

## **Author's response to reviews**

**Title:** Exposure from the Chernobyl accident had adverse effects on erythrocytes, leukocytes, and, platelets in children in the Narodichesky region, Ukraine. A 6-year follow-up study.

### **Authors:**

Eugenia Stepanova ([profstepanova@i.ua](mailto:profstepanova@i.ua))  
Wilfried Karmaus ([karmaus@sc.edu](mailto:karmaus@sc.edu))  
Marina Naboka ([marinavn1@yahoo.com](mailto:marinavn1@yahoo.com))  
Vitaliy Vdovenko ([xrisk06@yahoo.com](mailto:xrisk06@yahoo.com))  
Tim Mousseau ([mousseau@sc.edu](mailto:mousseau@sc.edu))  
Viacheslav M Shestopalov ([marinavn1@yahoo.com](mailto:marinavn1@yahoo.com))  
John Vena ([jvena@gwm.sc.edu](mailto:jvena@gwm.sc.edu))  
Erik Svendsen ([svendsee@gwm.sc.edu](mailto:svendsee@gwm.sc.edu))  
Dwight Underhill ([dmunderhill@gmail.com](mailto:dmunderhill@gmail.com))  
Harris Pastides ([Pastides@gwm.sc.edu](mailto:Pastides@gwm.sc.edu))

**Version:** 3 **Date:** 17 March 2008

**Author's response to reviews:** see over

## Point-by-point response to the reviewers' critique

We are grateful to the reviewers for their thoughtful insights into our research. We have incorporated many of their helpful suggestions to improve our manuscript. The reviewers recognized the significance of our work.

### 1. Reviewer's report

#### Minor Essential Revisions:

1. Please clarify the methods for obtaining the soil exposure estimates. Was this the average of several soil measurements in each town or only one soil measurement? Please clarify whether the dates of the soil measurements were the same as the dates of the hematologic measurements (1993-1998).

#### Response:

We added to the manuscript (page 6, para 1):

“Soil measurements represent the average of numerous measurements in each village over several years, in particular 1991-1993. These averages were officially named ‘certifications’ and documented in publications by the Ministry of Health.”

2. I suggest that the authors consider including some information about the prevalence of anemia in the Narodichesky region before the Chernobyl accident. This will help the reader to evaluate the findings.

#### Response:

No data is available on the prevalence of anemia in the Narodichesky region before the Chernobyl accident. In most regions, such data is normally not collected. For instance, in the US, only NHANES can provide some information; however, even a breakdown on states is questionable. We are also not aware that European bio-monitoring data, which was compiled only in the last ten years, covers all European regions.

3. The map shown in Figure 1 was difficult for me to evaluate. It seemed to show a combination of actual measured values in towns and modeled values for large areas. If so, this should be clearly explained. Also, perhaps it was because I was not using a color printer, but the shades used for the different levels of pollution seemed very similar. The shade for 10-20 kBk/m<sup>2</sup> looked like the shade for 555-1480 kBk/m<sup>2</sup>. I suggest several improvements to the map:

- a. Please show the location of Chernobyl.
- b. Please clarify whether the larger shaded areas are depicted that way because of actual measurements or because of modeling.
- c. There are only 35 towns listed but the text indicates that there were 38. Please show those 3 towns on the map. I could not find N. Dorogin or Hristinovka on the map.
- d. The spelling of the towns on the map differed from the spelling of the towns on Table 1. For example, Selec is Selets, Narodichi is Narodichy, Basar is Bazar, and Rossohy is Rozsohivske. Please try to be consistent.

#### Response:

Thank you for these helpful suggestions. We have improved the map and have included

- the distance to the Chernobyl site
- We added to page 8, para 2: “The average measurements shown for each settlement within colored zones are based on contamination modeling. Average values and the colored zones were retrieved from published data [21, 22].”
- We have included all settlements and their English names.

These corrections also revealed that some villages (which included a total of 3 children) were outside of the Narodichesky region. We added to page 8, para 2: “Three villages were at the border or outside of the Narodichesky region (Bogdanovka, Gunichu, and Ovruch, total of 3 children).” We decided not to eliminate these three children from the data.

4. Page 5 refers to Figure 1 but this figure is missing.

**Response:**

Figure 1 is the map, which was included.

5. In Table 1, the header notes that there were 1210 children but the numbers add to only 1209. Please clarify.

6. In Table 2, the number of children is noted to be 1251. Please clarify why it is different from Table 1.

**Response to question 5 and 6:**

We apologize, and thank you for noticing it. This was an oversight.

To determine the first participation (1,210 children), we sorted by number and year of participation and then excluded children who did not have a blood-draw in the first year. However, there were about 37 children without blood collection in the first year of participation, who had blood drawn in a consecutive year. Proc Mixed in SAS did it correctly. Hence, we changed to: (1) exclusion of children in a year with no blood-draw, then (2) first participation. This resulted in 1,247 children.

Now, all analyses of the full sample have 1,247 children and 4,981 repeated observations.

7. On page 10, the authors state that “53% of all red blood cell counts were below the critical clinical age-specific reference.” The authors may want to refrain from using the word “critical” in this context because it could be misinterpreted by readers. These are reference ranges but values outside the reference range may or may not be of critical importance depending on the clinical context.

**Response:**

We revised the description and eliminated the word ‘critical’ (page 10, para 4 and page 11, para 1):

“Fifty-three percent of all red blood cell counts were below the clinical age-specific reference values, as were 19% of the hemoglobin measurements, 0.7% of the platelet and 26.6% of the white blood cell counts. The proportion of values below the clinical limit decreased from 1993 to 1996. The white blood cell counts also showed improvement, but an increasing proportion of mild leukocytosis (white blood cell count > 11×10<sup>9</sup> cells per L; 1993: 0.1% , 1997: 3.2%, 1998: 2.0%).”

We also eliminated table 6 since we added one table (new table 6) and the old table 6 is not very informative.

8. On page 2, the abstract states that all children were **obliged** to participate in the yearly medical examination, but page 11 states that approximately 75% participated. Please explain why 25% did not participate if it was obligatory. Alternatively, please consider using a word other than obligatory.

**Response:**

We added to page 11, para 2:

“Officially, participation in the exams was obligatory. However, authorities did not enforce participation.”

**Discretionary Revisions:**

9. In the Discussion section of the manuscript, I would suggest that the authors consider extrapolating backwards from the values that were observed in 1993 to 1998 to estimate red and white blood cell counts, platelet counts, and hemoglobin values in 1986. Also, consider providing estimates of the dose of radiation based on the hematologic parameters.

**Response:**

We discussed these two suggestions. However, using extrapolated estimates will increase uncertainty. In addition, we do not know, whether the hematologic outcomes are linear responses to the soil contamination levels. Hence, we choose not to use this approach.

10. I would also suggest that the authors consider including additional discussion that compares these hematologic findings with other hematologic findings, such as those reported by Dr. Akleyev and colleagues (Akleyev AV, Kossenko MM, Silkina LA, Degteva MO, Yachmenyov VA, Awa A et al. Health effects of radiation incidents in the southern Urals. *Stem Cells* 1995; 13 Suppl 1:58-68.)

**Response:**

We appreciate this reference. The report showed a number of similarities to our findings. We added to page 15:

“However, similar effects were reported for the River Techa accident, which happened in 1957.[40] The exposure was characterized by gamma irradiation due to <sup>90</sup>Strontium and <sup>137</sup>Cs. As in children residing in Narodichesky region, adverse hematologic effects were detected. A normalization of the hematologic outcomes occurred only 13 years after the accident.[40]”

## 2. Reviewer's report

What I would have liked also is telling what the non-cancer effects were, lower IQ?, cardiac problems? Some of this is mentioned in the article [Ionizing radiation and children's health: Conclusions.](#) By Chris Busby and Alexandra Fucic. Acta Paed. 2006; 95 Suppl 453 pp 81-85.

### **Response:**

We referenced Busby et al. 2006 in the discussion section on page 14, last para.

Another aspect would have been interesting and might be done in future is to relate the number of platelets with the trombopoietin level. An inverse relation is proof of a toxic effect in bone marrow. Theoretically you cannot exclude a higher breakdown now.

### **Response:**

We agree that this would be of importance for future investigations. However, currently we do not have the data nor the funds to determine thrombopoietin.

Anyhow I like the article very much, it demonstrates a problem with hematopoiesis and I hope there will be a further follow-up. Discrepancies between recovery of the different classes of haematological cells, one more than the other, might be related to levels of different growth factors. For instance during maturation the number of erythrocytes might be decreased in favour of the number of platelets and vice-versa.

### **Response:**

We agree and this is another aspect for future analyses.

### **Few small remarks:**

Page 3: alinea 2 r.7 the second [from](#) [must](#) be deleted after 820 and also [a](#) a few words later.

**Response:** Thanks, we corrected this.

On page 11 alinea 2, r: 6 the word [in](#) between levels and of must be deleted.

**Response:** Thanks, we corrected this.

### 3. Reviewer's report:

1. Is the question posed by the authors new and well defined?

The question of a potential action of protracted exposure at low doses on the blood counts is scientifically interesting. Contradictory and incomplete statements are found in literature. The data deserve intensive attention. However, it cannot be read from the manuscript whether any hypothesis studied was defined before looking into the data. So, the statistics is explorative.

#### **Response:**

On page 5 we wrote:

“This motivated us to assess the association between residential soil density of <sup>137</sup>Caesium (<sup>137</sup>Cs) and hemoglobin concentration, and erythrocyte, platelet, and leukocyte counts in repeated measurements taken from 1993 to 1998.”

These are hypotheses since we are not fishing with multiple outcomes but focus on a few. It is needless to state that we expect adverse effects (i.e., reduction in cell counts).

2. Are the methods appropriate and well described, and are sufficient details provided to replicate the work? \see comments below

3. Are the data sound and well controlled?  
see comments below

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?  
The data are not fully given in the manuscript and there is no indication where they are or will be deposited.

#### **Response:**

The data is fully described in the text. We can share the data after careful examination of a proposal to share and after contacting internal human subject review boards, but cannot deposit data because of privacy protection. Is not acceptable to deposit data if individual participants can be identified.

5. Are the discussion and conclusions well balanced and adequately supported by the data?  
see comments below

6. Do the title and abstract accurately convey what has been found?

The title represents a statement of a result which, based on my other comments, is not yet justified. I should prefer a title like: Investigation of adverse effects on erythrocytes, leukocytes, and platelets in children in the Narodichseky region, Ukraine, as the consequence of the exposure from the Chernobyl accident. A 6-year follow-up study.

#### **Response:**

We do not see the difference to the current title:

“Exposure from the Chernobyl accident had adverse effects on erythrocytes, leukocytes, and, platelets in children in the Narodichesky region, Ukraine. A 6-year follow-up study.”

“As a consequence” equally posits some ‘causality’. Hence, we did not change the title.

7. Is the writing acceptable?

yes

General comments:

1. The study is an explorative statistical investigation of data which were obtained for other reasons and which were now used to interpret them in form of a follow-up study. The data existed before the hypotheses were formulated and they represent with a time-coverage from 1993 to 1998 just a subset of a larger time series of data. The actual timeframe was chosen somewhat arbitrarily according to budgetary reasons (page 11) and since this time contains data for children which were borne before and after the accident.

**Response:**

The study does not represent a statistical exercise. The general hypotheses that radiation may have adverse effects initiated the data collection. After the accident, the data was collected to monitor these effects on an individual level and not for other reasons. The clinical exams were not driven by the idea of conducting scientific analyses. However, scientific analyses were not excluded when the children were examined.

We agree that the actual time frame is chosen somewhat arbitrarily, as stated on page 11. However, we also stated on page 12, para 1: "The 1993 to 1998 period was chosen since all exams were established during this timeframe. Another advantage of this period is that it includes a comparable number of children who were born before the accident in 1986 and thereafter. This facilitates a comparison of exposures occurring after birth in children born before the accident in 1986 with exposure in children born after the accident (persistent <sup>137</sup>Cs exposure)."

Hence, given budget limitations, this time frame is appropriate.

2. The data are not really what one would like to have in a follow-up since the composition of the investigated population changed and evidently there are no systematic repetitions of individual blood counts but only random ones. There are no other parameters on the health status of the children available nor are there data on lifestyle or social conditions.

**Response:**

In epidemiology, we have different cohort studies, stable cohorts and dynamic cohorts. Both have advantages and disadvantages. This is a dynamic cohort.

There are a number of other health outcomes available (see page 6), but not other data on lifestyle or social condition.

However, we carefully explained that the design represents a natural experiment (page 13). Thus, confounding is unlikely since radiation was distributed randomly over all groups of the population. Hence, confounding is not a major issue.

3. There is a lot of statistics, but not any adequate medical or radioecological discussion of the blood data respectively of the exposures.

**Response:**

We agree that we present the state of the art to analyze such data. Our repeated measurement analysis also controls for baseline effects.

It is good practice in epidemiology to gather findings first, before making conclusions. In particular, it is wise to abstain from biological explanations. Biological explanations

make sense if there are more findings. We have presented knowns and unknowns in the introduction. In the discussion, we have emphasized what our study adds to the state of evidence.

- Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

5. General: One should be careful distinguishing between radiation exposure and what the authors call exposure or soil irradiation.

**Response:**

The word exposure comes from “exponere”, which means to put (pone) somebody in a setting in which he/she can intake foreign (ex-ternal) substances.

There is a lot of fruitless discussion on radiation exposure without gain in biological understanding. We present soil radiation or contamination density, which is by definition an exposure.

6. Page 3, paragraph 1: The statement “2,293 villages and towns with a population of 2.6 million inhabitants were contaminated” is not correct. Since the fall-out was spread all over the northern hemisphere, any statement about contaminations has to be made on the basis of a lower limit of deposition densities. Otherwise, everything gets confused. Usually such counts are done for deposition densities > 37 kBq m<sup>-2</sup>, but any other deposition density may be applicable, if it is well defined in a statement.

**Response:** The sentence is perfectly correct:

“In the Ukraine, 2,293 villages and towns with a population of 2.6 million inhabitants were contaminated.”

We focused on the Ukraine, not on other parts of the world.

7. Page 4, last 4 lines: Here the authors point to a critical aspect, namely the fact that repeated re-examinations may be biased due to the fact that more children are re-examined if their first result was above some critical value. I agree with that. However, I would not argue here with “regression to the mean” it could be simply due to a recovery from a health condition. An argumentation with regression to the mean is usually based on arguments about measurement uncertainties, rather than about changes in physiological conditions.

**Response:**

No, “regression to the mean” is a result of not examining the whole population. A follow-up exam will always find some variation, which is fine if a value can vary from a below threshold level to an above threshold level and vice versa. However, if only those with a below-threshold level are re-examined, the second value will always be higher than the first (and vice versa). This is what the direction “regression to the mean” addresses. See epidemiological or biostatistical textbooks.

8. Page 5, line 1: This statement is a claim, which is not justified by any reference.

**Response:**

The statement is correct. You cannot find any publication about this study in PubMed or any other search machine. There is no reference available, since there are no publications. One cannot expect that somebody reviewed the findings by the

“Chernobyl Sasakawa Health and Medical Cooperation Project” if there are no peer-reviewed publications to review.

9. Page 6, lines 1 and 2: I do not think that a strong correlation between the Cs-137 deposition density and the Cs-137 activity concentration in the body can be deduced from ref. 23. A correlation coefficient of 0.7 is not really a strong correlation. The Cs-137 deposition density may serve as a proxy for the individual exposure, but not as a very good one. It is well known in radioecology that e.g. the internal exposure depends strongly on the soil type one is living on. Also to type of living (rural versus urban) has a major influence on the internal exposure depending on the contribution of locally produced food.

**Response:**

In the scientific literature using human subjects, a correlation coefficient of 0.7 is considered a strong correlation. We also clearly state, that we used soil contamination as a proxy (page 6):

“Since residential and individual <sup>137</sup>Cs levels are highly correlated, we used the contamination in the residential area as an approximation of individual exposure [23].”

10. Pages 6 and 7: No clinical reference ranges are given for red blood cell counts, haemoglobin and thrombocytes, just lower limits. There is no indication whether the data represent normal ranges, what percentages of normal populations are covered. If one deals with adverse effects, I would expect some more detailed discussion in a medical sense what means “normal” and what might be the reasons for abnormalities in a population not exposed to radiation and on potential differences between different populations. Though the main conclusions of this manuscript are not based on a comparison of the data of the investigated population with normal values, I would expect a discussion of this point. I also wonder that no data on children from Ukraine living in areas with Cs-137 deposition densities below 37 kBq m<sup>-2</sup> were given or used.

**Response:**

Regarding hematologic radiation effects, there is ample evidence for leucopenia, thrombocytopenia, and anemia. Hence, only lower clinical references are important. We corrected the sentence on page 6/7 to:

“For descriptive purposes, values beyond clinical reference levels were defined as following [24]:”

The reference levels are based on clinical (pediatric) textbooks. Interestingly, red and white blood cell counts seem to have comparable levels in different human populations around the world. Thus, there is not a need to consider specific subgroups.

We choose not to establish our own references levels, since we cannot compete with standardized reference levels.

11. Page 7: The description of the statistical modelling is inadequate. Just to give references to software packages is not sufficient. The reader can expect a little presentation of what was really done with the statistics. It is also not said how normality of (which) data was tested. It is a well known fact that deposition densities and resulting radiation exposures are logarithmic normal distributed. The discussion of potential confounders is not exhaustive.

**Response:**

We carefully described the statistical methods (see below). We also referred to the textbooks that present the state of the art (page 7 and 8):

“Data were analyzed using the statistical package SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA). We had six repeated measurements of red and white blood cell markers. To determine whether the measurements agreed over time, we estimated intraclass correlation coefficients for the repeated measurements.[25]

To investigate the effect of residential  $^{137}\text{Cs}$  radiation, we used linear models for repeated measurements (PROC MIXED).[26] This statistical method compensates for the repeated measurements of each child over the six cross-sectional models (1993-1998). For all markers, we estimated the adjusted means, controlling for confounders.

For the statistical estimation, the regular maximum likelihood method was applied. This model requires that the random effects and the error vector are normally distributed, which was found to be the case for all markers. For the within-subject association, modeling started with an unstructured covariance model which required least constraints. For the repeated measurements, the initial model used serial correlation structures (Gaussian). Based on the Akaike information criterion (AIC), we could simplify the random effect to variance component, but could not simplify the repeated measurement matrix.

The residential measurements of  $^{137}\text{Cs}$  were ranked into five groups of nearly equal size (PROC RANK), with each rank treated as an indicator variable, facilitating assessing an exposure-response relationship. As confounding factors, the statistical models included gender, age, and year of measurement. Age was categorized into 18 indicator variables of one-year intervals, with the first group 0-1.5 years old. We used indicator variables for age and year of observation to investigate whether their relationships with the outcome variables were linear. If not, these indicator variables adjusted for non-linearity – for instance as we did with erythrocyte counts and age in girls. To determine whether the change in the distribution of the blood markers over the time of the observation depends also on the residential radiation level, we included an interaction term of time (year of measurement) and  $^{137}\text{Cs}$ .”

We clearly stated that we used quintiles for the soil contamination. There is no need to consider log-normal distribution for the predictor variable.

Using five groups (quintiles) is also conservative with regard to any measurement error, since quintile are less affected by specific measurement. What counts, is being one of five ranked groups.

Above, we have also described, why we do not need to consider additional confounders (natural experiment).

12. Page 7 and 8: I would expect some statements about the quality control and experimental uncertainties of the blood analyses.

**Response:**

On page 9, we describe the intraclass-correlation coefficients (ICC) for the four markers over the six years. ICC is defined as the between-subject minus the within-subject variance divided by the sum of the two variances. The ICC quantifies the proportion of total outcome variance that is due to inter-individual variation. Each repeated measurement is a control of the prior measurement. There is variation, which can be expected, however the results document substantial stability of the measurements.

13. Page 8, table 1 and figure 1: Table 1 and figure 1 are concordant. Different spelling for names is used frequently. Not all villages of table 1 are in figure 1, not all villages of figure 1 are in table 1. The Cs-137 deposition density for N. Dorogin (Noviy Dorogin) is 29 kBq m<sup>-2</sup> in table 1 and 169 kBq m<sup>-2</sup> in figure 1. The latter discrepancy affects the question whether the lowest deposition density is 29 kBq m<sup>-2</sup> or not (page 8). Also for Gresla (Grezya) the data are differing. Nothing is said about the uncertainties or the variability of the Cs-137 deposition densities in the villages. For Hristinovka (which is not given in figure 1) table 1 gives 879 kBq m<sup>-2</sup>. I saw already much higher data for this village: Health Physics 84 No. 4 (2003) 502.

**Response:**

We apologize for using different names. We have revised the map and made it congruent with table 1.

The value for N. Dorogin is 169 kBq/m<sup>2</sup> and the surrounding area is in the range of 100-185 kBq/m<sup>2</sup>.

The uncertainty is addressed by ranking the levels into five groups (quintiles, see above).

Hristinovka is now included in figure 1. It covers three contamination areas: 185 to more than 1,400 kBq/m<sup>2</sup>. Hence, values may vary.

We took the measurements documented for the village of Hristinovka.

The values in the paper by Handl et al., Physics 84 No. 4 (2003) 502, provide contamination levels of

River shore: 4,218 879 kBq m<sup>2</sup>.

Meadow: 738 kBq m<sup>2</sup>

Arable land: 680

Forest: 3,501.

The average value of 879 kBq m<sup>2</sup>, which we used, is closer to the values for meadow and arable land area than to river shore and forest, which is reasonable for this village.

14. Page 8, table 1: The sorting of the exposure in quintiles is problematic. If the Cs-137 deposition density is taken as a proxy for the radiation exposure, the quintiles used result in an extremely non-linear exposure coordinate. The quintiles also are apparently not based on the number of children (consequently quintile 3 and 4 are terribly unbalanced). The same is true for the number of repeated measurements. I cannot see the rationale behind the choice of the quintiles. Where they defined before starting the analysis or after looking into the data?

**Response:**

The use of quintiles is appropriate and the best approach. Quintiles are not used as 0-1-2-3-4 values but as indicator (dummy) variables (see page 7-8 of the manuscript). Hence, we compare children in quintile 1 to 4 with those in quintile 0.

We also explained that the quintiles were determined statistically. And we mentioned on page 8 that the groups are not perfect (18.9%, 20.1%, 9.3%, 31.1%, 18.9%). The reason is that some village contributed a large group of children that cannot be separated in the middle since they have the same soil exposure (also described in the text).

15. Figure 1, legend: The figure does not represent the quintiles used for grouping of exposures in this manuscript, but rather a more general classification of the Cs-137 deposition densities in the Narodici district.

**Response:**

On purpose, the figure does not represent quintiles, but the original values. The quintiles are presented in table 1. The map does not use a “more general classification of the Cs-137 deposition densities”, but average values measured in the village. Thus, the map facilitates a comparison of the exposure modeling in the surrounding area to the averages of the actual measurements in the villages.

16. Page 8 and general: It is a pity that children born in 1986 were omitted from the study. But, we have to live with it. At least, there are data for children born before and after the accident. But, no adequate discussion of the differences in the exposures of the children born before and after the accident is given. The Narodici area is in the western plume from the Chernobyl emissions and those children borne before the accident will have suffered a significant exposure due to short-lived radionuclides. This early exposure cannot be judged on the basis of the Cs-137 deposition densities.

**Response:**

We agree that the exposure to short-lived radionuclides such as <sup>131</sup>Iodine cannot be judged on the basis of the Cs-137 soil contamination. For this reason, we introduced the variable, born before vs. born after the accident. We stratified for this variable.

To clarify this, we added on

page 8:

“In addition, we investigated statistical differences in children born before or after the accident.”

and on page 11/12:

“The variable “born before” addresses two aspects, first, whether the child was exposed to other short-lived radionuclides immediately after the accident (for instance <sup>131</sup>Iodine, in children born before 1986), and, second, whether the child was exposed to <sup>137</sup>Cs during pregnancy and in its development (born after).”

17. Page 8, last sentence: I should like to see a histogram of the number of repeated measurements over the years.

**Response:**

We agree that it is necessary to document the number of repeated measurements and added to page 9:

“In 1993, we had 886 measurements; 786 in 1994; 787 in 1995; 783 in 1996; 1,030 in 1997; and 709 in 1998.”

18. Page 9, paragraph 1 and Figure 2: Again, a correlation coefficient of 0.72 does not indicate a high correlation. I see considerable differences in figure 2 between the red blood cell count and the haemoglobin. There is no adequate (medical or analytical) discussion of that.

**Response:**

A rank correlation of 0.72 is a high correlation. Hence, about 50% of the variance in one variable is explained by the other. There is not need and it is not justified to

come up with explanations for random differences between red blood cell count and hemoglobin.

19. Page 9, paragraph 2: This paragraph is unclear formulated with respect to the ICC and the 5 % confidence level. It does not tell the classes between which the correlations were looked at.

**Response:**

The description of the intra-class correlation is perfectly clear (see above, point 12). There are no classes. It is the name the method (literature is provided). ICC assesses the reproducibility for continuous measures – comparable to what kappa does for categorical scales. In our case, each child is its own class. ICC then measures the agreement in each child over the six years.

20. Page 9, 3rd paragraph: with respect to the choice of quintiles a critical remark has already been made. Now, there are mean values extracted for residential exposure classes which are very unbalanced. No indication of an uncertainty of the means is given. Always, if a mean is given some statement about its uncertainty as a square root of the variance should be given. See the ISO Guide for the Expression of Uncertainty in Measurement (ISO, 1995).

**Response:**

On page 9 we state the complementary role of table 4 and the figures 2 and 3. Table 4 provides information on statistical certainty (probability) and figures 2 and 3 illustrate the trends over time and exposures.

We have considered providing standard errors around the mean in figures 2 and 3. However, we found that the figure will be too crowded and impossible to read.

We did not identify that any year and exposure deviated from the overall trend.

Hence, table 4, in combination with figures 2 and 3, provides all relevant information.

Since one exposure level in one village changed, after our Ukrainian colleagues check the map (figure 1), we reran all analyses. We did no longer found a time\*exposure interaction for white blood cell counts, only a significant exposure effect. Now figure 5 provides the effect of the exposure. Table 6 provides the effect of the exposure for children born after the accident. In Table 5 and 6 we included the 5-95% values, which is preferable compared to mean and standard deviation.

21. Page 9, paragraph 4 and figure 2: The authors describe an increase of the data over the years. There is not an adequate discussion of the deep dip in the red blood cell counts in 1997, which appears also in the haemoglobin but not as strongly. In this context the question of quality control of the analytical procedures and about the tendencies in a control group with minimal exposure would be useful. It is astonishing, that the dip does not occur in the red blood cell counts of children living in the 266-310 kBq m<sup>-2</sup> regions, but it appears also in the haemoglobin.

**Response:**

We concur that there is no adequate discussion of the dip in 1996 and included the following text on page 10, para 1 of the revised manuscript:

“Figures 2 and 3 show lower values in 1996 in all exposure groups.”

And on page 12/13 of the revised manuscript:

“The results also show decreased erythrocyte counts and hemoglobin concentrations in 1996 in all exposure groups (Figures 2 and 3). It is possible that there was an instrument bias in 1996. Another explanation is that the winter in 1996

was colder. Thus, children may have suffered from a reduced supply of vitamins that are essential for the hematopoiesis. Since the decline occurred in all exposure groups, this represents a non-differential misclassification. Non-differential misclassifications tend to produce results that underestimate effects and do not present a threat to validity of the findings.”

22. Page 9, last paragraph: If there is a detailed discussion on an improvement over the years, the deep dip in figure 2 has to be discussed in detail, not just in one sentence.

**Response:** See above, point 21.

23. Figure 2 and 3: For all the mean values in the different years and exposure classes I request the authors to give uncertainties for the means. If this does not fit into the figures tables are needed.

**Response:** See above point 20.

24. Page 10, paragraph 1: The discussion about the highest **exposure group** having a lower radiation exposure than the second highest **exposure group** is strange and not justified by data. In addition, the problem is that the non-linear grouping by the quantile. If the authors are correct that the Cs-137 deposition density is a proxy for the radiation exposure, then they should not try to discuss that the proxy is wrong. If the proxy is wrong, all the statistics used is not adequate. Compare in this context also the discussion on page 12, paragraph 1. Figure 4 of ref. 23 demonstrates the weakness of this argument.

**Response:**

The main characteristic of a proxy is that it approximates the real value. Hence, there is a need to discuss the condition under which the proxy is more or less appropriate. Science is a pragmatic approach. Here we discuss that given the local setting of a village with more non-agricultural work (hospital, administration) the meaning of soil measurements may be different. We believe that this appropriate.

In additional analyses (no data shown), we excluded all villages with less than 50 children. The exposure effect did not change when we analyzed only children residing in larger settings.

Figure 4 of reference 23 does not demonstrate the weakness of this argument. On the contrary, if there is a close-to-perfect agreement between individual assessments and soil measurements, there is a need to discuss which factors may modify this agreement.

25. Page 10, paragraph 3: Here a comparison of the blood counts with critical clinical data is performed without a detailed discussion what is normal and what are potential reasons (confounders) for deviations from normal.

**Response:**

We have provided the lower limit of the clinical references on page 7 of the old manuscript (see point 10 of this reviewer). We stressed the setting of a natural experiment and the reason why no other confounders are needed above and in the manuscript.

26. Page 11, paragraph 2: On should not call this a cohort study. Evidently, there is no real control on the data if children leave and enter the **cohort** by chance.

**Response:**

See point 2 of reviewer 3.

We checked whether non-participation was related to prior blood values (see page 11, para 2 of the old manuscript).

27. Page 11, last paragraph: The authors state: "Originally, we planned to analyze radiation exposure using individual effective equivalent dose in millisievert (mSv), which incorporates total external and internal doses, including direct measurements with whole body counts [27], but there are uncertainties in the time periods in which the measurements were taken and their documentation. In addition, the individual effective equivalent dose was not calculated for every child and exposure models for biological dosimetry were changed several times." Currently we have more trust into residential exposure. This is not good epidemiological practice if the design of a study is not fixed before beginning it. It appears to be a general draw-back of this study that it is not clean from the design and a sound fixing of the conditions before beginning the evaluation.

**Response:**

The design of the study was fixed before the analyses. We only considered the appropriateness of two different exposure variables.

We believe that there is a general problem in understanding epidemiology, or what it should be. Words such as 'clean' are inappropriate.

28. Page 12, paragraph 1: Where does the statement "mean individual effective equivalent doses over increasing levels of residential exposure groups: 13.6, 19.6 mSv" come from? I could not substantiate it. About which time span are they integrated? If the numbers are meaningful, their origin should and according uncertainties should be given.

**Response:**

We clearly stated on page 11:

"Originally, we planned to analyze radiation exposure using individual effective equivalent dose in millisievert (mSv), which incorporates total external and internal doses, including direct measurements with whole body counts [27], but there are uncertainties in the time periods in which the measurements were taken and their documentation. In addition, the individual effective equivalent dose was not calculated for every child and exposure models for biological dosimetry were changed several times."

We have individual doses, but - compared to repeated soil measurement - we need more information to trust these values. We addressed this problem in the statement of page 11.

29. Page 12, last paragraph: The confounder discussion is not convincing. There is not any proof that potential confounders are randomly acting. The life-style in the Narodici region is very much different than in other places in Ukraine, e.g. rural self-sustained versus urban life. This criticism is meant for any discussion about deviations from normal. One might accept it only regarding the comparison of the 5 groups among each other.

**Response:**

We do not need to test this. There is never proof in science.

There is no evidence to show that, for instance the wind has distributed radiation in particular to those children who had a low birth weight. Hence, if the exposure is randomly distributed, for instance, independent of drinking during pregnancy or smoking, then there is no exposure-confounder association. If there is no exposure-confounder association, then there is no confounding.

The analytic work only compared the five groups of poor rural children to each other. The issue that we compared the value with clinical reference value was for descriptive purposes (stated on page 7), but not part of the statistical analyses.

30. Page 13, paragraph 1, last sentence: I cannot really call this study a randomized natural experiment. It is an explorative analysis of blood count data which were determined independently, non-systematic, and not randomized. Just one possible causing agent was looked at.

**Response:**

See above point 29.

31. Page 13, two last paragraphs: Here the authors derive far reaching conclusions which are not justified by the data and their analysis in this manuscript. In addition, they claim generally higher non-cancer morbidity for children in areas with chronic exposures. The biological parameters presented in this study do not support their statement.

**Response:**

We do not derive far-reaching conclusions. We do not claim higher non-cancer morbidity in exposed region. We generally state that the non-cancer morbidity is higher (world-wide), which is correct.

See for instance the reference suggested by reviewer 2 (Busby Fucic. Acta Paed. 2006; 95 Suppl 453 pp 81-85.)

In addition, the biological outcomes in this study show that problems in red and white blood cell and low platelet counts are very frequent.

32. Page 13, last paragraph: The statement about the different action of fission products and other radionuclides those from atomic bombs versus those from the Chernobyl accident lack any scientific foundation.

**Response:**

The characterization "lack any scientific foundation" shows the bias of the review 3.

We clearly cited:

Baverstock K, Williams D: The chernobyl accident 20 years on: an assessment of the health consequences and the international response. *Environ Health Perspect* 2006, 114(9):1312-1317.

Page 1312: "This dramatic contrast between the two incidents is in part due to the different types of radiation exposure, but both show that the effects of massive exposures to radiation are immensely complex. In comparing the health effects after Chernobyl with those after the atomic bombs, it must be remembered that apart from workers in or close to the power plant, the Chernobyl accident involved mainly exposure to radioactive isotopes, and the atomic bombs primarily involved direct exposure to  $\gamma$ -rays and neutrons."

There are many more publications available on this issue with similar conclusions; however, we focused on conclusions by known experts.

33. Table 2, year of first participation: Do the data mean that 20.7 % (1997) and 14.3 % (1998) of all observations were only for the time span 1997/1998 respectively 1998? For the 1998 data this would mean that 3.4 % of the children participating contributed in one year with 14.3 % of the observations?

**Response:**

It is correct that 3.4% of the children participated only in the last year. This does not mean that they contributed all observation in 1998, since other children continued to

participate in 1998. Mixed models can deal with the problem that the cluster size is “1” (no repeated measurement). However, in our case this affects 3.4% of the children.

34. Table 2, quintiles of the area irradiation: I calculate 9.1 % children participating for the region 266 – 310 kBq m<sup>-2</sup>. In addition, for the total number of observations I calculate slightly different number. These latter may be rounding errors. The term “area irradiation” is another confusing one.

**Response:**

1. We recalculated all numbers and made some corrections.
2. We change area irradiation to area contamination.

35. Table 1 and table 2: While in table 1 a total of 1251 children are mentioned, this number is 1210 in table 2.

**Response:**

We corrected this. See reviewer 1, point 5 and 6.

36. Table 3: The authors give medians and min- and max-values for the blood counts. If normality of the data can be assumed the arithmetic mean and a standard deviation would be adequate. It would be nice to have arithmetic means, medians and some well defined quantiles instead of the data presented in table 3.

**Response:**

Median and 5-95% value are more appropriate if the univariate distribution is not normal. (The multi-variable distribution (after controlling for age, sex) was normally distributed).

37. Table 3: What do the p-values of 0.00 indicate here?

**Response:**

It should have been <0.001. We corrected this.

38. Table 4: For what mathematical expressions are the quantities called numerator and denominator here. Where do the numbers 3692 and 3690 come from? There is no description which tests are being made here. Definitely, this table needs clarification until one can judge about it.

**Response:**

DF stands for degrees of freedom. Mixed models provide the numerator and denominator DF. We corrected table 4 and wrote numerator degrees of freedom and denominator degrees of freedom.

39. Table 5: It appears to me that these are estimated data for children borne after the accident. This is, however, nowhere stated. For what were the means adjusted? What means “lower” and “upper”?

**Response:**

Thank you, we corrected this.

40. Table 6: It is hard to accept the statements made by the authors on the basis of table 6 without a detailed discussion of “what is normal in Ukraine” or without other health indicators.

**Response:**

See point 10.

Additionally, we deleted the descriptive table 6, since we described the main findings in the text.

**- Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)**

41. Page 1, 3rd affiliation: typo, write **Radioecological**.

**Response:** Thanks, we corrected this.

42. Page 2, line 6 from bottom and elsewhere: The term **exposure time** is not a good one. The authors mean **time-integrated exposure**

**Response:** Residential exposure is correct. See point 5 of reviewer 3.

43. Page 3, paragraph 1, last sentence: write **It** has been exposed to radiation both externally and **internally**

**Response:** Good point; we used the revised sentence.

44. Page 4, paragraph 1: The deposition densities are not precisely given. I understand that it should read: **It** provinces with <sup>137</sup>Cs soil deposition densities of less than 37 kBq (1 kBq m<sup>-2</sup> = 1 Ci km<sup>-2</sup>) and deposition densities between 37 kBq m<sup>-2</sup> and 555 kBq m<sup>-2</sup>.

**Response:** The conversion is correct:

1 kilobecquerel (kBq) ~ 27 nanocurie (nCi)

1 curie (Ci) = 37 gigabecquerel (GBq)

1 curie (Ci)/km<sup>2</sup> = 37 (kBq)/m<sup>2</sup>

However, we clarified the second part of the sentence on page 4, line 4:

“provinces with <sup>137</sup>Cs soil contamination density of 37 kBq/m<sup>2</sup> or less (37 kilo Becquerel/m<sup>2</sup> = 1 Curie (Ci)/km<sup>2</sup>) and contamination densities between 38 and 55 kBq/m<sup>2</sup>.”

45. Page 4, line 15: Write **at** different points in time **at**

**Response:** Thank, we corrected “points”.

46. Page 5, line 14: I suppose it should read **The Human Subject Committee**

**Response:** Thank, we corrected “Committee”.

47. Page 5, line 4 and 3 from bottom: I do not know whether insects are of any importance in radionuclide transfer into the human food-chain. I would write: **It** where it is taken up by plants and animals and transferred into the local food supply.

**Response:** We removed the word “insects”.

48. Page 6, line 2 and elsewhere: I should not use the term “contamination” as it is used throughout this paper. Mostly is meant “Cs-137 deposition density”, “Cs-137 contamination level” may also be acceptable.

**Response:**

Our Ukrainian colleagues strongly emphasized the term contamination. Hence, we keep contamination. Deposition is a process, and contamination is the result.

49. Page 11, last paragraph: What do the authors mean with “biological dosimetry”? This term is completely misleading here, since normally it is used for deriving a dose from observations of biological parameters.

**Response:**

We changed it into individual dosimetry (p. 12).

50. Page 14, abbreviations: Write “1 Sievert = 1 Joule per kilogram” One could omit the abbreviations totally, since they are IUPAC convention or SI-units.

**Response:**

Not everybody is familiar with the abbreviations. We will include them.

51. Page 15, Acknowledgement: What means “(?)”.

**Response:**

We removed the question marks.  
This was a left-over from a question to a co-author.

52. Table 1: “Soil exposure” is a misleading term. Cs-137 deposition density would be better.

**Response:**

We changed it to “soil contamination”.

53. Table 2: Are the age group data for both sexes?

**Response:**

Data are provided below and do not indicate any differences. In addition, sex and age was controlled for in all models.

Sex and ages for all children.

There are no differences.

Table of agecls by Sex

agecls(age in 1-year classes: 1st 0-1.5, then		Sex(Sex)		Total
Frequency,	Col	Pct		
1	50	46	96	
2	43	59	102	
3	50	59	109	
4	51	53	104	

5	,	51	,	54	,	105
6	,	47	,	51	,	98
7	,	11	,	9	,	20
8	,	65	,	55	,	120
9	,	70	,	55	,	125
10	,	62	,	48	,	110
11	,	59	,	45	,	104
12	,	29	,	28	,	57
13	,	23	,	29	,	52
14	,	7	,	11	,	18
15	,	5	,	2	,	7
16	,	4	,	2	,	6
17	,	8	,	4	,	12
18	,	0	,	2	,	2
Total		635		612		1247

The FREQ Procedure

Statistics for Table of agec1s by Sex

Statistic	DF	Value	Prob
Chi-Square	17	16.6727	0.4767
Likelihood Ratio Chi-Square	17	17.5575	0.4173
Sample Size = 1247			

Sex and ages for all repeated observations.  
There are no differences.

Table of agec1s by Sex

agec1s(age in 1-year classes: 1st 0-1.5, then		Sex(Sex)		Total
Frequency,	Col Pct	F	M	
1		50	48	98
2		85	95	180
3		115	129	244
4		132	139	271
5		139	157	296
6		150	155	305

7	5.83	6.43	298
8	5.52	6.47	372
9	7.39	7.55	409
10	8.25	8.17	424
11	8.75	8.26	437
12	9.45	8.05	400
13	8.60	7.43	429
14	8.95	8.26	360
15	7.58	6.85	270
16	5.80	5.02	112
17	2.26	2.24	70
18	1.32	1.49	6
Total	2571	2410	4981

The FREQ Procedure

Statistics for Table of agec1s by Sex

Statistic	DF	Value	Prob
Chi-Square	17	20.9639	0.2279
Likelihood Ratio Chi-Square	17	21.2181	0.2167
Sample Size = 4981			