Author’s response to reviews

Title: Effects of long-term low-level radiation exposure after the Chernobyl catastrophe on immunoglobulins in children residing in contaminated areas: cohort and cross-sectional studies

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Version: 2
Date: 27 April 2014

Author’s response to reviews: see over
Responses to Reviewers’ Comments

Manuscript # 5715070901056325

Effects of long-term low-level radiation exposure after the Chernobyl catastrophe on immunoglobulins in children residing in contaminated areas: prospective and cross-sectional studies.

We appreciate the thoughtful and thorough review provided by the reviewer Dr. DeWitt and Dr. Akiba. We have tried to carefully address each recommendation. We believe our manuscript is stronger as a result of the modifications outlined below. We thank the editorial staff and the reviewer for their contributions.

Below we provided point-to-point responses (in purple ink type) to all comments. Modified text is highlighted in yellow in the revised manuscript.

Response to the comments by Dr. DeWitt

Minor Essential Revisions

1. The introduction is a bit lengthy. Much of the background material regarding immunoglobulin levels from people residing in nearby cities could be moved to the discussion and serve as a basis of comparison to the current study.

Authors’ response: We agree that the background section is fairly long; however, for each immunoglobulin, it gives a comprehensive overview of the studies that have been conducted after the Chernobyl accident. We believe that the background section will help the reader to understand the complexity of the problem. We moved the short description of function of each immunoglobulin into the discussion section. Also, the comparisons with relevant studies were moved to the discussion section (pages 21-22).

2. While reporting and evaluating absolute values of serum immunoglobulins can be somewhat informative, the analysis may be improved by evaluating relative amounts of immunoglobulin classes to each other. IgG comprises the greatest amount of immunoglobulin in serum, followed by IgA, then IgM, and then IgE. Shifts in the ratios of immunoglobulins can indicate shifts in cell populations and in cytokine signaling. If it is possible, evaluating ratios might give the authors a
better or more telling indication about shifts in adaptive immunity and therefore potential health risks.

**Authors’ response:** We found three other studies that looked at ratios of immunoglobulins. We believe that providing a ratio is very exploratory. In addition, we cannot simply take the ratios of the immunoglobulins measured in g/L since the molecular weights of the immunoglobulins are different (the molecular weight of IgA is 385 kilo Daltons; IgG’s molecular weight is 150-170 kDa and IgM is approximately 970 kDa). Only the ratio of the molecules (μmol/L) could indicate a shift among IgA, IgG, and IgM. However, if we start introducing ratios, we would also need to run other statistics with ratios and add a lot to the tables. This would further increase the length of the paper, which is already very long. Although our findings regarding the ratios are interesting, we could not follow this suggestion, but will publish these in additional papers.

3. Although the authors did not measure specific immune-related health outcomes (i.e., rates of infection such as otitis media, flu, etc.) other than allergy/asthma/eczema, the discussion would be greatly improved if the authors could comment on the particular role that each immunoglobulin class plays in adaptive immunity. This is done somewhat in the introduction, but if put into the context of the results, would enhance the discussion.

**Authors’ response:** The prevalence of reported bacterial infections and antibiotic use in the study population were very low; therefore, they were not used in the analyses. We moved the description of the role of different immunoglobulin classes from the Background to the Discussion.

4. For some of the results, it may not be appropriate to say that cesium induced “immunosuppression”. For example, in looking at Figure 3, values for IgA range from a low of about 1.65 g/L to a high of just under 2 g/L. It is unlikely that this difference indicative of immunosuppression. Similar conclusions could be made by looking at the graphs of the other immunoglobulins.

**Authors’ response:** We do believe that exposure to high levels of ionizing radiation is related to decreased immune response which has been demonstrated
by the previous studies conducted soon after the Chernobyl catastrophe. Under conditions of long-term exposure to the relatively low (as compared to high doses immediately after the nuclear incident) radiation exposures, the immune system is probably able to compensate, therefore, the immune response in the group with the highest exposure is lower than in all other exposure groups. Although IgA levels are within the normal range, they are the lowest in the highest exposure group. We agree that our language was too strong and made changes (page 5, 19, 21). However, we kept the terms ‘increase’ and ‘decrease’ to describe time trends.

5. The graphs in Figure 3 are challenging to read as they have gridlines as well as the cesium values printed on the graphs. It is recommended that the quintile values be included in a legend rather than printed on the graphs themselves. It is also recommended that the x-axis include years rather than the numbers 1-6.

Authors’ response: Thank you for your comment. The changes have been made and the figure 3 now looks better.

Discretionary revisions:

1. Note: “data were” and “data are” technically are correct; “data is” and “data was” technically are incorrect.
   Authors’ response: Thank you for pointing it out. The mistake was corrected.

2. Were the levels of IgG measured/reported total IgG or were different isotopes evaluated? Was IgM measured as a monomer or dimer or total?
   Authors’ response: We evaluated only total IgM and total IgG. We added the term “total” to the “Measurements of immunoglobulins A, G, M, and E”, page 11.

3. Did the authors evaluate IgE in relation to pets in the home? Given that they found that IgE was decreased in relation to indoor allergens, the presence of pets could influence this response.
   Authors’ response: Specific IgE responses were determined to a mix that included antigens present in cat and dog dander. It is known, that exposure to cats and dogs only explains a small proportion of specific IgE to indoor allergens. Some
publications even demonstrate that dog or cat exposure during the first year of life is related to a lower risk of allergic sensitization.


The aim is to identify factors other than exposure to cats or dogs that explain the occurrence of specific IgE response. To this end, we adjusted for exposure to cats and dogs in the household. Thus, we can identify other factors that can contribute to IgE response to specific indoor allergens. Our analysis shows that residential exposure to $^{137}$Cs was related to the identification of specific IgE to indoor allergens.

Response to the comments by Dr. Akiba

The information presented in this manuscript seems important.

In the analyses of data for 1993-98 data and for 2008-2010, authors divided study subjects into five and three groups, respectively, based on soil $^{137}$Cs contamination levels, which are largely determined by geography. Since authors did (could) not use individual radiation doses when evaluating the relationship between radiation exposure and serum immunoglobulin levels, this study should be regarded as a kind of an ecological study.

Authors' responses: An ecological study, for instance the consumption of red wine and the rate of myocardial infarction in different countries, is based on means per country and does not establish the exposure at an individual level. Hence, in aggregate ( ecological) studies, a direct relationship between myocardial infarction and red wine drinking is not established. It could even be that all cases with myocardial infarction drank more red wine although red wine consumption was lower within a specific country.

In contrast to this, our study established a level of residential exposure to $^{137}$Cs soil contamination for every child. However, the individual contact in the residential area may vary within limited margins. This is then called a non-differential misclassification. We have described the relation between soil measurements and individual body count measurements elsewhere. See: J Expo Sci Environ Epidemiol. 2013 Sep 25. doi: 10.1038/jes.2013.60. Individual whole-body concentration of $^{137}$Cesium is associated with decreased blood counts in children in the
Authors should present the results of the whole body counting of study subjects even if the data are not available for all of them. (when doing so, authors should present its median and range, at least.)

**Authors’ response:** The individual whole body count was not available in the longitudinal cohort 1993-1998, and to keep the exposure in this investigation comparable, we used the residential exposure in all analyses. However, we have shown that the residential exposure explains the individual body count with a path coefficient of 0.39 (p=0.0001) (Lindgren et al. 2013). In the current publication we never used whole body counts as exposure.

In the previous paper (Stepanova et al. 2008 Environ Health PMID: 8513393), authors used five groups of residential exposure (<116, 116-, 165-, 266-, 350+ kBq/m2), which appear to be the same dose grouping as the one used in the present study. The individual effective equivalent doses for those exposure categories were 13.6, 11.7, 16.7, 34.9, 19.6 mSv, respectively. Also in the present study, authors should present the average (or median) individual effective equivalent dose for each exposure category of soil Cs-137 contamination (if they are different from the previous study).

**Authors’ response:** To be comparable, we used the same residential exposure groups measured in kBq/m². We have followed the literature and saw that radiation exposure classifications seem to change every 10-20 years. As a measurement unit of residential exposure we used kBq/m². For the prior publication we had to convert Curie (Ci/km²) to Becquerel. We believe that one reason to change the exposure about every 20 years is to confuse the scientific community. We do not want to fool our colleagues but achieve maximum comparability among studies. We also addressed this as exposure not as a dose, since - given the Latin origin “exponere” is describes the milieu outside the body. Originally, we planned to analyze radiation dose using individual effective equivalent dose in millisieverts (mSv), which incorporates total external and internal doses, including direct measurements with whole body counts. However, this exposure measure includes so many individual uncertainties and changes
over time that we rejected such an assessment as non-reliable. It does not help but only confuses since it integrates so many other influences beside the external exposure, which was our focus.

The relationship of this study with the one reported by Svendsen et al. (Svendsen ER et al. 137Cesium exposure and spirometry measures in Ukrainian children affected by the Chernobyl nuclear incident. Environ Health Perspect. 2010;118:720-5. PMID: 20100677) is not entirely clear. In the study of Svendsen et al., the soil 137Cs contamination levels were divided into five categories. (The mean radiation levels of five categories were 90.7, 128.2, 195.5, 308.6 and 355.5. kBq/m2. The range of radiation level for each category was not reported.) Then, they investigated the association of soil contamination levels with spirometry measures for 415 children of the contaminated Narodichesky region who were examined from 1993 to 1998. The frequencies of respiratory problems were determined based on repeated measurement data obtained from their longitudinal study. It is desirable to examine the association of immunoglobulin levels with the frequency of respiratory problems, which were identified by Svendsen et al.

**Authors’ response:** As explained in the paper by Svendsen et al, only children of a specific height (reflecting age) could participate in the lung function tests. Thus, more children provided a blood sample than had a spirometry. Given these conditions, the sample sizes between these two analyses differ. In this analysis we focused on the larger sample of children who provided serum samples to measure immunoglobulins. The suggestion to examine the association between immunoglobulin levels and the frequency of respiratory problems is good and will be addressed in the future papers focused on pulmonary health problems.

Fig3: IgA showed no clear dose response in any single year during 1993-1998. The significant interactive effect between time and radiation exposure levels on IgA levels appears to stem from the observation that the second highest exposure group showed a time trend different from the other groups. Authors failed to identify the factors involved in this trend over time.

**Authors’ response:** Yes, we failed to identify factors involved in this time trend. We are not omniscient but can improve our understanding (the truth) step-by-step. We discussed that there is a ∩-type relationship between 137Cs exposure and IgA. This ∩-type relationship is responsible for the interaction of time and exposure overall, and not only the time*exposure interaction of the second
highest exposure group. Note, this is an exposure*time interaction and not a time-trend.

Below is the SAS output when we ran the mixed linear model without the second highest exposure group. Note, that in Table 2 we had 4 degrees of freedom, here we only have 3 degrees of freedom for “gr_cs137_kBq_m2” (originally 5 groups, one group removed 4 groups  DF = 3).

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>gr_cs137_kBq_m2</td>
<td>3</td>
<td>10.18</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>0.35</td>
<td>0.5558</td>
</tr>
<tr>
<td>agecls</td>
<td>17</td>
<td>13.80</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>time</td>
<td>5</td>
<td>23.56</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>time*gr_cs137_kBq_m2</td>
<td>15</td>
<td>3.23</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

As can be seen, the interaction effect does not result from some peculiarities of the second exposure group, since we find a significant interaction effect even after the exclusion of this group.

Fig3: IgM and IgG levels increased evidently during 1993-98. Are there any possibilities that their increases were due to technical problems? Another factor possibly involved in this observation is nutrition since blood counts of RBCs, platelets and WBCs were also increased during this period (as reported by their previous paper: Sepanova et al 2008). Authors should discuss the factors involved in this time trend, including technical problems and nutrition.

Authors’ response: Technical problems with measurements of IgG and IgM are unlikely since the lab analyses from 1993 to 1998 were performed using the same method and the same personnel. The increase in levels of IgG and M from 1995 through 1998 could have been related to decreased supply of contamination-free food and related increased consumption of contaminated local produce and forest plants and game.

We made changes to the paper on page 20:

“In the dynamic cohort 1993-1998, for all immunoglobulins, A, G and M, was observed a similar secular trend with dip or plateau around 1995 and consequent increase over time (Figure 3). Reduced levels in 1995 may be related to changes in supply of radiation-free food provided by the Ukrainian government to residents of radionuclide contaminated areas after the Chernobyl incident. In 1995, due to deteriorated financial situation, the Ukrainian government stopped delivery of
uncontaminated food to the residents, and radionuclide-free meals at schools were reduced down to two per day. As a consequence, the residents increased their consumption of locally grown produce and forest goods contaminated with radio cesium. Technical causes that might contribute to these changes are unlikely, since during this period, laboratory analyses were conducted by the same personnel using the same techniques.”

Fig3: In the case of IgM, the secular changes are much larger than the differences among exposure groups if the second highest exposure group (266-310 kBq/km2) is excluded. The IgM levels of this group (the second highest exposure group) were evidently higher than other groups. Authors did not discuss the possibility that the IgM levels in this group may be affected by factors other than radiation. Authors should discuss this problem.

Authors’ response: There is no reason to consider that changes in methods affected the IgM determination in the second highest exposure group (266-310 kBq/km2). There may be numerous other reasons why the second highest exposure group has the highest IgM values. However, we are not aware of any other specific reason why only the second highest exposure group would be affected. We discussed this issue with regard to a \(\cap\)-type association.

In the previous paper (Stepanova et al. 2008 Environ Health PMID: 8513393), authors reported that the counts of RBCs, platelets and WBCs were lowest in the second highest exposure group (266-310 kBq/km2). Authors did not make any discussions regarding the similarity (the direction was opposite but the pattern was similar) in the results of IgM in the present study and blood cell counts in their previous report.

Authors’ response: Biologically, the red blood cell and the platelet counts have little connection with immunoglobulin concentration. In addition, we distinguish different roles of the humoral and the cellular responses. Following this, there are only weak correlations (correlation coefficients <0.05) between leukocyte, red blood cell, basophil granulocyte, and monocyte counts with IgA, IgG, and IgM. There may be some association with eosinophil count and with specific lymphocytes, such as CD4, CD8, CD22 cells, but results on these cells have not been published yet. Inclusion of these cells would go beyond the scope of this paper that focuses only on the humoral immune response.
It is correct, that there may be pattern similarities in the second highest exposure group, but these cannot yet be explained by a "common cause" for the different blood markers. Thus, we do not need to discuss these.

Fig3: In the case of IgG, the secular changes are larger than the differences among exposure groups if the highest exposure group is excluded. The IgG levels of this group were evidently lower than other groups. Authors did not discuss the possibility that the IgG levels in this group may be affected by factors other than radiation.

**Authors' response:** There are some “secular trends” as noticed above for IgM and also for IgG. We controlled for age the analyses; hence the age-effect was largely removed from the effect of the years (“secular trends”). As we mentioned before for IgM and here IgG, there is no problem if the “secular trend” is larger then the effect related to soil concentration. The secular trend also includes changes of exposure or changes of effects over time, since for all three immunoglobulins the interaction of exposure * year was significant (Table 2). In this case, as shown below, if we exclude the highest exposure group, there is no longer an interaction effect of years with soil exposure.

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
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<tbody>
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<td>gr_cs137_kBq_m2</td>
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<td>0.92</td>
<td>0.4318</td>
</tr>
<tr>
<td>time</td>
<td>5</td>
<td>15.37</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>0.46</td>
<td>0.4986</td>
</tr>
<tr>
<td>agecls</td>
<td>17</td>
<td>6.12</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

But there is no reason to exclude the highest exposure group since we cannot present biased results. Sure, there could be other factors that may have affected the highest exposure group only and that may have led to our findings. However, there is no single reason to consider that other factors could have altered the effect in the highest exposure group only.

In the present study, the highest and the second highest groups of soil 137Cs contamination had immunoglobulin levels different from the other groups. Authors should divide the highest group into several areas, and examine area differences of immunoglobulin levels. Authors should conduct a similar analysis for the second highest group. If area differences are found, authors should present the results, creating a new table.
The majority of children in the highest exposure group resided in the same large village Narodichi, and all but one child in the second highest residential exposure group resided in another village Selec, therefore, the environmental conditions and nutrition within each of these groups/villages were homogenous. There are no known differences in local diet at the village level since this is one area with the same ethnicity of the residential population.

In page 16, authors wrote as follows: “In addition, we tested whether IgA, IgG, or IgM serum concentration co-varied with antibiotic use in the last 12 months and found no significant or suggestive association (data not shown). Therefore we discarded antibiotic use as a potential confounder.”

Comments: I understand that factors without statistical significance (and factors without suggestive significance) can confound the results. Therefore, I am afraid the decision made by authors was not correct. According to the most famous textbook of modern epidemiology (Modern Epidemiology, 2nd edition, edited by Rothman and Greenland p256), many epidemiologists think that adjustment for a variable should be made if the estimate of parameter of association is changed by 50% or larger by the adjustment for that variable.

Authors’ response: The original selection of potential confounders for the study is based on the literature and tests of bivariate associations between exposure and confounder, confounder and outcome. The final selection of confounders is based on 10% rule. According to conventional rules, a confounder should be a risk factor for the outcome (see Modern Epidemiology, 3d ed. Edited by Rothman and Greenland, p. 194). A potential confounder must be associated with the outcome (we do not discuss significance here) to be a confounder. This condition was not met for antibiotic use, since we detected no association between IgA, IgG and IgM and antibiotic use. To check confounding by antibiotic use, we additionally used 10% rule. Removal of antibiotic from the statistical model did not change the estimate of the exposure variable (137Cs soil contamination levels) beyond 10%. We added use of 10% rule to assess confounding to the methods section and removed the sentence about discarding the antibiotic from the paper, page 16.

In ll20-22, p13, authors wrote as follows: “For the 2008-2010 data, we additionally adjusted for exposure to environmental tobacco smoke (ETS), active smoking, antibiotic use, exposure to
cats and dogs, and coal, coke, wood or gas used as a fuel in stoves for heating and cooking. We adjusted for indoor allergens and irritants.

Comments: the criteria for selecting those covariates are not clear.

Authors' response: ETS, active smoking, antibiotic use, exposure to cats and dogs, indoor use of coke/wood/gas stoves are known factors that may affect immune responses. There a thousands of articles that can be found in PubMed. It is reasonable to adjust for these confounders. The problem is not whether we adjusted for too many variables. If these were not confounders (10% rule) we could have ignored these anyway. There would be a problem if we forgot to adjust for important confounders.

In the discussion (pp18-19), authors wrote as follows: “There is no clear linear pattern, but various convex-curve-like associations.” However, Models 1 and 2 in Table 4 (cross-sectional study 2008-2010) apparently showed different shapes of dose-response for association between radiation levels and Ig A. It was U-shaped in Model 1 and linear in Model 2.

Authors' response: In both models we see a U-shaped association, it is not as strong in Model 2, but it is still not linear. See below.

Extract from Table 4.

<table>
<thead>
<tr>
<th>Radiation (kBq/m²)</th>
<th>IgM (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;116</td>
<td>1.76 (1.43-2.09)</td>
<td>0.0067</td>
</tr>
<tr>
<td>116 – 164</td>
<td>1.25 (0.88-1.63)*</td>
<td>0.0067</td>
</tr>
<tr>
<td>165 - 265</td>
<td>1.95 (1.77-2.13)</td>
<td></td>
</tr>
<tr>
<td>&gt;85</td>
<td>2.02 (1.81-2.23)*</td>
<td></td>
</tr>
</tbody>
</table>

Gender | Gender

IgM levels in the dynamic cohort (N=617) increased during the period 1993-1998. In the longitudinal analysis (N=25) from time 1 (1998-1997) to time 2 (2008-2010), IgM levels decreased. Authors did not make any discussions regarding those observations. Anyway, authors should present average IgA, IgM and IgG levels for all the children (N=650) and those aged 8-17 (n=523) examined during 2008-2010 so that readers can understand the time trend from the 1990s to 2000s.

Authors Response: Yes. This is an interesting examination. We added to the Results section (page 19): “Interestingly, IgM levels particularly in the two highest exposure groups (266-310 and 350-879 kBq/m²) increased between 1993 and 1998
(Figure 3, sample size n=617). However, in the small longitudinal cohort (n=25) with measurements in 1997/98 and 2008-2010, IgM levels decreased in the highest exposure group (in this case: 165-253 kBq/m²). The latter group had the least increase from 1993-1989 (Figure 3). However, both trends may not be comparable since the age in the 1993-1998 cohort was 1 to 17 years and in the longitudinal cohort it was 8-15 years in the first medical exam and 19-27 years in the second.“

The mean IgM levels are presented in Table 6. It is obvious, that at the first medical exam the levels are comparable to those in Figure 3 for 1993-1998. We added “The mean serum concentrations of IgA, IgG and IgM in children 8-17 years in the cross-sectional study 2008-2010 were 1.86 g/l, 11.6 g/l and 1.14 g/l, respectively”, page 15.

Authors found that IgE specific against indoor allergens, but not outdoor allergens, were statistically significantly less frequent in children residing in villages with higher soil concentrations. They suspect that 137Cs radiation reduces the population of mite and mold species, reducing the indoor allergen but not outdoor allergen burden. The IgE assay was conducted using two mixtures of allergens: indoor (Dermatophagoides pteronyssinus, Dermatophagoides farina, Aspergillus fumigates, Aspergillus niger, cat and dog dander) and outdoor (seasonal weeds and flowers). It is not entirely clear why they selected those antigens. If they conducted the CAP-RAST tests (or similar tests) to examine various antigens, including food antigens, more insightful information could have obtained.

Authors' response: All selected antigens were the most common indoor and outdoor antigens in that region. Food antigens were not included since food allergies are much less frequent compare to aeroallergens. We made changes on page 11. We would have preferred not to rely on mixtures of allergens, but had budget limitation. Not including food allergens is a minor problem, but it would have been of interest to better understand different indoor allergens. For this reason we wrote in the conclusion (page 24):

“Surprisingly, higher soil radiation levels were related to fewer indoor allergen sensitizations (specific IgE), a finding which needs to be replicated in future studies.”
The amount of radioactive fallout in a particular area is affected by weather and terrain (wind, rain, the pass of wind, etc), and the extent of soil contamination can be affected by the nature of soil, drifted dust deposits, wind and waterfall. Those factors might have also affected the daily lives of residents (before and after the accident) that are related to various determinants of serum immunoglobulin levels. Therefore, it seems difficult to imagine that study subjects were randomly exposed to radiation. Authors wrote that they conducted a random sampling of study subjects.

Authors’ response: We agree that wind and weather conditions were the primary factors affecting the fall-out. However, the region covers 1284 square kilometers (diameter about 60-80 km) and has the same whether, wind, and climate conditions. Within this district, radiation was allocated randomly. We consider this statistically as the random act of nature. Since there is no differences in the climate, temperature, wind, or whether conditions between the villages in this area, the villages Narodichi, Basar, or Davidki had the same living conditions. The radiation in this area hit everybody randomly, irrespective whether parents were smokers, lived, and worked on a farm or lived in an apartment. We added the following sentences to the paragraph about “Study population” on pages 8-9: “There are no differences in climate, temperature, wind, or other weather conditions between the villages in this area that may have affected the fall-out. Additionally, the fall-out was not related to differences in the resident population, occupation, or living conditions.”

In page 5, authors wrote as follows: “Another study linked serum immunoglobulins to internal doses of 137Cs in children accumulated over 14 years after the Chernobyl incident [14].” Comments: I think this statement is important to understand the backgrounds of the study made by authors. However, since the reference 14 is written in Russian, it is not easy for readers to read it. Authors should describe more details about this paper. In particular, authors should describe how internal doses were measured or estimated. The unit of internal dose should also be presented. If available, authors should state the median and the range of the dose.

Authors’ response: The requested information was added, page 5: “In the study by Chebanenko et al. (2004), whole body concentration (WBC) of 137Cs was measured in Bq using gamma-spectrometer (Whole Body Counter SKRINNER-3M). This measurement was used for calculation of the internal dose of 137Cs in mSv. According with their WBC, children were divided into 3 groups: I -
1,087-13,956 Bq (mean 4540±210 Bq), internal dose 0.150-1.147 mSv (mean 0.412 mSv); II - 1,046-10,060 Bq (mean 3820±260 Bq), internal dose 0.106-0.899 (mean 0.209 mSv); III - 0-960 Bq (mean 410±60 Bq), internal dose was not reported.”

Minor points

In the analysis of 1993-98 data, authors divided study subjects into five groups based on soil contamination levels. The means (or medians) of those five categories (<116, 116-, 165-, 266-, 350+ kBq/m2) should be presented.

**Authors’ response:** The means of soil contamination levels were added to Table 1.

In the analysis of 2008-2010 data, authors divided study subjects into three groups based on soil contamination levels. The means (or medians) of those three categories (<116, 116-164, 165-265 kBq/m2) should also be presented.

**Authors’ response:** The means of categories of residential exposure to $^{137}$Cs were added to Table 1.

The definition of dynamic cohort is not entirely clear. In a broad sense, a cohort is the population followed for a certain period. Therefore, I guess a “dynamic cohort” is an “open population” followed for a certain period. However, if a part of population is not followed at all, that subpopulation is not a cohort. In this study, were the newly-added members followed for a certain period? –Authors might have planed to follow them-up. However, that is irrelevant to the study reported in this manuscript.

**Authors’ response:** The term dynamic cohort was described in “A Dictionary of Epidemiology” edited by John M. Last, Professor of Epidemiology, and is commonly used. “Dynamic population” is a prospective population, in which participants join, leave, or re-join the cohort. All participants in our dynamic cohort study of 1993-1998 had at least 2 examinations.

The definition of “linear mixed models” is not entirely clear. Is it a mixed effect model? (it appears to be merely a linear model with an interaction term).

**Authors’ response:** Linear mixed model is defined in multiple statistical textbooks. It is a regression model that allows analysis of repeated measures by using a
covariance matrix that compensates for the non-independence of the repeated observations. We added a reference to this method to the Statistical analysis section, page 13.

Results described in the abstract section are not clear enough. In Abstract, authors wrote as follows: “Residential soil contamination in 2008 highly correlated with the individual body burden of 137Cs.”

Comments: In the abstract, authors should use a term much more easily understood instead of “the individual body burden”.

Authors’ response: The term “individual body burden” is a term used in many papers regarding the current radioactive burden within the body, in our case the whole body count.

In Abstract, authors wrote as follows: “Serum IgG and IgM concentrations increased between 1993 and 1998. Children with higher 137Cs soil exposure had lower serum IgG levels, which, however, increased in the small cohort assessed between 1997 and 2008.”

Comments: Isn’t it 1997-2010?

Authors’ response: Thank you for pointing this out. The corrections have been made.

In Abstract, authors wrote as follows: “Children with higher 137Cs soil exposure had lower serum IgG levels, which, however, increased in the small cohort assessed between 1997 and 2008.”

Comments: I guess that “between 1997 and 2008” is “between 1997 and 2010”.

(or, I may be confused)

Authors’ response: Thank you for pointing this out. The corrections have been made.

Line 4, page 5: The meaning of “incident” is not clear.

Authors’ response: We added a word “Chernobyl” before the “incident”, page.

The bottom line, page 5: the meaning of “suppressed-increased” is not entirely clear.
Authors' response: We changed the term to “suppressed-increased time pattern”, since it refers to the initial decrease after the exposure followed by an increase, as described in the sentences preceding this term.

In page 6, authors wrote as follows: “During the first 1.5 months after the Chernobyl accident IgM serum levels in children 1-14 years residing in Braginsky region of Belarus with density of soil contamination $137$Cs $3\text{Ci/km}^2$ ($111 \text{ kBq/m}^2$), seemed to be slightly increased [4, 9].”
Comments: I am afraid that there are typographical errors in the underlined part of this sentence.

Authors' response: The PDF file with your comments that we received did not have any underlining in the sentence in question. We corrected the sentence (page 6): “During the first 1.5 months after the Chernobyl accident IgM serum levels in children 1-14 years residing in the Braginsky region of Belarus with a $137$Cs soil contamination density of $\text{Ci/km}^2$ ($111 \text{ kBq/m}^2$), seemed to be slightly increased [9,11]”

In page 6, authors wrote as follows:
“Hence, it seems that long term exposure to ionizing radiation after the incident results in higher IgE levels in children residing in contaminated areas.”
Comments: In the introduction, authors did not make any discussion regarding potential confounders and biases. Therefore, the arguments made by authors do not seem convincing.
BTW, the meaning of “incident” is not clear, either.

Authors' response: 1. We added to the introduction on page 8:
“However, the prior studies did not take into account confounders such as cat and dog exposure that may be associated with the exposure to $^{137}$Cs in the soil and may affect serum IgE levels.”
2. We added a word Chernobyl before the “incident”, page 8.

In page 15, authors wrote as follows:
“In addition, using individual measurements, after adjusting for age, gender, weight, and month of measurement, we determined whether the residuals of the individual radiation levels (not explained by residential exposure) correlated with the area soil contamination level. “
Comments-1: Authors should clearly state the meaning of individual measurements. (Are they the results of whole body counting?) It is necessary to present the unit of radiation levels.
Comments-2: Authors should explain how the adjustment was made. --In the statistical model, individual measurements were log-transformed? Age and weight, month of measurements were categorized? If so, were they used as categorical variables or continuous variables? Were any interaction term (for example, gender*age) included in the model?

**Authors’ response:** 1. Thank you. We added some explanations on page 15.

“In addition, using individual measurements of whole-body burden of $^{137}$Cs (continuous, Bq) in a linear regression model we adjusted for age (years), gender, weight (kg), and month of measurement and determined the residuals. These were normally distributed. Then the residuals of these individual measurements (not explained by residential exposure) were correlated with the area $^{137}$Cs soil contamination (kBq/m$^2$). Indeed, the correlation in 26 villages was high ($r=0.64$, Figure 2). Finally, using the same approach, we correlated the residuals of the individual whole-body burden of $^{137}$Cs with the radiation values in milk (Bq/L) for 2008. The correlation coefficient was lower (0.42, $p=0.04$, 25 villages).

The linear regression model to estimate the residual included no interaction terms. We are not aware of any interaction term of combinations of age, gender, weight, and month of measurement that would make sense.

In page 15, the authors wrote as follows: “In the dynamic cohort, 617 children had repeated measurements between 1993-1998, providing 2,407 measurements of IgA, IgG and IgM. The analyses focused on soil contamination (quintiles) and adjusted for gender and age of the child, year of the exam, and the interaction between year and soil contamination.”

Comments: The statistical model used by authors is unclear.

**Authors’ response:** The description of the model was edited, page 15:

“In linear mixed models, we predicted mean levels of each immunoglobulin (the outcome) based on quintiles of $^{137}$Cs soil contamination (the exposure) and adjusted for gender and age of the child, year of the exam, and the interaction between year and soil contamination.”

In page 15, authors wrote as follows: “For IgA, the main effect of soil radiation and its interaction with the year of the exam were statistically significant ($p=0.039$, Table 2).”
Comments: This sentence is not clear. Is the P value of 0.039 for the main effect or for the interaction term? – I guess that it is for the interaction between the main effects of soil radiation and the year of exam.

Authors’ response: Thank you for pointing it out. Both effects were statistically significant. We added on page 16: “(p=0.034 and p=0.039, respectively, Table 2)”.

In p16, authors wrote as follows: “In addition, active smoking was significantly higher in older children residing in areas with the lowest 137Cs soil contamination.”

Comments: Authors should present a p value for the observed association

Authors’ response: Since we did not put into the text p-values for the other confounders mentioned earlier in this section, adding p-value for the active smoking is not necessary. Instead, we added comparison of percentages “(12.3% vs. 7.3%)”, page 17.

In p17, authors wrote as follows: “Controlling for age, gender, dog and cat exposure, IgE responses to indoor allergens were statistically significantly lower in children residing in the 116-265 kBq/m2 area (p=0.013, Table 5). There was no statistically significant difference for IgE specific to outdoor allergens.”

Comments: the statistical analysis used for calculating the p value of 0.013 is not clear. If authors conducted a t-test or a similar test, authors should describe whether the p value was adjusted for multiple comparisons or not.

Authors’ response: We added: Controlling for age, gender, dog and cat exposure in logistic regression analyses, IgE responses to indoor allergens were statistically significantly lower in children residing in the 116-265 kBq/m2 area (p=0.013, Table 5). We also added ‘Multivariable logistic regression” to the footnote of Table 5.

P values of 0.0067 for 137Cs in soil in Table 4: The method used for this p value is not entirely clear. Since the footnote says “t-test in linear regression models”, it is considered to be a sort of a p for trend. However, I am not sure whether such a small p value for trend can be obtained from such a U-shaped dose response.

Authors’ response: For both models was used F-test. The typo was corrected. Thank you.
In the footnote of Table 4, authors wrote as follows: “Multivariable generalized linear model adjusted for gender, age and ETS.”

Comments: It is not clear whether this description is for model 1 or model 2 or both. This footnote seems to be at variance with the description in ll20-22 in p13.

Authors should present a figure.

Authors’ response: The description “Multivariable generalized linear model adjusted for gender, age and ETS” refers to both models (the same superscript “a” is placed near adjusted means for both models in the heading of Table 4). For the cross-sectional analyses of 2008-2010 data we had information on several potential confounders; however, not all of them were included into final statistical models dependent on the results of tests for confounding. The text in the Statistical analyses section has been corrected accordingly “we considered additional adjustment for…”, page 13.

“Overtime” should be “over time”.

Authors’ response: Thank you for pointing it out. The changes have been made, pages 6 and 16.