Author's response to reviews

Title: Pollutant effects on genotoxic parameters and tumor-associated protein levels in adults: a cross sectional study.

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Version: 3 Date: 7 May 2008

Author's response to reviews: see over
Covering letter

The editors wrote: However, the manuscript still lacks in clarity and style and therefore needs further revision. Authors should only capitalize the first word, and proper nouns, in the title (remove unnecessary capitals; Pollutant effects on genotoxic parameters and tumor-associated protein levels in adults: a cross sectional study).
Answer: These changes were made.

The editors wrote: In the Abstract, the Background section should provide a justification for the study, not just a description of the local area.
Answer: This section was changed from

“Flanders is densely populated, with intensive traffic, important metallurgic and petrochemical industries and farming with intensive use of pesticides close to habitation.”

to

“This study intended to investigate whether residence in areas polluted by heavy industry, waste incineration, a high density of traffic and housing or intensive use of pesticides, could contribute to the high incidence of cancer observed in Flanders.”

The editors wrote: Second sentence is a run-on (authors are packing too much information into one sentence), must be split, suggestion: Subjects were 1583 Flemish residents, aged 50-65, from nine different areas with varying/dissimilar pollution levels. Cadmium, lead, p,p’-DDE, hexachlorobenzene, PCBs and dioxin-like activity (Calux test) were measured in blood, and cadmium, t,t’-muconic acid (ttMA) and 1-hydroxypyrene (1-OHP) in urine?.
Answer: The sentence was changed to “Subjects were 1583 residents aged 50-65 from 9 areas with different types of pollution. Cadmium, lead, p,p’-DDE, hexachlorobenzene, PCBs and dioxin-like activity (Calux test) were measured in blood, and cadmium, t,t’-muconic acid and 1-hydroxypyrene in urine.”

The editors wrote: Please try to use abbreviations as sparingly as possible in the abstract.
Answer: Abbreviations were removed in the abstract.

The editors wrote: The Conclusions need to be rephrased to indicate how the results are interpreted.
Answer:
Implications for cancer risk were added to the conclusions.
Conclusions in the abstract have been rephrased to:
“Levels of internal exposure, and residence near waste incinerators, in cities, or close to important industries but not in areas with intensive use of pesticides showed positive correlations with biomarkers associated with carcinogenesis and thus probably contribute to risk of cancer”.

Conclusions in the main text were rephrased to:
“Although we found the levels of genotoxic parameters and of tumor-associated proteins quite homogenous in Flanders, residence near waste incinerators, or in cities, or close to important industries showed a positive correlation with biomarkers associated with carcinogenesis. **Thus, residence in those areas probably contributes to the risk of cancer.** Whereas for the 'fruit area' with intensive use of pesticides favourable results were obtained, in some other rural areas the levels of these biomarkers were not lower than in the rest of Flanders. In addition, we observed more intense biological effects occurring in persons residing near point sources of pollution. This suggests that the relatively high values of internal exposure measured in most people in Flanders, independent of their area of residence, are in part due to emissions of point sources, the effects of which, in terms of both internal exposure and biological effects, can only be detected in people residing at short distance. Levels of internal exposure occurring in the general population in Flanders showed positive correlations with biomarkers associated with carcinogenesis and **probably contribute to the risk of cancer.”**

**The editors wrote:** Abstract is now a few words over the limit.  
**Answer:** The abstract now has a word count of 350.

**The editors wrote:** The Background (p.4) should explain how this part of the biomonitoring study is linked to other parts, preferably with literature citation of the components, e.g. the results on dioxin already is available along with reference 5.  
**Answer:** This text was added to explain the relationship of this part with the other parts of the Flemisch Environment and Health Study: “This program comprised measurements of internal exposure on each of these age groups. Concerning the neonates, follow-up studies concerning neuropsychic development, asthma and allergy were performed. Concerning the adolescents, effect biomonitoring comprised measurements of hormone levels in boys and sexual maturation of boys and girls. For adults, effect biomonitoring entailed genotoxic tests and measurements of tumor associated protein levels (reported in the present paper) and also measurements of the expression of selected genes. Also, for both adolescents and older adults a study was performed on the relationship between carcinogenesis-related biological effects and 36 polymorphisms in 23 genes involved in xenobiotic metabolism, DNA repair and oxidative stress. This biomonitoring program (2002-2006) already resulted in several publications: internal exposure to pollutants in adolescents was described by Schroyen et al.; dietary exposure to dioxin-like compounds in adolescents, mothers aged 18 to 44 years and adults aged 50 to 65 years was reported by Bilau et al.; the association of thyroid hormone concentrations with levels of organochlorine compounds in cord blood of neonates was reported by Maervoet et al. A detailed report on the internal exposure to pollutants of the adults participating in the study reported here will be published elsewhere. All public information on the project as well as an overview of these data on internal exposure is already available on a website. Several publications on yet unpublished results are in preparation. “
The editors wrote: Use ?pollution level? in place of ?pollution pressure? (perhaps this is a language issue but ?level? seems to make more sense).

Answer: “Pollution pressure” was replaced in the manuscript by “level and type of pollution” or to “pollution level”.

The editors wrote: Pg 6, remove semicolon (;) in ?Ghent port? has mainly metallurgic industries; however,??.

Answer: Semicolon was changed to comma.

The editors wrote: Last paragraph, ?Flanders, is a industrial..? add ?n? to ?a? (Flanders, is an industrial..), also add ?an? before ?area? (As an area with a high..).

Answer: These changes were made.

The editors wrote: Please clarify what is meant that rural areas are ‘not devoid of environmental pollution’ and ‘higher exposures due to certain local habits’.

Answer: The text was changed to clarify: “These rural areas are, in Flanders, certainly not devoid of environmental pollution and might (see discussion) even show some higher exposures [5,24] due to certain local habits such as burning waste, inappropriate use of pesticides and consumption of self-grown food [25].”

In the discussion this is further elaborated:” Remarkably, results for rural Flanders were not significantly better for any of the biomarkers of effect than for the rest of Flanders. For micronuclei and DNA-strand breaks, relatively high values were observed, significantly elevated above those observed in the area with the lowest value. Correspondingly, relatively high levels of internal exposure to some pollutants were observed in residents of rural areas in Flanders in both the pilot and the subsequent biomonitoring studies in Flanders. Indeed, in the Flemish pilot biomonitoring study, women aged 50-65 residing in the rural area of Peer had higher levels of cadmium and dioxin-like activity in their blood or serum, and higher levels of cadmium and 1-hydroxypyrene in their urine than women residing in the city of Antwerp [24]. Also, adolescents residing in rural areas had blood levels of cadmium and organochlorine pollutants above Flemish reference values [5].

Concerning effect biomarkers, in the rural area of Peer men were found to have a lower sperm quality and lower testosterone levels (Dhooge et al 2007), and women aged 50-65 showed higher HPRT mutant frequencies than residents of the industrial city of Antwerp. Taken together these results indicate that, at least in some respects, internal exposure and biological effects related to environmental pollution are no less in rural areas than in other Flemish areas. We do not know how this comes about, except for the fact that certain local habits such as burning waste, inappropriate use of pesticides and consumption of self-grown food[25] could be involved. Indeed, consumption of self-grown vegetables, which is more frequent in rural areas, has been associated with a higher exposure to pesticides and cadmium and also with lower sex hormone levels and with lower sperm quality (discussed by Dhooge et al. [25]).”
The editors wrote: Please explain all abbreviations when first used (and also in the list of abbreviations).
Answer: abbreviations are now explained at first use, and also in the list.

The editors wrote: In pg 7, second to last sentence, check spelling (pollutant).
Answer: This was corrected.

The editors wrote: In pg 8, the following sentence could be modified ?All laboratory analyses were performed blindly in ??, perhaps substitute ?using blinded methods? or ??were performed by blinded researchers??.
Answer: This was changed to “using blinded methods”

The editors wrote: Please rephrase the first sentence in the first full paragraph on p. 11.
Answer: This sentence has been edited to: “The following is a summary of the data on which our selection of biomarkers of effect was based”.

The editors wrote: Numbers up to ten should be spelled out.
Answer: on p11. “3 years “ was changed to “three years”, on page 9 “10 times” was changed to “ten times”.

The editors wrote: The first paragraph on pg 22 needs to be broken up (really one run- on sentence).
Answer: This sentence was broken down to: “Both Antwerp and Ghent levels of CEA and of micronuclei were significantly elevated above the level observed in the area with the lowest value. In Ghent DNA strand breaks were significantly increased above the level observed in the area with the lowest value, as well as above the level observed in the rest of Flanders.”

The editors wrote: Second paragraph, suggestion: ?Remarkably, results for rural Flanders were not significantly better for any of the biomarkers of effect than for the rest of Flanders. For micronuclei and DNA-strand break,?? (period after ?Flanders?, comma after ?break?).
Answer: this sentence was changed to “Remarkably, results for rural Flanders were not significantly better for any of the biomarkers of effect than for the rest of Flanders. For micronuclei and DNA-strand breaks, relatively high values were observed, significantly elevated above those observed in the area with the lowest value.

The editors wrote: In pg 23, suggestion ?In terms of DNA strand breaks, a difference of almost a factor of three was observed. This suggests that the relatively high values of internal exposure measured in most people in Flanders, independent of their area of residence, are in part due to emissions of point sources. Although these do not lead to
detectable increases in internal exposure nor to detectable biological effects in the larger area where they are located, do lead to significantly higher internal exposure and associated biological effects in people residing at short distance.

**Answer:** In terms of DNA strand breaks, a difference of almost a factor of three was observed. This suggests that the relatively high values of internal exposure measured in most people in Flanders, independent of their area of residence, are in part due to emissions of point sources. Although they do not lead to detectable increases in internal exposure nor to detectable biological effects in the larger area where they are located, they do lead to significantly higher internal exposure and associated biological effects in people residing at short distance.

**The editors wrote:** Last sentence in pg 25, add ?a? before ?number?.

**Answer:** Number was changed to “the number”.

**The editors wrote:** In pg 26, remove ?well? in ?So it is well possible..?.

**Answer:** The word “well” is now removed.

**The editors wrote:** In pg 27, spell check (?indoor stoking?).

**Answer:** Indoor stoking refers to “burning of materials in a stove”, so this was not a spelling mistake. To avoid confusion however, this was changed to “indoor stoking of diverse organic materials”

**The editors wrote:** In references 42 & 51, check format (source unclear).


**The editors wrote:** Since tables were included in the text, keep titles above each table (though this is not stated in the instructions).

**Answer:** Titles were moved to above each table.

**The editors wrote:** Please check the unit for lead in Table 2.

**Answer:** The error in the unit of Lead concentration was corrected to µg/L.

**The editors wrote:** In figures, minimize white space as much as possible. They should be designed so that they can be considerably reduced in size, so the numbers and legends need to be in much larger size.

**Answer:** The figures were redone, to minimize white spaces and maximize character size.
The editors wrote: Please review text for missing punctuation (particularly commas and/or periods) in the first few sections.

Answer: The text has been reviewed and edited for missing punctuation.

Also, some typing errors have been corrected.