

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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Author's response to reviews: see over

Covering letter

The editor suggested abbreviating the title, e.g., to "Pollutant Effects on Genotoxic Parameters and Tumor-Associated Protein Levels in Adults: A cross sectional Study". This was done.

The editor wrote: The abstract should contain some more background on the exposure setting, and the conclusions need to be expanded within the permissible word count. Further on the editor also wrote: These changes must also be reflected in the abstract.

Answer: In the abstract, the text on the background was changed to: *"Flanders is densely populated, with intensive traffic, important metallurgic and petro-chemical industries and farming with intensive use of pesticides close to habitation."*

In the abstract, the text on the Results was somewhat condensed and the text on the conclusions was changed and expanded to:

"Levels of internal exposure, and residence near waste incinerators, or in cities, or close to important industries but not in areas with intensive use of pesticides showed positive correlations with biomarkers associated with carcinogenesis. In some rural areas, the levels of these biomarkers were not lower than in the rest of Flanders. "

The editor wrote: The Background section is too brief and does not reflect the overall purpose and the a priori hypotheses to be tested. What was the research strategy, and how did it lead to the design of the present study?

Answer: The background section was expanded substantially from 209 words to 399 words to reflect the overall purpose, the research strategy and the a priori hypotheses to be tested.

Following text was included:

"Because of the important role of somatic mutations in carcinogenesis {Deman, 2001 188 /id}, we included effect biomarkers for genotoxicity: oxidative DNA damage measured through a metabolite in the urine and DNA strand breaks and micronuclei measured in peripheral blood cells. As we did previously [4], we also included tumor-associated protein levels measured in blood. In our present study we measured prostate-specific antigen (PSA), carcinoembryonic antigen (CEA) and p53 levels (p53) in serum. During the long latency period after initiation of carcinogenesis and/or under the impact of tumor promoters, some cells in the body might express certain aspects of the tumoral phenotype, which may result in the release, in body fluids, of macromolecules associated with this phenotype being released into body fluids. An increased concentration of such molecules in body fluids might, to some extent, reflect a higher exposure to cancer-inducing or cancer-promoting agents [6-10] or an increased risk of cancer [11-18]. Using these biomarkers associated with carcinogenesis or with risk of cancer we aimed at investigating whether residence in Flemish areas with specific types of pollution pressure, in particular stemming from heavy industry, waste incineration, a high density of traffic and housing or intensive use of pesticides, could contribute to cancer risk.

Our project also intended to test the hypothesis that low levels (such as these occurring in the general population in Flanders) of internal exposure to pollutants known or suspected to cause cancer were associated with increases in levels of oxidative DNA damage, of DNA strand breaks, of micronuclei or of tumor associated proteins."

The editor wrote: the Methods section needs to describe why the areas and populations were selected in light of the overall hypothesis to be tested.

Answer: The section on the selection of study areas was expanded from 351 words to 535 words to explain why areas and populations were selected. A table giving more information on characteristics and emission data for the 9 study areas was provided (the new table 1).

This section now reads:

“As areas with a high level of pollution pressure stemming from heavy industry, the harbour areas of Antwerp (“Antwerp port”) and Ghent (“Ghent port”), and the “Albert canal” and “Olen” industrial basins were chosen. At the start of the project the ports of Antwerp and Ghent were considered together as one industrial zone, but in view of differences in type of industry and in view of the results obtained (e.g. for the adolescents,[20]), we thought it adequate to consider the results for the ports separately. “Antwerp port” is an important industrial site with huge petrochemical industries, chemical and plastic industry and production of pesticides (n=163 adults between 50-65 years old recruited in this area); however, only part of the participants from Antwerp port, such as those from Burcht, resided in zones that have an important exposure to industrial emissions, whereas others, from the municipality of Beveren, resided at a distance of several kilometers to the west of the main industrial installations. “Ghent port” has mainly metallurgic industries; however, all adult participants (n=36) resided in the municipality of Evergem at the south west of the main industrial installations. “Albert canal” is an industrial zone with chemical and plastic industries and production of electricity amidst rural areas (n=196). “Olen” is an industrial zone with a large non-ferrous smelter, chemical, plastic and automobile industry amidst rural areas (n=203). As areas with a high level of pollution pressure stemming from a high density of traffic and housing the cities of Antwerp and Ghent were chosen. “Antwerp”, the largest city in Flanders, is a industrial city with 404,000 inhabitants and very dense traffic (n=197 adults between 50-65 years old recruited in this area). “Ghent”, the second largest city in Flanders, is a smaller industrial city with 213,000 inhabitants (n=198). As area with a high level of pollution pressure stemming from intensive use of pesticides, the “fruit area“ around Sint Truiden was chosen, comprising a rural region with intensive apple or pear cultivation (n=193). As area with a high level of pollution pressure stemming from waste incineration, neighborhoods close to waste incinerators in 6 municipalities, spread out over the whole of Flanders (n=198) were chosen; these neighborhoods comprised a limited area, with a mean surface of 6.2 km² mainly under the wind of a waste incinerator. For comparison we included “rural areas”. These rural areas are, in Flanders, certainly not devoid of environmental pollution and might even show some higher exposures due to certain local habits. However, with a lower population density, less traffic and no local heavy industry they constitute an interesting point of comparison. In our study “rural areas” comprised 24 municipalities, spread out over 9 contiguous areas in the western half of Flanders with no highways and no industries reported in the emission inventory of the Flemish environmental protection agency (n=199).

Table 1 summarizes characteristics, including some emission data, of the different study areas and figure 1 shows their position in Flanders. The surface of the studied area is 3,036 km², corresponding to 22% of the total surface of Flanders (13,521 km²). The 65 selected municipalities correspond to 20% of the total Flemish municipalities. Except for ‘rural Flanders’ and for ‘waste incinerators’, all study areas were contiguous geographical entities.

The new table 1 reads:

<i>Area of interest</i>	<i>Surface (km²)</i>	<i>Number of inhabitants*</i>	<i>Characteristics and sources of pollution</i>	<i>Pesticide use (kg/km²)**</i>	<i>Industrial Emission to air (per year)***</i>	<i>Industrial Emission to surface water (per year)***</i>
<i>Agglomeration of Antwerp</i>	<i>81</i>	<i>404241</i>	<i>Metallurgic industry, large non-ferrous smelter, important highways, huge</i>	<i>13.3</i>	<i>80 kg PAH</i>	

			traffic			
Antwerp Harbor	179	64510	Huge petrochemical industries, chemical and plastic industry, production of pesticides	117	15 kg Cd 837.5 kg PAH 93729 kg benzene	15 kg Pb 4.03 kg Cd 4.1 kg pesticides 3.2 kg PAH 640.9 kg benzene
Agglomeration of Ghent	100	213025	Metallurgic and automobile industry, intensive traffic	27.7	150 kg Pb	24.7 kg Pb
Ghent Harbor	202	65554	Mainly metallurgic industries	99.2	34500 kg Pb 542.1 kg Cd 1823 kg PAH 225 kg benzene	1690 kg Pb
Area with fruit cultivation	362	95829	apple and pear orchards: more than 10 hectares per km ²	617		
Rural area	1181	153770	Less than 250 inhabitants per km ² . No highways crossing the municipalities. No industries reported in the emission inventory of the Flemish Environmental protection agency.	233		
Industrial zone of Olen	183	68068	Large non-ferrous smelter, delineated in function of modelled, calculated immission of at least 0.9ng lead per m ³ from the non-ferrous smelter. Chemical, plastic and automobile industry. Rural areas	34.6	810 kg Pb 87 kg Cd 4050 kg benzene	88 kg Pb 39.71 kg Cd
Albert canal industrial zone	711	64763	Chemical and plastic industries, rural areas, production of electricity	20.8	430 kg benzene	59.5 kg Pb 67.36 kg Cd
Surroundings of incinerators	37	56405	Waste water and waste treatment. Delineated in function of modelled, calculated immission of at least 1.2mg smoke per m ³ from the waste incinerator	80.1	14 kg Cd	48.7 kg Pb 1.89 kg PAH
Total	3036	1186165			35460 kg Pb 658,1 kg Cd 2740.5 kg PAH 98434 kg benzene	1925.9 kg Pb 111.1 kg Cd 4.1 kg pesticides 5.09 kg PAH 640.9 kg benzene

* Based on the number of inhabitants of 1998; ** Belgian ministry of Economic affairs and Institute for Social and Economic Geography, Catholic University of Leuven; *** Emissions of pollutants reported by the emitting companies themselves to the Flemish Environmental Protection Agency (www.vmm.be).

The editor wrote: The same applies to the genotoxicity markers and other outcomes, why were they considered particularly useful for this study?

Answer:

A new section on the selection of biomarkers of effect was introduced.

This section reads:

“Selection of biomarkers of effects

Follows a summary of the data on which our selection of biomarkers of effect was based.

Increased serum levels of Prostate Specific Antigen (PSA) are found in the vast majority of men with prostate cancer. A Finnish study found, for men under 65 years of age, a sensitivity of 93% and a specificity of 96% for PSA as a diagnostic test for prostate cancer using a limit value of 4 ng/ml [25]. Serum PSA level also allows to assess risk of prostate cancer. Of men with a normal PSA level of 2.1 to 4.0 ng/ml, 1.6% to 5.5% were diagnosed with prostate cancer within one year, whereas only 0.06% to 1.02% of men with a PSA level of 1.1- 2.0 ng/ml did so and only 0.01% of men with a PSA level lower than 1 ng/ml was diagnosed with prostate cancer in a period of 3 years [26]. Increased serum PSA levels were found in men exposed to various pollutants or toxic substances (see discussion).

Serum levels of Carcinoembryonic Antigen (CEA) are increased in many types of cancer, in smokers and in persons exposed occupationally or environmentally to pollutants [27-29].

Serum CEA levels measured years before clinical diagnosis showed a positive correlation with risk of cancer {Jarvisalo, 1993 353 /id;Palmqvist, 2003 79 /id}. In our pilot biomonitoring study [4] we found a positive correlation between CEA serum levels on the one hand and blood lead levels, an index reflecting internal exposure to several pollutants, and HPRT mutant frequency in peripheral blood cells on the other.

Increased serum levels of mutated p53, or of anti-p53 antibody, have been found in cancer patients {Attallah, 2003 388 /id} and in workers exposed to carcinogenic substances [6,9,10,33,34]. Anti-p53 antibodies or increased levels of mutant p53 protein can predate the diagnosis of cancer [35-37]. In our pilot biomonitoring study we found a positive correlation between anti-p53 antibody levels on the one hand and blood lead levels and an index reflecting internal exposure to several pollutants on the other At the start of the project we gave preference to p53 serum level above anti-p53 antibody serum level, because it was reported that increased levels of p53 would occur in more persons than increased levels of anti-p53 antibodies [6].

Measurement of the number of micronuclei per 1000 binucleated peripheral blood cells (micronuclei) is one of the best established biomarkers of chromosome damage [38]. An increased frequency of micronucleated cells is a biomarker of genotoxic effects that can reflect exposure to agents with clastogenic (chromosome breaking; DNA as target) or aneugenic (aneuploidogenic; effect on chromosome number; mostly non-DNA target) modes of action [39]. An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans [40]. This test reflects genetic damage that occurred over a longer period of time and is relatively stable over a 12 month period [41]. Sensitivity is rather low. We estimated that difference of the order of 30% is needed in order for the test to reach a power of the order of 0.9 in discriminating between two groups of 100 persons [42]. The micronucleus test is simpler, cheaper and is less time consuming than the chromosome aberration assay that was too expensive in the context of our project.

Measurement of DNA-strand breaks in peripheral blood cells in terms of the median value of the percentage of DNA in the tail using the comet assay was selected to reflect recent damage to DNA. The alkaline single-cell gel electrophoresis (SCGE) or Comet assay is an easy, reliable and rapid method to detect DNA single strand breaks, alkaline labile sites and delayed repair sites of DNA. It is able to detect low levels of DNA damage, requires small sample sizes and has a rather low cost [42]. The inter and intra-assay coefficients of variation are of the order of 20% [43]. We estimated that the assay has a power of the order of 0.90 to discriminate between two groups of about 30 persons showing a mean difference of 10% [42].

*The measurement of 8-hydroxy-deoxy-guanosine in urine per gram creatinine (HDG.) was selected to assess recent oxidative stress. 8-hydroxy-deoxy-guanosine is excreted in the urine after excision of this mutagenic oxidative adduct from DNA and reflects the amount of oxidative damage to DNA and its repair. Its measurement **is affordable and** has inter- and intra-assay coefficients of variation of 8 to 24% [44]. We estimated that the test has a power of the order of 0.9 to discriminate between two groups of about 100 persons showing a mean difference of 12% [42].”*

The editor wrote: The Conclusions can then be sharpened in the light of the a priori anticipations and the method interpretations

Answer: In the discussion, the section on “differences in association with area of residence” was expanded (see below in the answer to remarks of the Reviewers), a new paragraph was added to the

section on “Exposure effect relationships” (see below in the answer to remarks of the Reviewers) and the conclusions were expanded and sharpened.

The conclusions now read:

“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. 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.”

The first reviewer wrote: Much too much emphasis is put on the issue of statistical significance and control of confounding.

Answer: In the results several sentences repeating the control for confounding were deleted. A row mentioning confounding factors taken into account was added to table 4.

The first reviewer wrote: Some vocabulary could also be checked, such as the use of "pressure" when in fact most probably referring to "density" or the use of the politically correct "gender" when in fact referring to "sex" because dealing with biology and not with culture.

Answer: We checked the use of the word “pressure” and remain convinced that it is the correct term here.

The term gender was replaced by the term “sex”

The first reviewer wrote: I am not sure one has to say twice that the 2 portuary areas originally considered as 2 zones only became one. It is perfectly honest to say so, but may be it is really needed.

Answer: We remain convinced that, in the sake of clarity, it is preferable to clearly mention that the port area was split, this to explain why less participants reside in each of the two separate areas.

The first reviewer wrote: In the analysis, when looking at and/or controlling for smoking, 2 dimensions could and should be considered: daily dose and duration. In terms of etiology for cancer, duration carries much more weight than daily dose and by lumping the 2 together, there may be some dilution and therefore poorer control of confounding. May be the 2 dimensions of control could be kept; otherwise it could worth looking at which difference does it make to control just for duration rather than for the total number of cigarettes smoked over a life-time.

Answer: We performed statistical analyses with duration of smoking as confounding factor, and also with both duration of smoking and daily dose of smoking as confounding factors.

There was not much difference in the results, so we preferred, as to correction for confounding in the case of effect parameters that might reflect exposures over a long period of time, to use lifetime smoking in terms of units (cigarettes, cigars, pipes) smoked during the entire lifetime as we did in our original manuscript. .

The first reviewer wrote: Some definition need to be given and then one should check if they really apply to the case at hand. For ex. authors should define "confounding" and also simple terms such as "normal" in the first paragraph on biomarkers of effect or more technically on the same page explain the meaning of "at least 30% DNA".

Answer:

-Confounding was defined as follows:

"A confounding factor is a variable that correlates with both the dependent and the independent variable and controlling for confounding is necessary to avoid false positive conclusions that the dependent variable is correlated with the independent variable."

-In the first paragraph on biomarkers of effect the meaning of the term "normal" was clarified as follows:

"The within-run precisions of the methods applied on samples in the normal, non-pathological range were..."

-The sentence containing "at least 30% DNA" was clarified as follows:

"In those positive controls at least 30% of DNA had to migrate into the tail area to control the functioning of the electrophoresis."

The first reviewer wrote: more background should be given along with references to explain why some PCBs and not others were selected to "reflect exposure to PCBs in general"

Answer: The sentence in question now reads: *"As we did in our previous studies [4, 20], we used the parameter "marker PCBs" (the sum of serum concentrations of PCBs occurring in the highest concentrations: PCB 138 + PCB 153 + PCB 180) in our analysis to reflect exposure to PCBs in general."*

The first reviewer wrote: You should not write about "questioned exposure" but rather about "exposure as assessed from questionnaire".

Answer: The relevant changes were made.

The first reviewer wrote: When you discuss the CEA level, do not forget to mention also ovarian cancer.

Answer: We searched the literature for data indicating a link between CEA levels and risk of ovarium cancer, but did not find such data. CEA levels are known to be elevated in ovarium cancer patients, as in many other types of cancer, but no link with increased risk of subsequent ovarium cancer has been described for women with somewhat higher CEA levels.

The first reviewer wrote: be sure categories are clearly non overlapping. I am not sure all graphs are needed.

Answer: We think the graphs add meaningful information. The categories do not overlap.

The second reviewer wrote: The paper needs major revision to clarify hypotheses of relationships and testing these. Eg relationships of adverse effects related to

living close to industrial locations and waste incinerators. The paper should also discuss the implications of the findings.

Answer: Through the entire manuscript we placed more emphasis on hypotheses and the possible implications of our findings as described in more detail below.

-As mentioned above, the background section was expanded substantially from 209 words to 399 words to reflect the overall purpose, the research strategy and the a priori hypotheses to be tested.

-As mentioned above, the section on the selection of study areas was expanded from 351 words to 535 words to explain why areas and populations were selected. A table giving more information on characteristics and emission data for the 9 study areas was provided (the new table 1).

-As mentioned above, a new section on the selection of biomarkers of effect was introduced.

-In the Results, a new section on the extent of exposure was introduced to give more information to the reader concerning the intensity of exposure and thus to facilitate appreciation by the reader of exposure-effect associations.

This section reads:

“Range of internal exposure

Table 2 shows crude data concerning measured internal exposure in the whole study population. A detailed report on the impact of area of residence and of other factors on the internal exposure will be published elsewhere.”

Table 2

	<i>n</i>	<i>median</i>	<i>p10</i>	<i>p90</i>
<i>Cadmium blood (µg/l)</i>	1579	0,480	0,160	1,240
<i>Cadmium urine (mg/g creatinin)</i>	1581	0,615	0,323	1,304
<i>Lead (µg/l)</i>	1579	39,180	20,180	76,350
<i>HCB (ng/g fat)</i>	1577	55,920	28,170	121,360
<i>PCB99 (ng/g fat)</i>	1577	10,810	1,660	24,350
<i>PCB118 (ng/g fat)</i>	1577	25,850	11,710	50,680
<i>PCB156 (ng/g fat)</i>	1577	18,900	11,010	31,890
<i>PCB170 (ng/g fat)</i>	1577	40,280	24,910	64,410
<i>Sum marker PCBs (138+153+180) (ng/g fat)</i>	1577	345,480	213,230	532,150
<i>Calux assay (pg TEQ/g fat)</i>	1437	22,898	5,430	45,713
<i>p,p'-DDE (ng/g lipid)</i>	1577	487,330	141,600	1587,300
<i>1-OHP (µg/g creatinin)</i>	1575	0,143	0,032	0,684
<i>ttMA (mg/g creatinin)</i>	1391	0,082	0,017	0,334

-In the discussion, the section on “differences in association with area of residence” was expanded from 437 words to 781 words to discuss the implications of our findings in terms of adverse effects related to residence in certain areas.

This section now reads:

“As in the pilot campaign of the Flemish human biomonitoring program [2,4,48] we found significant differences in effect-biomarkers in association with area of residence. Such differences were found for CEA, micronuclei, DNA-strand breaks and oxidative DNA damage.

In the “Olen” and “Albert Canal” industrial areas micronuclei values were significantly higher than in the area with the lowest value, suggesting that both petrochemical and non-ferro industries might contribute to the risk of cancer of people residing in their vicinity.

However we found no evidence indicating that the risk of cancer of the whole population of large areas of the order of 200 km² is increased above the risk of the rest of the Flemish population by the heavy industry situated in those areas. *This may in part be explained by the fact that many participants resided in zones located to the west of the industrial sites in both port areas. The dominant wind pattern in Belgium is from west to east, which means that residents living west of the point sources are less exposed. Within Antwerp port, the district of Burcht situated close to an industrial site showed much more DNA-strand breaks than the district of Beveren situated about 6 kilometers further to the west of the industrial sites. Our findings suggest that a detectable increase in risk might be restricted to those groups, such as the inhabitants of Burcht, residing within a few kilometers of important point sources. Our observations indicate that residence near waste incinerators might be associated with an increase in the risk of cancer. Indeed, all three biomarkers of genotoxic effects were significantly increased, not only above the level observed in the area with the lowest value, but also above the level observed for the rest of Flanders. For each of these biomarkers the highest level was observed in residents of "waste incinerators". In addition, the level of CEA was above the level observed in the area with the lowest value.*

Our observations also suggest that residence in cities might be associated with some increase in the risk of cancer, as in both Antwerp and Ghent levels of CEA and of micronuclei were significantly elevated above the level observed in the area with the lowest value, whereas in Ghent also DNA strand breaks were increased above as well the level observed in the area with the lowest value as above the level observed in the rest of Flanders.

Interestingly, we found no evidence that residence in an area where intensive use of pesticides occurred increases risk of cancer. On the contrary, although the level of oxidative DNA damage observed for these residents was significantly increased compared to the level observed in the area with the lowest value, the levels of micronuclei, of DNA strand breaks, of CEA and of PSA were significantly lower than those observed for the rest of Flanders. In terms of our observations, the "Fruit" area appeared to be the area with the most favorable results.

Remarkably, results for rural Flanders were for none of the biomarkers of effect significantly better than for the rest of Flanders, and for micronuclei and DNA-strand breaks relatively high values were observed, significantly elevated above those observed in the area with the lowest value. Taken together with previous observations pointing in the same direction [4,20,49,50], 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 consumption of self grown food could be involved[49].

Our study was not designed to detect differences in internal exposure or in biological effects in the immediate surroundings of sources of pollution, and was only meant to observe differences between large areas with different types of pollution pressure. However, we did observe higher internal exposure (Schroijen et al 2007; unpublished results) or more intense biological effects (this paper and also unpublished results on gene expression) near point sources of pollution reaching marginal or even full statistical significance, although only a low number of people were studied around these point sources. In terms of DNA strand breaks a difference of almost a factor 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 that, although they 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. Distance from nuclear power plants

showed a pronounced negative correlation with childhood leukaemia in a recent German study [51]. “

In the discussion, a new paragraph was added to the section on “Exposure effect relationships” to clarify possible implications of our findings:

“Our results indicate that levels (see table 2) of internal exposure to some environmental pollutants as they occur in the Flemish population show indeed a positive correlation with some biomarkers of genotoxic effects and with the levels of some tumor-associated proteins. Although quite low, these levels might contribute to the relatively high risk of cancer observed in Flanders [52]. As discussed below for each of the effect biomarkers such positive correlations were observed for blood lead levels with serum levels of CEA; for urinary cadmium levels with serum levels of CEA and PSA; for urinary 1-hydroxypyrene levels (a biomarker for PAH exposure) with oxidative DNA damage and serum levels of CEA; for urinary muconic acid (a biomarker for benzene exposure) with oxidative DNA damage and serum levels of CEA; for serum levels of PCB 118 with the amount of DNA-strand breaks and the number of micronuclei in peripheral blood cells; for serum level of marker PCBs with p53 serum levels; for serum level of HCB with p53 serum levels; for serum level of DDE with serum levels of p53 and CEA; for an index of internal exposure with serum levels of CEA and p53.”

-As mentioned above, the conclusions were expanded and sharpened.

-Finally, in the methods, the results (essentially in table 5 and figure 6) and the discussion mention was made of our observations concerning associations between an Index of exposure and some effect biomarkers. Similar associations were mentioned in a previous paper as described in the first version of our manuscript (van Larebeke et al 2006, reference 4 in our revised manuscript.).

Under methods, in the section on Statistics, the Index of exposure is described as follows:

“We calculated for each biomarker of exposure a standard or z score for each individual by dividing the difference between the value for that individual and the mean value for the entire subject population by the standard deviation for the entire subject population. We calculated for each subject an index of internal exposure I_{ex} defined as the arithmetic mean of the z scores for

blood lead concentration, sum of serum concentrations of marker PCBs (138, 153 and 180), serum concentration of PCB 118 (considered to reflect exposure to dioxin-like PCBs [47]), HCB and p,p'-DDE, dioxin-like activity in serum measured through the calux bioassay, urinary excretion of cadmium, 1-hydroxypyrene and of t-t'-muconic acid per g creatinin ($I_{ex} = (z_{blood\ lead} + z_{sum\ of\ serum\ marker\ PCBs} + z_{PCB118} + z_{HCB} + z_{DDE} + z_{dioxin-like\ activity} + z_{urinary\ Cd} + z_{1-OHP} + z_{ttMA})/9$).”

Associations with this Index of internal exposure are described for p53 in figure 6 in our revised manuscript and for CEA in table 6 of our revised manuscript.