicated (209 peoples over 35 years old) but was chosen by “convenience”.

Answers:
Convenience sampling is a non-probability sampling technique where subjects are selected because of their convenient accessibility and proximity to the researcher. The method used in the study means we could have good cooperation with the community health center to collect samples and do the test. Then the participants were the ones who had signed informed consent and were randomly chosen. Totally, we had 267 participants and used 209 inhabitants with completed information.

5) The data “on food intake was collected using the food frequency questionnaire (FFQ). The FFQ was quote from National Nutrition Survey and verified to have a good reliability and validity”. More details on the questionnaire should be provided and/or literature references.

Answers:

6) Concerning the cadmium level in foods etc. “A total of 1680 food items cadmium were detected and combined with the intake of the inhabitants”, this part should be more carefully described and should be reported in methods instead of in results.

Answers:
I have rewritten this part and added references. (Page 6, line 32)

7) The Monte Carlo simulation model is adequate (but without references); it was described by Metropolis N. and Ulam S (1949 - The Monte Carlo method. Journal American Statistician Association 44:335–341).

Answers:
Thanks for the reviewer’s good evaluation and kind suggestion. I wrote detailed description and added the references both in method part and discussion part. (Page 7, row 17, Page 13, row 25)
8) This method was used in heavy metals exposure determination by Danieli P.P., Serrani F., Primi R., Ponzetta M. P., Ronchi B., Amici A., (2012. Cadmium, lead and chromium in large game: a local scale exposure assessment for hunters consuming meat and liver of wild boar. Arch. Environ. Con. Tox. 63:612-627), and in other studies. All of them have been ignored by the authors. Answers:
I added several references. The method I used followed the guideline of “FAO/WHO: Dietary Exposure Assessment for Chemical Substance in Food. 2005.” and “Guiding Principles for Monte Carlo Analysis” from EPA. Also different software was used for probabilistic distribution in this study.

9) Concerning the number of peoples included in the study criticism could be raised; are 209 peoples representative of 17-20 million inhabitants of the area? For instance, Danieli et al 2012 (reported above) included in their study 107 questionnaires (250 people) on a total of 4000 hunters. de Winter–Sorkina R, Bakker MI, van Donkersgoed G, van Klaveren JD (2003 - Dietary intake of heavy metals (cadmium, lead and mercury) by the Dutch population. Report no. 2003.016. Rikilt Institute of Food Safety, Bilthoven, Netherlands) included 6250 peoples, on a total of 6 million of inhabitants. Are the data sound? Answers:
Considering the cost-effectiveness, the formula of sample size estimation was list below:

\[ n = \left( t_{1-\alpha} \times \frac{S}{\delta} \right)^2 \]

- \( n \): sample size
- \( \alpha \): testing level
- \( S \): the sample standard error
- \( \delta \): the allowed error
- \( t_{1-\alpha} \): 1- \( \alpha \) percentile corresponded to the probability density curve of t distribution.

In this study, \( \alpha \) was set as 0.05; We picked up food items such as rice, pork, vegetables and what local inhabitants ate as the main food. These data was chosen as the parameter of the sample standard error. According to the Chinese national dietary research in 2000 for adults, we assumed the sample standard of rice, pork, vegetable and fish were 114.3, 54.33, 98.98 and 85.58 respectively. The allowed error was assumed to be 10-20. Then the sample size was about 200-400.

10) The data are interesting as they point out on cadmium exposure for people of a specific area. I don’t know if data on that area are available in literature, but the results are not transferable to other areas and nothing new is reported in the paper. The scientific interest is limited. Answers:
I have changed the units and compared the local data with other countries in discussion part. (Page 11, row 17)

11) In addition, no data are provided for children and it is well known that cadmium burden is particularly important before 12 years of age. The information concerning children is considered necessary when analyzing cadmium exposure. In addition the information on other heavy metals is available by the same experimental schedule only with supplementary analysis, why other heavy metals have not been considered? Does the manuscript adhere to the relevant standards for reporting and data deposition?

Answers:
The food frequency questionnaire is not suitable for children, because parents or teachers are the one we asked about the children or students’ eat habbit. It will cause bias and spend more time. Duplicate portion method cost a lot and cannot be used in large samples. Besides blood samples of children is not easy to collect. Furthermore, the study was the little part of the foundation, other heavy metals were not considered.

12) In my opinion the authors should refer to international guideline on exposure/risk assessment data reporting (e.g., ATSDR, EFSA, other) Are the discussion and conclusions well balanced and adequately supported by the data?

Answers:
I added discussion and references in the revised version and especially followed the guideline of “FAO/WHO: Dietary Exposure Assessment for Chemical Substance in Food. 2005.” and “Guiding Principles for Monte Carlo Analysis”.

13) Although there are several inconsistencies on the aims and the conclusions the authors present data discuss the topic reported in the title. However criticism can be made on the deficiencies reported above. A wide literature exists on this topic and authors seem not to have deeply considered existing knowledge when discussing the results. Are limitations of the work clearly stated?

Answers:
Several references were added and extended in the discussion. Please read the revised version.

14) Some limits are reported, but several limits reported in the revision should be considered. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?

Answers:
I add information in the limits. Please read the revised version. (Page 14, row 8)

15) No, many references are omitted mainly concerning methods. Do the title and abstract accurately convey what has been found?

Answers:
I have rewritten the abstract. Please read the revised version. (Page 2, row 15)
16) No, the Authors consider the dietary cadmium exposure in citizens aged over 35 years in Shanghai, China (also including smoke; is this a dietary source? In the background they indicate “non-occupational” exposure may be this definition is more appropriate. In the abstract-conclusion is reported “there was no greater health risk among adult residents in Shanghai, China as a result of recent total cadmium exposure”. I think that the purpose is confused!)

Is the writing acceptable?

Answers:
The aim of the study has been answered on question 1. Besides, the tobacco is one of the inhaled resource, I have corrected the definition in the article. (Page 7, row 5). The meaning of calculating the tobacco cadmium exposure is to help understand the contribution of dietary cadmium exposure in the total cadmium exposure.

17) A part some typewriting mistakes (e.g. repeating/repeating) English is quite good but a mother tongue revision or professional editing is advisable. As a general consideration the paper provide a very little contribute to scientific knowledge and should be rejected. All the suggestions should be considered as Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached).

Answers:
Your comments are very useful; I have learned a lot and re-edited the article carefully. Please read the revised version.

Comment from reviewer 3:

Minor Essential Revisions

1-“This might indicate the improvement in reducing cadmium contamination”.
It is difficult to compare temporal evolution with 2 different spatial scales (a city Vs a country)

Answers:
Thanks for reviewer’s suggestion. I added food cadmium data in Shanghai on Table 2 coming from Chinese national food survey to make the data comparable. (See Table 2)

2-Could you please clarify how you calculate the mean water cadmium exposure with censored data.

Answers:
Considering the low contribution of the water cadmium and the censored data in this study, I assumed 0.025µg/L as the average mean between 0 to 0.05µg/L (LOD). As the result, the mean water cadmium exposure was 0.03µg/day which accounted for
0.2% of the total environmental. (Page 8, row 20)

3-Please remove multiple spaces/ add necessary space (a lot) and strange symbol used as a coma.
Answers:
Thanks for reviewer’s suggestion. I have changed it in the revised version.

4-Try to keep only one temporal aggregation about intake exposure (day, week or month).
Answers:
I have united the data in the revised version.

5-You are talking about population over 35 years but in the figure 2A the youngest person is 39.
Answers:
I changed title as “Exposure Assessment of Dietary Cadmium: Findings from Shanghainese over 40 years, China” when I found the number of participants under 40 was just 2 and then excluded them.

6-Provide a histogram of the different intake contribution. Don’t give all the contribution in the result part.
Answers:
Thanks for reviewer’s suggestion. I added figure 1 as” Contribution of Cadmium Factors to The Daily Cadmium Exposure (%) ”.

7-Provide a table with statistical concentration analysis results of the different food items.
Answers:
Thanks for reviewer’s suggestion. I added table 2 as “Cadmium level of Local Food (mg/kg) and Daily Intake of Local Food(g/day)”.

8-Provide a table with statistical distributions parameters used in the probabilistic analysis.
Answers:
Thanks for reviewer’s suggestion. I added table 5 as “Probabilistic Distributions and Statistics of Daily Cadmium Exposure (µg/day)”.

9-Could you please justify why you use a non parametric test (Mann-Whitney U test).
Answers:
Because the data was non-normal distribution and I added illustration in the revised version. (Page 7, row 24)
10-Could you please explain how you dealt with censored data.
Answers:
For the water detection, I have answered in question2.
For the tobacco detection, I assumed the cadmium was 1.5 mg Cd/kg, based on Qian et al. [12].
Other data followed the reference “GEMS/Food-EURO, Reliable Evaluation of Low-Level Contamination of Food, 1995 EUR/ICP/EHAZ.94.12/WS04, Germany”.

11-Some results look strange:” The tobacco cadmium exposure was 3.93±8.99 µg/day.” Could you change the presentation of this result (negative value is not possible).
Answers:
The number is not wrong because it is not symmetrical distribution. The description of using standard deviation is not appropriate. I changed it with median and 95 percentile.

12-Figures 1: They are not legible.
Answers:
I have changed it in the revised version.

13-Please indicate letter A to E in figure 1.
Answers:
I have changed it in the revised version.

14-Figure 1 in the text refers to the real figure 2.
Answers:
I have changed it in the revised version.

15-Figures 2: please provide the R² values on the figures.
Answers:
I have changed it in the revised version.

16-Is the body weight not a sensitive parameter in the sensitivity analysis?
Answers:
Yes, it is. But the contribution of it was just 1-3% as I have calculated. It followed the dietary and tobacco factors.

17-I think it’s also not necessary to provide so many decimal for each value…Follow the classical rule of scientific result presentation.
Answers:
I have changed it in the revised version.

18-More risk will be found with youngest age classes (especially for [2-7]). It is difficult to conclude: “The findings indicate that the population was not at
greater risk”.
Answers:
“The population” means the middle and senior population. I made the detailed instruction in the revised version. We did not collect children data because the food frequency questionnaire using in children is not suitable. Parents or teachers are the one we asked for what their children or students eat. It will cause bias and spend more time. Duplicate portion method cost a lot and cannot be used in large samples. Besides blood samples of children are not easy to collect.

Major Compulsory Revisions
19-Innovation should be more described. The paper is too short and should be more developed on the discussion of the different methodology result (point estimation and probabilistic estimation)
Answers:
I have changed it in the revised version. (Page 13, row 15)

20-Please, explain the novelty of this study and the improvement of the assessment regarding Gao et al. (2000).
Answers:
I have changed it in the revised version. (Page 13, row 19)

21- What is the representativeness of the study group with regard to the general Shangainese population over 35 years? Why didn’t you use a statistical adjustment with sociodemographic or socioeconomic data?
Answers:
We did not ask for the financial situation of inhabitants because financial situation had poor accuracy according to the survey we did before. And considering the cost-effectiveness, the formula of sample size estimation was list below:

\[ n = \left( t_{1-\alpha} \times S/ \delta \right)^2 \]

\( n \): sample size
\( \alpha \): Testing level
\( S \): The sample standard error
\( \delta \): The allowed error
\( t_{1-\alpha} \): 1 - \( \alpha \) percentile corresponded to the probability density curve of t distribution.

In this study, \( \alpha \) was set as 0.05; We picked up food items such as rice, pork, vegetables and which local inhabitants ate as the main food. These data was chosen as the parameter of the sample standard error. According to the Chinese national dietary research in 2000 for adults, we assumed the sample standard of rice, pork, vegetable and fish were 114.3, 54.33, 98.98 and 85.58 respectively. The allowed error was assumed to be 10-20. Then the sample was about 200-400. It could represent the dietary intake of middle and senior age inhabitants.
22-Some additional parameters should be also integrated in the questionnaire for a better characterization of the individual exposure (as proximity to an industrial site, homegrown consumption…).

Answers:
I added education and marriage condition in table 1. Factories have been slowly moved to the nearby cities around Shanghai in recent 10 years, so there were just few factories located in suburbs which were also far away from where we did the study. We did not ask about the homegrown consumption which is a good question for knowing the resources of the food. In further study, we will consider it as one of the factors.

23-Conclusions: Please make a summary of what has been done, the strength to combine both approaches and the weaknesses to work on those limited data. Open the conclusion with longer term perspectives.

Answers:
I have changed it in the revised version. (Page 14, row 31)

Discretionary Revisions
24-Write one or two sentences on the difference between external and internal dose characterization.

Answers:
I have changed it in the revised version. (Page 12, row 32)

25-Heavy metal: prefer ‘trace metal’ term.

Answers:
I have changed it in the revised version. (Page 4, row 2)

Comment from reviewer 4:

1. Abstract.
   a. MER Weekly cadmium exposure values and % PTWI values have too many significant figures. The chemical analyses do not warrant that level of accuracy or precision.

   Answers:
   Thanks for reviewer’s suggestion. I have changed it in the revised version.

   b. MCR Mean values of cadmium in urine and blood are VERY low, e.g., 0.0052 µg Cd/L for BCd in total population. There appears to be a mistake. For example, mean values for cadmium in western countries are in range of 0.5 µg Cd/L for urine and blood in non-smokers and can be 1.5 µg Cd/L and higher in smokers.
Answers:
Thanks for reviewer’s comments. The data was 100 times lower as I checked up the lab record. I have changed it in the revised version.

c. DR Conclusions. Should say, “there was no increase in health risk” changed.
Answers:
I have changed it in the revised version.

2. MCR Sentence structure throughout the manuscript needs the attention of an editor.
Answers:
I have changed it in the revised version.

3. P5, Methods. Additional information is needed regarding the methods, for example:
a. MER A reference should be given for the food frequency questionnaire from the National Nutrition Survey, so the reader can gain insight into the questionnaire.
Answers:

b. MCR Methods should be described regarding the approach taken to estimate cadmium intake from tobacco.
Answers:
I added the method of tobacco estimation. (Page 7, row 5)

c. MCR In addition to the code number for the cadmium analyses, details of the methods used for analyzing urine and blood for cadmium need to be described to give the reader confidence that the results are accurate.
Answers:
As our team has published papers which had already described the method of urine and blood cadmium detection, in this paper I just made brief description on the method briefly and quoted the references [16,17]. (Page 5, row 32)

d. MCR Were certified standards of human blood and urine analyzed along with the subjects’ samples to validate the analytical methods? These standards are needed for this kind of study.
Answers:
Please read the answer for question 3c.

a. MCR The statement, “Moreover, we collected tobacco and water consumption to calculate the total cadmium exposure.” is very general and gives little insight into how intakes from tobacco and water were determined. The authors need to provide more detail on methods here.
Answers:
Please see the method part of revised version. (Page 7, row 5)

b. MCR “Tobacco cadmium was detected as 1.5mg/kg[12].” The meaning of this sentence needs to be clarified. I think the authors mean: “Tobacco cadmium concentrations were assumed to be 1.5 mg Cd/kg, based on Qian et al. [12].”
Answers:
Thanks for reviewer’s suggestion. I have changed it in the revised version.

5. MCR Table 2. This table provides cadmium intake values with little insight into how these values were derived. The data would be strengthened if two additional endpoints were presented. For each food item, it is suggested you provide the dietary intake value that you obtained from your subjects, along with a measure of uncertainty. Along with these intake values, the cadmium concentration used for each food item should be provided, with a measure of uncertainty. The latter two sets of data will allow the reader to compare the intake levels you obtained from your study subjects to others, and to compare the food cadmium concentrations you used from the SCDC dataset to others. As is, the results in Table 2 are presented with no insight into the datasets used to determine the cadmium intake values.
Answers:
I added table 2 for the level of food cadmium and the intake of each food items and added the detection method from SCDC in the method part. (Page 6, row 32)

6. MCR Table 3. As indicated in comment 1a. above, the units of UCd and BCd in this table look wrong. The values given appear to be too low to be correct.
Answers
Please read the answer for question 1c.

a. MCR “The smoking cadmium of male and female were 12.11µg/day and 1.26µg/day.” Again, the methods used to get these values and what they mean needs to be clarified. Do these values indicate amounts of inhaled cadmium?
Answers:
Yes, the tobacco cadmium was the main resource of cadmium inhalation. I added the institution in the method part. (Page 7, row 5)

b. MCR “The tobacco cadmium exposure was 3.93±8.99 µg/ day.” What was the route of intake of this tobacco cadmium? Chewing tobacco? Again, methods are missing.
Answers:
The tobacco cadmium was the main resource of cadmium inhalation. I quote the references for the level of tobacco cadmium.
Please read the revised version. (Page 7, row 5)

8. P8. MER Assessment of the Cadmium Exposure. The authors use too many significant figures for the results. For example, total cadmium exposure is not known to 5 significant figures (125.51 µg/week).
Answers:
Please read the revised version as I made the figures changing.

9. Figure 1.
a. MER The figure legends should give the meaning of Certainty, Certainty Max and Certainty Min.
Answers:
I added Table 5 as “Probabilistic Distributions and Statistics of Daily Cadmium Exposure (µg/day)”.

b. MER The x-axis needs to be labeled.
Answers:
Please See Figure 1 as I revised.

c. MER P9, end of 1st paragraph. It is not clear why the value of 93.13% given in the text differs from the Certainty value shown in Fig 1A of 97.65%. It seems these two values should be the same. This question applies to follow-on paragraphs on P9 also where values for % of population below the PTWI are quoted in the text and differ from values in Figure 1.
Answers:
Please read the revised version. I have checked up with the figures.

a. DR Para2. Good comparison of your results to those of other countries.
b. DR Para3. It would be good to also express here the Shanghai results from this report in the same units as those quoted for other countries (µg/kg bw/week), to provide for easier comparisons.
Answers:
Please read the discussion in red as I did the units changing. (Page 11, row 17)

11. MER P11, para 2. The authors should compare their values for cadmium intake from tobacco to those reported by others in the literature.
Answers:
Please read the revised version. (Page 12, row 11)