

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Exposure to air pollution and meteorological factors associated with children's primary care visits at night due to asthma attack: case-crossover design for three-year pooled patients
AUTHORS	Yamazaki, Shin; Shima, Masayuki; Yoda, Yoshiko; Oka, Katsumi; Kurosaka, Fumitake; Shimizu, Shigeta; Takahashi, Hironobu; Nakatani, Yuji; Nishikawa, Jittoku; Fujiwara, Katsuhiko; Mizumori, Yasuyuki; Mogami, Akira; Yamada, Taku; Yamamoto, Nobuharu

VERSION 1 - REVIEW

REVIEWER	Carlos E. Rodriguez-Martinez Universidad Nacional de Colombia, Colombia
REVIEW RETURNED	21-Jul-2014

GENERAL COMMENTS	<p>It is an interesting and well-written manuscript about the relationship between exposure to air pollution and meteorological factors, and children's primary care visits at night due to asthma attack. I only have the following minor comments:</p> <p>ABSTRACT</p> <p>1. Page 2, line 32: Please provide the p value for this measure of association (OR)</p> <p>METHODS</p> <p>2. Page 7, line 26. In case-crossover studies, control period data can be collected using two approaches: the pair-matched interval approach or the usual frequency. Please specify the approach used in this study</p> <p>3. Page 7, line 53: The text "and three-day mean concentrations of each air pollutant before PCVs". Does this sentence refers to the three control days?</p> <p>RESULTS</p> <p>4. Page 8, line 51: The text "The interquartile ranges (IQRs) of daily mean PM2.5,...". The interquartile range is ia measure of statistical dispersion, that is usually used when the median is the measure of central tendency, not the mean. Why do the authors report the mean instead of the median? Alternatively, if the data were distributed normally, why did not the authors report the mean and the standard deviation for these values?</p> <p>5. Page 9, line 22: Please report the p value for this measure of association</p> <p>DISCUSSION</p> <p>6. Page 11, line 51: The text "We consider the results of the present</p>
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	<p>three-year analysis to be more stable than those of the two-year or three-month analyses, due to the sample size of the present study being 1.5 times larger than that of the...". As sample size increases, random error decreases, but not necessarily increase the stability. Please review this sentence</p> <p>7. Authors do not present the practical implications nor the clinical utility of the findings of their study. Please add some information about it</p> <p>8. When using this case-crossover design, although there is complete control of between-person confounders, a key limitation of this study design is in the control of within-person confounding, which is still possible for multiple, correlated transient factors that change over time within a subject. For example, if a patient in this study was exposed to certain air pollution or meteorological factors and concurrently was using an inhaler of corticosteroids or albuterol, this confounding would be uncontrolled and would be a threat to the internal validity of the findings. Please mention this aspect in the limitations of the study.</p>
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REVIEWER	Evangelia Samoli Dep. of Hygiene, Epidemiology and Medical Statistics, Medical School, University of Athens, Greece
REVIEW RETURNED	30-Nov-2014

GENERAL COMMENTS	<p>The paper investigates the association of pollution and meteorological factor with emergency pediatric asthma night visits in Himeji visits in Japan, enriching a previous analysis of the group on the subject. Although this is very interesting topic and data coming from Asia are needed in order to confirm associations that that have been reported in US and Europe, this paper needs considerable more work as there are serious concerns for the methods applied and interpretation of identified associations.</p> <p>Major points</p> <p>Introduction</p> <p>1) The references especially in the Introduction are old. The authors should update their references (see also European studies such as Atkinson et al 2001 or WHO review REVIHAAP 2013)</p> <p>2) Some info on possible biological mechanisms for pollution/meteorological factors investigated is missing.</p> <p>Data</p> <p>1) Why did the authors restrict to night visits? Please provide a justification for such a choice.</p> <p>2) Please provide a better description of the area/diagnoses not referring the reader the previous work.</p> <p>Methods</p> <p>1) The authors should clarify in page 28 that the control days are also for the same year</p> <p>2) The authors could have controlled for national holidays by including a dummy variable. Have they tried the sensitivity of the associations for that inclusion? Do they believe that the baseline population is different on holidays?</p> <p>3) I believe that the way meteorological parameters are entered in the models is not adequate and the authors should investigate the sensitivity of the results to alternate control. There is no prior knowledge to include factors such as pressure and wind speed. Is</p>
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	<p>there a possible underlying mechanism or suspected confounding that would not be captured by temperature and humidity control? The authors include all these terms linearly in the models, while many alternatives have been suggested in the literature from piecewise association to splines, as the association with the health outcome is not expected to be linear. Also the lag structure of pollutants and at least temperature should be the same, hence when 3 days are investigated 3 days lags should be included for these parameters in the models. Furthermore, there is a lot of discussion on the optimal control of O3 and temperature effects in models investigating these associations due to their interdependence. Hence I do not find it appropriate to include them both in rather simple models and report the estimates for both from these models. Depending on the focus the model may be different, as for ex stricter control for meteorology when pollution is looked at. Different exposures may have different patterns of association with outcomes and this is not accounted by the use of a common model. See also work by Gasparini and Armstrong. I believe that either the authors should restrict on the association with pollution or meteorology or fir many more models for better investigation of the association reported.</p> <p>4) The authors should report the correlation between pollutants and meteorology, in order also to elaborate the validity of a 3 pollutants model</p> <p>5) In the last paragraph of the methods the authors say that they included dummy variables for seasonal effects. Presumably also their interaction with parameters of interest is included and this should be clarified.</p> <p>Results</p> <p>1) A plot of the PCV would also be informative.</p> <p>2) It would be more easy to follow if the authors comment on association by pollutant, for ex first O3 then PM and do not switch from one commentary to the other</p> <p>3) It is impressing that the effect of 3day O3 in spring is not changed in the 3polluatn model, and if this is still the case after analysis proposed this should be further commented.</p> <p>Discussion</p> <p>1) As mentioned before the authors need to be update their references and also include possible mechanisms of effects.</p> <p>2) For better comparability with previous studies the authors could also investigate the effects in broader periods of the year and not strictly seasonal.</p> <p>3) Strong confounding between temperature and ozone makes it hard to distinguish effects especially in the model as used and commented upon in my previous comments. The authors should restrain from conclusions. Anyway the discussion on meteorology is limited and should be either revisited after different analysis or completed discarded.</p> <p>4) Discussion end of page 12. Different age groups display different time-activity patters hence the comparison between ages should either take it into consideration or restrict comparison within the same age groups. The authors should consider that most previous studies support stronger associations during hotter periods.</p> <p>5) The discussion of the authors in the second paragraph of page 13 imply a non-linear association with NO2. The authors should elaborate.</p> <p>6) Multiple comparisons for seasonal effect estimates is a possible limitation as discussed but this is limited by the consistency of identified associations. I believe that under this perspective the mention to the precautionary principle should be less stressed as it</p>
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	<p>is in the scientific scope of this type of research to back up the results.</p> <p>Minor points</p> <p>1) The paper needs editing. Some major examples include the word “elements “ for meteorological parameters/factors, or the word “relationship” that refers to estimations of associations. Some editing is also needed in line 16 page 14 “...precautionary principle” as it is a concluding statement. See also comment above.</p> <p>2) In the strength/limitations the authors should note that their work refers to pediatric asthma ER at night and in the same part the statistical multiple comparisons is not the main limitations that should be reported here, but the small sample size or the use of night visits only during the week.</p> <p>3) “Course of one year” in text and tables should be replaced by “Annual effects”.</p>
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VERSION 1 – AUTHOR RESPONSE

RESPONSE TO REVIEWER 1:

We wish to express our appreciation to the Reviewer for his or her insightful comments, which have helped us significantly improve the paper.

Comment 1

Page 2, line 32: Please provide the p value for this measure of association (OR)

Response: In accordance with the reviewer’s suggestion, we have now provided the p-values for the association. (P3)

Comment 2

Page 7, line 26. In case-crossover studies, control period data can be collected using two approaches: the pair-matched interval approach or the usual frequency. Please specify the approach used in this study.

Response: In the present study, we used the M-to-one matched intervals approach for data collection. In accordance with the reviewer’s suggestion, we have now added the following to the text to clarify the method used: (M-to-one matched approach used in the present study)(P8 L33)

Comment 3

Page 7, line 53: The text "and three-day mean concentrations of each air pollutant before PCVs". Does this sentence refers to the three control days?

Response: We apologize for the confusion; no, it doesn’t. This description refers to the mean concentrations of each air pollutant over the three days before a PCV, which were analyzed as an exposure index. To improve clarity, we rewrote the text as follows: We examined associations of daily mean concentrations (day of and day before PCVs) of each air pollutant before PCVs with the risk of PCVs at night due to asthma attack. We also examined associations of three-day mean concentrations of each air pollutant before PCVs with the risk of PCVs at night due to asthma attack. (P8 L4)

Comment 4

Page 8, line 51: The text "The interquartile ranges (IQRs) of daily mean PM2.5,...". The interquartile range is a measure of statistical dispersion, that is usually used when the median is the measure of central tendency, not the mean. Why do the authors report the mean instead of the median? Alternatively, if the data were distributed normally, why did not the authors report the mean and the standard deviation for these values?

Response: We agree with the reviewer and, in accordance with their suggestion, have now described mean and standard deviations in Table 2. Our description of IQRs may have provoked a misunderstanding, so we have now deleted that text.

Comment 5

Page 9, line 22: Please report the p value for this measure of association

Response: We agree with the reviewer and, in accordance with their suggestion, have now added p-values to the text where appropriate. (P9)

Comment 6

Page 11, line 51: The text "We consider the results of the present three-year analysis to be more stable than those of the two-year or three-month analyses, due to the sample size of the present study being 1.5 times larger than that of the...". As sample size increases, random error decreases, but not necessarily increase the stability. Please review this sentence

Response: In accordance with the reviewer's suggestion, we mended the sentence as follows: We consider the results of the present three-year analysis to have smaller random error than those of the two-year or three-month analyses, due to the sample size of the present study being 1.5 times larger than that of the two-year analysis and approximately 2.5 times that of the three-month study. (P11 L55)

Comment 7

Authors do not present the practical implications nor the clinical utility of the findings of their study. Please add some information about it

Response: We agree with the reviewer and, in accordance with their suggestion, have now added the following sentence to the Conclusion: Informing local residents of air pollution levels and meteorological parameters may help prevent asthmatic events in the area. (P14 L51)

Comment 8

When using this case-crossover design, although there is complete control of between-person confounders, a key limitation of this study design is in the control of within-person confounding, which is still possible for multiple, correlated transient factors that change over time within a subject. For example, if a patient in this study was exposed to certain air pollution or meteorological factors and concurrently was using an inhaler of corticosteroids or albuterol, this confounding would be uncontrolled and would be a threat to the internal validity of the findings. Please mention this aspect in the limitations of the study.

Response: We agree with the reviewer and, in accordance with their suggestion, have now added the following sentences: First, our study design is limited with respect to control of within-person

confounding, which is still possible for multiple, correlated transient factors that change over time within a subject. For example, if a patient in this study was exposed to certain air pollution or meteorological factors and concurrently was using an inhaler of corticosteroids or albuterol, this confounding would be uncontrolled and would be a threat to the internal validity of the findings. (P14 L13)

RESPONSE TO REVIEWER 2:

We wish to express our appreciation to the Reviewer for his or her insightful comments, which have helped us significantly improve the paper.

Comment 1

The references especially in the Introduction are old. The authors should update their references (see also European studies such as Atkinson et al 2001 or WHO review REVIHAAP 2013)

Response: We agree with the reviewer and, in accordance with their suggestion, we have reviewed the U.S. EPA's "Provisional Assessment of Recent Studies on Health Effects of Particulate Matter Exposure (2012)"[2], WHO regional office for Europe's "Review of evidence on health aspects of air pollution – REVIHAAP project: final technical report (2013)"[4] and Atkinson's recent published systematic review (2014) [6], adding these reports as references. Further, we also incorporated the reviewer's recent study investigating the short-term effects of air pollutants on pediatric asthma emergency admissions. [14] (P4 L11)

Added References

2. U.S. EPA. Provisional Assessment of Recent Studies on Health Effects of Particulate Matter Exposure. U.S. Environmental Protection Agency, Washington, DC, 2012. Available from <http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247132> [accessed 2nd December 2014].
4. The WHO European Centre for Environment and Health, WHO Regional Office for Europe. Review of evidence on health aspects of air pollution – REVIHAAP project: technical report. WHO regional office for Europe, Copenhagen, Denmark. 2013. Available from http://www.euro.who.int/__data/assets/pdf_file/0004/193108/REVIHAAP-Final-technical-report-final-version.pdf?ua=1 [accessed 2nd December 2014].
6. Atkinson RW, Kang S, Anderson HR, Mills IC, Walton HA. Epidemiological time series studies of PM_{2.5} and daily mortality and hospital admissions: a systematic review and meta-analysis. *Thorax*. 2014;69:660-665.
14. Samoli E, Nastos PT, Paliatatos AG, Katsouyanni K, Priftis KN. Acute effects of air pollution on pediatric asthma exacerbation: evidence of association and effect modification. *Environmen Res*. 2011;111:418–424.

Comment 2

Some info on possible biological mechanisms for pollution/meteorological factors investigated is missing.

Response: We have now added the following sentences to describe possible biological mechanisms for pollution/meteorological factors: Children are considered to be particularly susceptible to air pollution and meteorological factors because their lungs are still growing and their immune and pulmonary systems are developing, making them vulnerable to infection with respiratory pathogens. (P4 L11)

Comment 2

Why did the authors restrict to night visits? Please provide a justification for such a choice.

Response: Because the setting of this study was an emergency clinic with nighttime consultation hours, subjects of this study were those who visited the clinic between 9 p.m. and 6 a.m. (P6 L13)

Comment 3

Please provide a better description of the area/diagnoses not referring the reader the previous work.

Response: In accordance with the reviewer' suggestion, we have now added the following sentences to the Methods: The city is 534 km² in area, and its population of the city was about 540,000, some 80,000 of whom were aged less than 15 years. (P6 L17)

Comment 4

The authors should clarify in page 28 that the control days are also for the same year

Response: We agree with the reviewer and, in accordance with their suggestion, have now clarified that the control days were in the same year as the date of a PCV. (P7 L42)

Comment 5

The authors could have controlled for national holidays by including a dummy variable. Have they tried the sensitivity of the associations for that inclusion? Do they believe that the baseline population is different on holidays?

Response: In the present study, reference (control) days were same day of the week in the same month as the date of a primary care visit. When a national holiday falls on a weekday, differences can be observed in people's behavior and traffic density, both of which relate to air pollution, as the luxury of a holiday may increase exposure by allowing more people the freedom to go outside, and increased numbers of people driving and using public transportation can increase levels of air pollutants. We therefore decided to exclude subjects who visited their physician on a national holiday and also exclude national holidays from the control day pool (references). Internal validity of this method was better than that using a dummy variable.

Comment 6

I believe that the way meteorological parameters are entered in the models is not adequate and the authors should investigate the sensitivity of the results to alternate control. There is no prior knowledge to include factors such as pressure and wind speed. Is there a possible underlying mechanism or suspected confounding that would not be captured by temperature and humidity control? The authors include all these terms linearly in the models, while many alternatives have been suggested in the literature from piecewise association to splines, as the association with the health outcome is not expected to be linear. Also the lag structure of pollutants and at least temperature should be the same, hence when 3 days are investigated 3 days lags should be included for these parameters in the models. Furthermore, there is a lot of discussion on the optimal control of O₃ and temperature effects in models investigating these associations due to their interdependence. Hence I do not find it appropriate to include them both in rather simple models and report the estimates for both from these models. Depending on the focus the model may be different, as for ex stricter control for meteorology when pollution is looked at. Different exposures may have different patterns of association with outcomes and this is not accounted by the use of a common model. See also work by Gasparrini and Armstrong. I believe that either the authors should restrict on the association with

pollution or meteorology or for many more models for better investigation of the association reported.

Response: While we appreciate this comment and understand the Reviewer's concern, we believe that seasonal stratified analysis is useful for understanding the association between air pollutants/meteorological parameters and adverse health effects. The "Conditional Poisson models" method proposed by Armstrong et al. is an alternative method for case-crossover analysis. [R1] Given that the present study was not a randomized controlled trial, we were unable to conclude any association between air pollutants/meteorological parameters and PCV. We believe in the importance of establishing a hypothesis of a potential association between air pollutants/meteorological parameters and PCV.

Reference

R1. Armstrong BG, Gasparrini A, Tobias A. Conditional Poisson models: a flexible alternative to conditional logistic case cross-over analysis. *BMC Med Res Methodol.* 2014 Nov 24;14(1):122. [Epub ahead of print] PubMed PMID: 25417555.

In addition, as the reviewer suggested, few studies have assessed the association between meteorological parameters and asthma attack. Therefore, in this study, we examined association between meteorological parameters and PCVs due to asthma attack.

Comment 7

The authors should report the correlation between pollutants and meteorology, in order also to elaborate the validity of a 3 pollutants model.

Response: In accordance with the reviewer's suggestion, we incorporated the correlation matrix into the newly added Table 3. (P22)

Comment 8

In the last paragraph of the methods the authors say that they included dummy variables for seasonal effects. Presumably also their interaction with parameters of interest is included and this should be clarified.

Response: The potential effects of season on the association between air pollutants and PCVs were adjusted using the M-to-one matched interval approach in the same month of the same year. We were therefore unable to examine the interaction between seasonal effects and parameters.

Comment 9

A plot of the PCV would also be informative.

Response: While we understand the Reviewer's wish to see a plot, we worry that plots may cause readers to misunderstand the subject distribution, given that those who visited primary care on national holidays were excluded. In addition, but perhaps more minor, the number of Figures and Tables allowed is limited by the journal.

Comment 10

It would be more easy to follow if the authors comment on association by pollutant, for ex first O3 then PM and do not switch from one commentary to the other.

Response: In accordance with the reviewer's suggestion, we restructured the Results section. (P9 L29 - P10 L51)

Comment 11

It is impressive that the effect of 3day O3 in spring is not changed in the 3polluatn model, and if this is still the case after analysis proposed this should be further commented.

Response: In accordance with the reviewer's suggestion, we have added the following sentence to the Discussion section to highlight the interest of this finding: Of note, the effect of 3-day mean ozone levels in spring in the single pollutant model did not differ markedly from that in the multi pollutant model. (P11 L17)

Comment 12

As mentioned before the authors need to be update their references and also include possible mechanisms of effects.

Response: In accordance with the reviewer's suggestion, we added the U.S. EPA's new review paper (2012) [2], WHO's new paper (2013) [4], and Atkinson's recent published systematic review (2014) [6] as references. (P4 L11)

Added References

2. U.S. EPA. Provisional Assessment of Recent Studies on Health Effects of Particulate Matter Exposure. U.S. Environmental Protection Agency, Washington, DC, 2012. Available from <http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247132> [accessed 2nd December 2014].
4. The WHO European Centre for Environment and Health, WHO Regional Office for Europe. Review of evidence on health aspects of air pollution – REVIHAAP project: technical report. WHO regional office for Europe, Copenhagen, Denmark. 2013. Available from http://www.euro.who.int/__data/assets/pdf_file/0004/193108/REVIHAAP-Final-technical-report-final-version.pdf?ua=1 [accessed 2nd December 2014].
6. Atkinson RW, Kang S, Anderson HR, Mills IC, Walton HA. Epidemiological timeseries studies of PM2.5 and daily mortality and hospital admissions: a systematic review and meta-analysis. *Thorax*. 2014;69:660-665.
14. Samoli E, Nastos PT, Paliatsos AG, Katsouyanni K, Priftis KN. Acute effects of air pollution on pediatric asthma exacerbation: evidence of association and effect modification. *Environmen Res*. 2011;111:418-424.

We also added the following to the Discussion: Samoli et al. also noted an association between ozone levels and risk of asthma admission among older children in summer [14]. (P4 L31)

Possible mechanisms of effects were added to the Introduction. (P4 L11)

Comment 13

For better comparability with previous studies the authors could also investigate the effects in broader periods of the year and not strictly seasonal.

Response: We did also analyze the data annually; we believe that seasonal stratified analysis is more useful for understanding the association between air pollutant/meteorological parameters and adverse health effects.

Comment 14

Strong confounding between temperature and ozone makes it hard to distinguish effects especially in the model as used and commented upon in my previous comments. The authors should restrain from conclusions. Anyway the discussion on meteorology is limited and should be either revisited after different analysis or completely discarded.

Response: While we do still believe our findings are useful in considering the possible associations between meteorological parameters and PCV due to asthma attack, we have clarified the limitations of our study in the Discussion to underscore the weaknesses in our study now and changed the text slightly as follows: Further, certain meteorological parameters—such as atmospheric pressure in spring, temperature in spring or summer, and hours of daylight in fall—may be associated with PCVs due to asthma attack. (P14 L44)

Comment 15

Discussion end of page 12. Different age groups display different time-activity patterns hence the comparison between ages should either take it into consideration or restrict comparison within the same age groups. The authors should consider that most previous studies support stronger associations during hotter periods.

Response: In accordance with the reviewer's suggestion, we have now added the following to the Discussion, with a new reference added as well: Strickland et al. also observed associations with several traffic-related primary pollutants during the warm season in a large study analyzing over 90,000 emergency department visits for pediatric asthma in relation to ambient air pollutant concentrations [32]. (P15 L15)

Added Reference

32. Strickland MJ, Darrow LA, Klein M, Flanders WD, Sarnat JA, Waller LA, et al. Short-term associations between ambient air pollutants and pediatric asthma emergency department visits. *Am J Respir Crit Care Med* 2010;182: 307-316.

Comment 16

The discussion of the authors in the second paragraph of page 13 implies a non-linear association with NO₂. The authors should elaborate.

Response: In winter, the Pearson's correlation coefficient between NO₂ and ozone was -0.69 , which accounts in part for the differences in associations observed among four seasons. We have therefore added the following sentence to the Discussion explaining the inverse association between NO₂ and PCV in the single pollutant model in winter: In winter, the Pearson's correlation coefficient between NO₂ and ozone was -0.69 . (P15 L40)

Comment 17

Multiple comparisons for seasonal effect estimates is a possible limitation as discussed but this is limited by the consistency of identified associations. I believe that under this perspective the mention to the precautionary principle should be less stressed as it is in the scientific scope of this type of research to back up the results.

Response: In accordance with the reviewer's suggestion, we deleted the sentence with respect to the precautionary principle.

Comment 18

The paper needs editing. Some major examples include the word “elements “ for meteorological parameters/factors, or the word “relationship” that refers to estimations of associations. Some editing is also needed in line 16 page 14 “...precautionary principle” as it is a concluding statement. See also comment above.

Response: In accordance with the reviewer’s suggestion, we have had the manuscript and this letter carefully reviewed by an experienced medical editor whose first language is English and who specializes in the editing of papers written by physicians and scientists whose native language is not English.

Comment 19

In the strength/limitations the authors should note that their work refers to pediatric asthma ER at night and in the same part the statistical multiple comparisons is not the main limitations that should be reported here, but the small sample size or the use of night visits only during the week.

Response: In accordance with the reviewer’s suggestion, we have revised the limitations to more accurately reflect the conditions of our study. (P14 L11)

Comment 20

“Course of one year” in text and tables should be replaced by “Annual effects”.

Response: In accordance with the reviewer’s suggestion, we replaced the text in the manuscript and tables. (Table 4, Table 5) (P23 - P25)

VERSION 2 – REVIEW

REVIEWER	Evangelia Samoli University of Athens, Grece
REVIEW RETURNED	20-Jan-2015

GENERAL COMMENTS	<p>Although the authors have addressed satisfactory the majority of my previous comments, comments 7 and 8 are not appropriately addressed. More specifically:</p> <ol style="list-style-type: none"> 1) I would like to see a couple of sensitivity results at least for the single pollutant models as reply to comment 7, while 2) the reply to comment 8 is still unclear to me. If the authors have performed stratified analysis per season this should be clearly mentioned, otherwise if an indicator with 4 levels (as described) was included in the annual model how did the effect of the pollutants changed without considering interactions? 3) Additionally, on the analysis, the authors should clarify whether OBC was measured in PM10 or PM2.5 and should include results from a model with both OBC and the relevant PM mass 4) The authors have revised the text in the Methods saying “..model adjusted for one-day/three-day mean atmospheric pressure “. If this means that when including 3days of pollutants they also include 3 days of meteorological parameters, it should be made clear, otherwise this is the appropriate analysis for this kind of data. 4) Even if the option of a plot of PCV (previous comment 9) provided as Supplementary material to the publication is not a choice, I would
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	<p>like to ask the authors to provide one for my understanding as a reply to my comment. Also please edit the reply to comment 16 to read more clearly in the paper.</p> <p>5) Finally, as a minor additional point I would like to clarify my previous comment 13 by which I suggested looking into 6 months periods instead of 3 -as this would also provide more power .</p>
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VERSION 2 – AUTHOR RESPONSE

Comment 1

Although the authors have addressed satisfactory the majority of my previous comments, comments 7 and 8 are not appropriately addressed. More specifically:

- 1) I would like to see a couple of sensitivity results at least for the single pollutant models as reply to comment 7, while
- 2) the reply to comment 8 is still unclear to me. If the authors have performed stratified analysis per season this should be clearly mentioned, otherwise if an indicator with 4 levels (as described) was included in the annual model how did the effect of the pollutants changed without considering interactions?

Response: We agree with the Reviewer’s comment on these points and apologize for any confusion. We suspect that our description of the statistical methods in our initial submission may have caused some misunderstanding and have therefore amended the 4th paragraph of the Statistical methods section in the Methods as follows:

Revised text: Because we used a time-stratified case-crossover analysis, which involves the M-to-one matched interval approach for the same month of the same year, the potential effects of season on the association between air pollutants and PCVs were not measured (i.e. season was the matching variable between cases and controls). In the annual model, we also adjusted for seasonal effects using the M-to-one matched interval approach for the same month of the same year.

Comment 2

- 3) Additionally, on the analysis, the authors should clarify whether OBC was measured in PM10 or PM2.5 and should include results from a model with both OBC and the relevant PM mass

Response: In accordance with the Reviewer’s suggestion, we have now added the following to the Air pollutants and meteorological parameters section in the Methods:

New text: In this study, OBC in PM2.5 was measured.

With respect to the effects of OBC on PCV, we added the following to the Particulate matters and NO2 section in the Results:

New text: No association was noted between OBC and PCV using the single pollutant model (Table 4), nor was any association noted between OBC and PCV using the multi-pollutant model, which was adjusted for PM2.5, NO2, ozone, and meteorological factors (new supplemental Table S1).

Comment 3

- 4) The authors have revised the text in the Methods saying “..model adjusted for one-day/three-day mean atmospheric pressure “. If this means that when including 3days of pollutants they also include 3 days of meteorological parameters, it should be made clear, otherwise this is the appropriate

analysis for this kind of data.

Response: We apologize for any confusion and have now clarified our meaning by adding the following sentence as an explanation to the Statistical methods section in the Methods:

New text: When three-day mean concentrations of air pollutants were explanatory variables, adjusted variables were also included as three-day mean values.

Comment 4

5) Even if the option of a plot of PCV (previous comment 9) provided as Supplementary material to the publication is not a choice, I would like to ask the authors to provide one for my understanding as a reply to my comment. Also please edit the reply to comment 16 to read more clearly in the paper.

Response: The number of PCVs for each respective month (January through December) were as follows: 62, 62, 81, 113, 150, 127, 127, 164, 116, 232, 133, and 80.

In accordance with the Reviewer's suggestion and with respect to our previous reply to Comment 16, we have now added the following sentence as an explanation in the Discussion:

New text: Because ozone is secondarily formed by the action of short-wavelength radiation from the sun on NO₂, when the concentration of ozone was increased, the concentration of NO₂ subsequently tended to be decreased.

Comment 5

6) Finally, as a minor additional point I would like to clarify my previous comment 13 by which I suggested looking into 6 months periods instead of 3 -as this would also provide more power.

Response: When using a 6-month interval as suggested (warmer months vs. colder months), we were still able to detect an association between ozone and PCV, with an OR (95% CI) of 1.13 (1.02-1.25) in warmer months, but not in colder months (OR: 1.06; 95% CI: 0.89-1.26). Further, associations between PM_{2.5} and PCV were not statistically significant (data not shown).

VERSION 3 - REVIEW

REVIEWER	Evangelia Samoli University of Athens, Greece
REVIEW RETURNED	16-Mar-2015

GENERAL COMMENTS	The authors still have not replied to my Comment 1a. Also I would suggest removing their response to my Comment 1b as it does not really add in the description of the Methods. Limitations of the study should be enhanced to address poor control of meteorology in the models for ozone.
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VERSION 3 – AUTHOR RESPONSE

Comment 1

The authors still have not replied to my Comment 1a. Also I would suggest removing their response to my Comment 1b as it does not really add in the description of the Methods.

Response:

1a) We agree with the Reviewer's comment on these points and apologize for any confusion. In accordance with the reviewer's suggestion, we incorporated the correlation matrix into the newly added Table 3 in the former manuscript (R1). Now, we added a description of results of single-pollutant model considered interaction between ozone and temperature as sensitivity analysis in the Results:

New text: In addition, when we considered interaction between ozone and temperature in the single-pollutant model, there were no interaction between ozone and temperature (p-values of the interactions in the same day of PCV, in the day before PCV, and for 3-days mean were 0.17, 0.21 and 0.11, respectively); the association between ozone and PCV remained significant.

1b) In accordance with the Reviewer's suggestion, we have removed the description that the reviewer suggested.

Comment 2

Limitations of the study should be enhanced to address poor control of meteorology in the models for ozone.

Response: In accordance with the Reviewer's suggestion, we have now added the following to the Limitation section:

New text: Fourth, association between concentrations of air pollutants and PCV might not be adequately adjusted for meteorological factors, because associations between meteorological factors and PCV are not clear.