

## PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form ([see an example](#)) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below. Some articles will have been accepted based in part or entirely on reviews undertaken for other BMJ Group journals. These will be reproduced where possible.

### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Longitudinal Assessment of Chlorpyrifos Exposure and Self-Reported Neurological Symptoms in Adolescent Pesticide Applicators
<b>AUTHORS</b>	Khan, Khalid; Ismail, Ahmed; Abdel-Rasoul, Gaafar; Bonner, Matthew; Lasarev, Mike; Hendy, Olfat; Al-Batanony, Manal; Crane, Alice; Singleton, Steven; Olson, James; Rohlman, Diane

### VERSION 1 - REVIEW

<b>REVIEWER</b>	Kimberly Yolton Associate Professor Department of Pediatrics Cincinnati Children's Hospital Medical Center Cincinnati, OH, USA
<b>REVIEW RETURNED</b>	28-Oct-2013

<b>GENERAL COMMENTS</b>	<p>If the paper is accepted for publication, prior to publication, it will require a hard edit to correct missing punctuation and some grammatical errors. Also, placement of in-text citation is inconsistent throughout.</p> <p>This manuscript presents interesting research on exposure to chlorpyrifos (CPF) among adolescent pesticide applicators in Egypt. The study includes serial biomarkers of exposure to CPF and neurological symptoms among Egyptian adolescent CPF applicators and non-applicators across the applications season. Several aspects of the study design are unclear in the write-up and require additional work to present the study adequately. Many details are missing and required to clearly describe the study. For example, a timeline of the research events is needed to relate the periods in which exposures were likely to be highest among the applicators and how these times relate with the data collection periods. Critical details of biological sample collection are missing. In addition, the methods are not clearly described. The initial description suggests that the authors collapsed and analyze the symptom and exposure data across the study period negating the longitudinal study design and resulting in a loss of potentially rich data linking CPF application to biological markers and neurological symptoms. However, when reviewing the results, they are presented related to specific exposure periods, indicating that the samples and symptoms were not collapsed over time. This is a problem with writing style.</p> <p>The findings suggest significant differences between applicators and non-applicators in their exposure and neurological symptoms across the CPF application season. The results are important to relay but need to be presented more clearly.</p> <p>The authors should check the work of VA Rauh for possible relevant</p>
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support of the associations between CPF exposure and child outcomes.

Abstract:

The first sentence in the results section is unclear as it states the pesticide applicators were compared with non-applicators “after several weeks of repeated CPF application.” Perhaps the authors wish to state that the comparison was made several weeks into the CPF application season.

Introduction:

Page 4, Paragraph 1 – Suggested clarification: “ ... due to their associations with ADVERSE neurological outcomes.”

Page 5, Paragraph 3 – Edit: “ ... (TCPy), IS a relatively specific metabolite of CPF exposure ...”

Page 5, Paragraph 2 - 2nd sentence is unclear. Do the authors mean to say that “ ... identification of effects of specific OP exposure is important ...” ??? Is the purpose to understand the impact of each specific OP?

Page 5, Paragraph 3 – Edit: “ ... repeated exposures to OPs are associated with reported neurological symptoms ...” Also, how is this different from “whether there are any associations between OP biomarkers and neurological symptoms ...”?

Page 6, Paragraph 3: The objective of the study should be clearly stated such as “The objective of this study was to determine whether occupational exposure to CPF is associated with self-reported neurological symptoms. We compared ...”

Methods:

Page 7, Paragraph 1 – Edit: “Recently, Fenske et al.<sup>26</sup> reported that ...”

A few questions/suggestions emerge with respect to the study methods.

1. On page 8, the authors indicate they used a 4-point likert scale to have study subjects rate the weekly frequency of neurological symptoms. However, since 90% of the responses were rated as 0-2, they collapsed the scale to never or at least once per week. This greatly diminishes the richness of their data and the ability to examine the frequency of symptoms. It would be helpful for the reader to know more details about the distribution of the subject responses to know whether the collapsing of the responses is warranted. The analysis will likely be more meaningful if the original response categories are retained. It may be reasonable to analyze the symptom counts, followed by frequency data, to provide the clearest picture of the neurological effects.

2. On page 8, the neurological symptom questionnaire is described as administered 32 times over a period of 8 months, “at least once per week.” The recall period for the questionnaire is 1 week. Responses were averaged across the data collection period which spanned pre-application, application, and post-application periods. This provides a very crude comparison of the symptoms between applicators and non-applicators across the entire time period and does allow for analysis of changes in symptoms at different times

	<p>across the growing season. From the results section, it appears that there was no averaging of the symptoms over time. This is promising, but obviously, this aspect of the methods description needs clarification.</p> <p>3. Urine TCPy values from 8 separate samples collected across the time of the study were analyzed as a cumulative measure. Again, this loses the prospective/longitudinal possibilities that the study design could allow and missed the opportunity to observe changes in exposure throughout the collection period.</p> <p>4. It would be helpful to know if the urine collection cups were pretested for pesticide residues. Also, how were the samples collected? Did subjects use alcohol wipes prior to providing their samples? Did they wear gloves to as not to contaminate the urine sample with pesticide residues that may have been on their hands? Also, where did non-applicators have their samples collected? If they needed to travel to the field station, it is likely they experienced some increased exposure during that trip.</p> <p>5. A clear timeline description of the study collection periods for symptoms data and urine and blood samples as it coincides with the CPF-application season is needed to help the reader understand the sequence of data collection as it relates to potential exposure. Some of these details are revealed in the statistical analysis section of the manuscript, but they should occur early on in the methods so the reader is not required to speculate.</p> <p>Results: The results suggest that both CPF applicators and non-applicators experienced increases in neurological symptoms as well as changes in biological markers of exposure across different periods of the application season. Covariates included in adjusted models need to be described explicitly in addition to being listed in the footnote of figures and tables.</p> <p>Discussion: The authors need to acknowledge the possibility that the frequent completion of the neurological symptoms survey (32 times over 8 months) could itself have had an influence on the increase in symptoms reported. This could help explain the increase in symptoms among the non-applicators across the study period and why these symptoms were not associated with TCPy levels.</p> <p>Tables and Figures: Figure 1: X axis needs to be clearly labeled for Day since baseline assessment. Covariates included in adjusted models need to be listed in the footnote for the table.</p> <p>Supplemental Table 1 should be included as one of the main central tables for the paper since the association between exposure and neurological symptoms is the primary research question.</p>
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<b>REVIEWER</b>	Peter P. Egeghy National Exposure Research Laboratory U.S. Environmental Protection Agency
<b>REVIEW RETURNED</b>	06-Nov-2013

**GENERAL COMMENTS**

This manuscript describes a study of adolescent agricultural pesticide applicators, evaluating presence of neurological symptoms and the relationship between those symptoms and biomarkers. The paper is well written. The authors do a good job of putting the study in appropriate context, reviewing literature that suggests that organophosphate pesticides have been associated with neurological symptoms, particularly among applicators and that adolescents may be more vulnerable to the effects. The purpose, methods (mostly), results, and discussion are presented well. The deficiencies that I see are related to the analytical chemistry methods, the explanation of observed results among the non-applicator controls, a lack of non-questionnaire assessments, and a discussion of appropriate interventions that should be taken.

With the analytical chemistry methods, the authors summarize a methods that has been previously published, but should give some more detail and be more clear. Specifically, I recommend mentioning that the method involves hydrolysis, extraction, and derivatization. Also, please clarify which assay is referred to by "the within-run imprecision of this assay" - I am assuming the gc/ms analysis and not the Jaffe reaction (P. 10, line 30). "Within-run imprecision" needs to be defined - does it refer to duplicate runs of a standard? The minimum detection level is stated as "0.0501 ng" (P. 10, line 41). First, MDLs are typically reported as a concentration rather than a mass. Second, it is difficult to believe that this method has resolution at the 0.1 pg level. Third, a more detailed description of the method in Fayssal et al. (2010) from the same laboratory lists the MDL as 0.5 ng/mL. Lastly, the meaning of the sentence beginning with "Finally, cumulative urinary TCPy..." (P. 10, line 43) is not clear.

With respect to the results among non-applicators, it is clear from Table 3 that the change in neurological symptoms is (1) erratic, but (2) generally follows the pattern (with respect to peak) seen in applicators. The erratic nature of the results among non-applicators calls into question the reliability of the questionnaire. Further, the authors report that the non-applicators reported a significant increase in symptoms at a time corresponding to the peak reporting of symptoms by the applicators. The explanation that this may be a result of in-home insecticide application is not supported by any evidence (e.g., that organophosphate insecticides are used instead of pyrethroid insecticides in residential environments). Further, it is not clear if the same domains of neurological symptoms are increasing in both the applicators and non-applicators.

The relationship between agricultural pesticide application and neurological symptoms as assessed by questionnaire seems to be well established (based on the review by the authors). The study could have been strengthened by adding some type of psychomotor tests (e.g., choice reaction time, visual vigilance, dual task, short term memory scanning), neurophysiological tests (e.g., eye blink reflex), or sensorimotor tests (e.g., postural sway) to groundtruth the questionnaires.

Lastly, this seems to be one of many in a series of similar studies that have now provided substantial evidence of an increase in neurological symptoms related to agricultural application of OP insecticides, yet no recommendations for appropriate interventions are suggested. Clearly, the cotton industry relies on pesticide application, so we know that will not magically stop no matter how many studies are performed. What steps should be taken from a

	<p>public health perspective to lower the exposure among applicators? What would be appropriate and acceptable? What should be done?</p> <p>Minor comments:</p> <p>In the box that describes the “Strengths and limitations of study”, the second bullet doesn’t make much sense as written, the third bullet should follow the first bullet, and the fifth bullet, "is another limitation study that" is not necessary (and poorly written)/</p> <p>In the box that describes the “What this paper adds”, the first bullet should be rewritten from the perspective of what the paper adds not the current state of the science: for example, "Results lead to a better understanding of how neurological symptoms vary ..." In the second bullet, the phrase "more likely to report increased symptoms" is awkward. I think what you are saying is that applicators are more likely to experience neurological symptoms.</p> <p>P. 5, line 25: "Because of their smaller body size, the biological doses of pesticides (for children and adolescents may be substantially higher than adults" Please explain how smaller body size leads to higher biological dose.</p> <p>P. 5, line 27: extraneous open parenthesis mark</p> <p>P. 7, line 30: "Recently, 26 reported that dermal exposure..." should be "Recently, Fenske et al.^26 reported that dermal exposure..."</p> <p>P. 8, line 45: extraneous closing parenthesis mark.</p> <p>P. 10, line 43: The meaning of the sentence beginning with “Finally, cumulative urinary TCPy...” is not clear.</p> <p>Sup Fig 3a (symptoms vs TCPy) is compelling and should be in the paper.</p> <p>P. 22, sentence beginning "Our study was conducted in..." is awkward</p> <p>Page 22, "Results of our study may be generalizable only to agricultural communities with similar sociodemographic characteristics" is meaningless as I suspect that agricultural communities are likely to have similar sociodemographic characteristics (at least with respect to SES).</p>
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### VERSION 1 – AUTHOR RESPONSE

Reviewer Name Kimberly Yolton

Institution and Country Associate Professor

Department of Pediatrics

Cincinnati Children's Hospital Medical Center

Cincinnati, OH, USA

Please state any competing interests or state 'None declared': None declared

1. If the paper is accepted for publication, prior to publication, it will require a hard edit to correct missing punctuation and some grammatical errors.

Response: We have edited the manuscript to correct missing punctuation and some grammatical error. We hope that the quality of the manuscript has been significantly improved after the edit and major revisions.

2. Many details are missing and required to clearly describe the study. For example, a timeline of the research events is needed to relate the periods in which exposures were likely to be highest among the applicators and how these times relate with the data collection periods.

Response: We have revised the Methods and Results sections and also included a figure in the manuscript indicating time intervals and sample collection period.

3. In addition, the methods are not clearly described. The initial description suggests that the authors collapsed and analyze the symptom and exposure data across the study period negating the longitudinal study design and resulting in a loss of potentially rich data linking CPF application to biological markers and neurological symptoms. However, when reviewing the results, they are presented related to specific exposure periods, indicating that the samples and symptoms were not collapsed over time. This is a problem with writing style.

Response: We have revised the Methods section and explained why we collapsed the scale responses. We believe that this does not diminish the richness of the data since more than 90% of the responses were rated either “0=never” or “1=once a week and 2=once in every 2-3 days”. This also allowed us to simplify statistical analysis and present the data in a more meaningful way. Even when we ran the analysis using the original codes for five responses (0, 1, 2, 3, 4, 5) we obtained similar study findings (data not shown).

4. The authors should check the work of VA Rauh for possible relevant support of the associations between CPF exposure and child outcomes

Response: Rauh et al. (2012) has been added as a reference in the introduction section while discussing the effect of CPF on brain development.

5. Abstract: The first sentence in the results section is unclear as it states the pesticide applicators were compared with non-applicators “after several weeks of repeated CPF application.” Perhaps the authors wish to state that the comparison was made several weeks into the CPF application season.

Response: We have re-constructed the sentence as per reviewer’s suggestion “When we compared reporting of symptoms between applicators and non-applicators at different time intervals over the 8-month study period, we observed both groups reporting the highest numbers of symptoms in the middle of the CPF application season.

6. Introduction: Page 4, Paragraph 1 – Suggested clarification: “ ... due to their associations with ADVERSE neurological outcomes.”

Response: We have revised the sentence as per reviewer’s suggestion.

7. Page 5, Paragraph 3 – Edit: “ ... (TCPy), IS a relatively specific metabolite of CPF exposure ...”

Response: We have revised the sentence as per reviewer’s suggestion.

8. Page 5, Paragraph 2 - 2nd sentence is unclear. Do the authors mean to say that “ ... identification of effects of specific OP exposure is important ...” ??? Is the purpose to understand the impact of each specific OP?

Response: We agree with the reviewer and have decided to delete the sentence.

9. Page 5, Paragraph 3 – Edit: “ ... repeated exposures to OPs are associated with reported neurological symptoms ...” Also, how is this different from “whether there are any associations between OP biomarkers and neurological symptoms ...”?

Response: We agree with the reviewer that these two components of the sentence look redundant. We have deleted the first one from this specific sentence.

10. Page 6, Paragraph 3: The objective of the study should be clearly stated such as “The objective of this study was to determine whether occupational exposure to CPF is associated with self-reported

neurological symptoms. We compared .....

Response: We have included a sentence describing the primary objective of the study in the paragraph mentioned by the reviewer.

11. Methods: Page 7, Paragraph 1 – Edit: “Recently, Fenske et al.<sup>26</sup> reported that ...”

Response: We have revised the sentence as suggested by the reviewer.

12. A few questions/suggestions emerge with respect to the study methods. On page 8, the authors indicate they used a 4-point likert scale to have study subjects rate the weekly frequency of neurological symptoms. However, since 90% of the responses were rated as 0-2, they collapsed the scale to never or at least once per week. This greatly diminishes the richness of their data and the ability to examine the frequency of symptoms. It would be helpful for the reader to know more details about the distribution of the subject responses to know whether the collapsing of the responses is warranted. The analysis will likely be more meaningful if the original response categories are retained. It may be reasonable to analyze the symptom counts, followed by frequency data, to provide the clearest picture of the neurological effects.

Response: We have revised the Methods section and explained why we collapsed the scale responses. Even if we run the analysis keeping the response categories as they our findings do not change (data not shown). Therefore, we have decided to present the data in the most simplified way so that they look meaningful to the readers.

13. On page 8, the neurological symptom questionnaire is described as administered 32 times over a period of 8 months, “at least once per week.” The recall period for the questionnaire is 1 week. Responses were averaged across the data collection period which spanned pre-application, application, and post-application periods. This provides a very crude comparison of the symptoms between applicators and non-applicators across the entire time period and does allow for analysis of changes in symptoms at different times across the growing season. From the results section, it appears that there was no averaging of the symptoms over time. This is promising, but obviously, this aspect of the methods description needs clarification.

Response: We agree with the reviewer that the description of outcome variables need more clarification. Therefore, we have revised the Methods section under the subheading “outcome assessment” to clarify two outcome variables that we have used in this manuscript.

14. Urine TCPy values from 8 separate samples collected across the time of the study were analyzed as a cumulative measure. Again, this loses the prospective/longitudinal possibilities that the study design could allow and missed the opportunity to observe changes in exposure throughout the collection period.

Response: We agree with the reviewer that we missed the opportunity to observe changes in exposure over time using the urinary biomarker data. The change in chlorpyrifos (CPF) exposure through the collection period has been discussed in a manuscript published by the same group of authors (Crane et al, 2013). We have cited our TCPy biomarker work at several places in the current manuscript. One of our limitations was that the study was under-powered (as far as biomarker data are concerned) to detect more subtle relationships within more narrow windows (i.e. within specific time periods).

15. It would be helpful to know if the urine collection cups were pretested for pesticide residues. Also, how were the samples collected? Did subjects use alcohol wipes prior to providing their samples? Did they wear gloves to as not to contaminate the urine sample with pesticide residues that may have

been on their hands? Also, where did non-applicators have their samples collected? If they needed to travel to the field station, it is likely they experienced some increased exposure during that trip.

Response: All urine collection cups were new and individually wrapped. They were opened at the time of sample collection. Therefore, pre-testing for pesticide residues was not required. This information has been added to the Methods section. Also, there was a remote possibility of contamination of the urine samples. Also, it was very unlikely that non-applicators got any substantial exposure while traveling to field stations for providing urine samples.

16. A clear timeline description of the study collection periods for symptoms data and urine and blood samples as it coincides with the CPF-application season is needed to help the reader understand the sequence of data collection as it relates to potential exposure. Some of these details are revealed in the statistical analysis section of the manuscript, but they should occur early on in the methods so the reader is not required to speculate.

Response: Figure XX has been modified and included in the main document to describe the study collection periods for symptoms data and urine and blood samples.

17. Results: The results suggest that both CPF applicators and non-applicators experienced increases in neurological symptoms as well as changes in biological markers of exposure across different periods of the application season. Covariates included in adjusted models need to be described explicitly in addition to being listed in the footnote of figures and tables.

Response: We have added a description of the covariates in the paragraph under subheading "Change in symptoms over time".

18. Discussion: The authors need to acknowledge the possibility that the frequent completion of the neurological symptoms survey (32 times over 8 months) could itself have had an influence on the increase in symptoms reported. This could help explain the increase in symptoms among the non-applicators across the study period and why these symptoms were not associated with TCPy levels.

Response: We have added a new paragraph in the discussion section (while discussing the limitations of the study) taking reviewer's suggestion into account.

19. Tables and Figures: Figure 1: X axis needs to be clearly labeled for Day since baseline assessment. Covariates included in adjusted models need to be listed in the footnote for the table.

Response: Footnote added; Axis re-labeled and figures have been modified as per reviewers' suggestions.

20. Supplemental Table 1 should be included as one of the main central tables for the paper since the association between exposure and neurological symptoms is the primary research question.

Response: Supplemental Table 1 has been included as Table 5 in the main body of the manuscript.

Reviewer Name Peter P. Egeghy  
Institution and Country National Exposure Research Laboratory  
U.S. Environmental Protection Agency  
Mail Drop E205-04  
Research Triangle Park, NC 27711



USA

21. With the analytical chemistry methods, the authors summarize a methods that has been previously published, but should give some more detail and be more clear. Specifically, I recommend mentioning that the method involves hydrolysis, extraction, and derivatization. Also, please clarify which assay is referred to by "the within-run imprecision of this assay" - I am assuming the gc/ms analysis and not the Jaffe reaction (P. 10, line 30). "Within-run imprecision" needs to be defined - does it refer to duplicate runs of a standard? The minimum detection level is stated as "0.0501 ng" (P. 10, line 41). First, MDLs are typically reported as a concentration rather than a mass. Second, it is difficult to believe that this method has resolution at the 0.1 pg level. Third, a more detailed description of the method in Fayssal et al. (2010) from the same laboratory lists the MDL as 0.5 ng/mL. Lastly, the meaning of the sentence beginning with "Finally, cumulative urinary TCPy..." (P. 10, line 43) is not clear.

Response: We have made revisions in the paragraph with sub-heading "Urine collection and analysis."

22. With respect to the results among non-applicators, it is clear from Table 3 that the change in neurological symptoms is (1) erratic, but (2) generally follows the pattern (with respect to peak) seen in applicators. The erratic nature of the results among non-applicators calls into question the reliability of the questionnaire. Further, the authors report that the non-applicators reported a significant increase in symptoms at a time corresponding to the peak reporting of symptoms by the applicators. The explanation that this may be a result of in-home insecticide application is not supported by any evidence (e.g., that organophosphate insecticides are used instead of pyrethroid insecticides in residential environments). Further, it is not clear if the same domains of neurological symptoms are increasing in both the applicators and non-applicators.

Response: We are not sure that the erratic nature of the symptoms reporting necessarily implies limited reliability. We would probably not expect the inaccuracy of the questionnaire to spuriously induce a relationship with pesticides applications (as we observed), but it could happen. When we examined the symptom reporting by each domain separately we observed the similar pattern of reporting across time for both applicators and non-applicators (data not shown). Furthermore, both applicators and non-applicators were from villages located in an agricultural region. Therefore, one explanation for the increase in symptoms among non-applicators may be environmental exposure in this group of participants who were living near agricultural fields that were being sprayed with CPF.

23. The relationship between agricultural pesticide application and neurological symptoms as assessed by questionnaire seems to be well established (based on the review by the authors). The study could have been strengthened by adding some type of psychomotor tests (e.g., choice reaction time, visual vigilance, dual task, short term memory scanning), neurophysiological tests (e.g., eye blink reflex), or sensorimotor tests (e.g., postural sway) to ground truth the questionnaires.

Response: Thanks for the comment. Future studies in the same geographic region include the use of additional psychomotor tests.

24. Lastly, this seems to be one of many in a series of similar studies that have now provided substantial evidence of an increase in neurological symptoms related to agricultural application of OP insecticides, yet no recommendations for appropriate interventions are suggested. Clearly, the cotton industry relies on pesticide application, so we know that will not magically stop no matter how many studies are performed. What steps should be taken from a public health perspective to lower the exposure among applicators? What would be appropriate and acceptable? What should be done?

Response: We have included the following in the conclusions section to address this specific issue. "Our study reinforces the need for the development and execution of intervention programs for the residents of agricultural communities, including pesticide applicators, in developing countries. Future interventions should include hygiene practices, behaviors and use of protective equipment, in both occupational and residential environments."

25. Minor comments:

In the box that describes the "Strengths and limitations of study", the second bullet doesn't make much sense as written, the third bullet should follow the first bullet, and the fifth bullet, "is another limitation study that" is not necessary (and poorly written)/

In the box that describes the "What this paper adds", the first bullet should be rewritten from the perspective of what the paper adds not the current state of the science: for example, "Results lead to a better understanding of how neurological symptoms vary ..." In the second bullet, the phrase "more likely to report increased symptoms" is awkward. I think what you are saying is that applicators are more likely to experience neurological symptoms.

P. 5, line 25: "Because of their smaller body size, the biological doses of pesticides (for children and adolescents may be substantially higher than adults" Please explain how smaller body size leads to higher biological dose.

P. 5, line 27: extraneous open parenthesis mark

P. 7, line 30: "Recently, 26 reported that dermal exposure..." should be "Recently, Fenske et al.^26 reported that dermal exposure..."

P. 8, line 45: extraneous closing parenthesis mark.

P. 10, line 43: The meaning of the sentence beginning with "Finally, cumulative urinary TCPy..." is not clear.

Sup Fig 3a (symptoms vs TCPy) is compelling and should be in the paper.

P. 22, sentence beginning "Our study was conducted in..." is awkward

Page 22, "Results of our study may be generalizable only to agricultural communities with similar sociodemographic characteristics" is meaningless as I suspect that agricultural communities are likely to have similar sociodemographic characteristics (at least with respect to SES).

Response: The above revisions suggested by the reviewer have been made.

## VERSION 2 – REVIEW

<b>REVIEWER</b>	Kimberly Yolton Cincinnati Children's Hospital Medical Center USA
<b>REVIEW RETURNED</b>	31-Dec-2013

<b>GENERAL COMMENTS</b>	<p>The authors have been responsive to the reviewers' comments, and the manuscript is greatly improved in clarity and overall quality by the changes that have been made.</p> <p>In the previous review, I inquired about pretesting of collection supplies for pesticide residues. The authors have responded that "all urine collection cups were new and individually wrapped ... Therefore, pre-testing for pesticide residues was not required." The investigators should understand that sample collection containers and testing supplies are often contaminated with environmental agents during the manufacturing process. The fact that collection cups are new and individually wrapped does not guarantee that they have not been contaminated during manufacture. The highest quality studies include lot testing of collection supplies to ensure no contamination has occurred prior to collection of study samples. No changes are needed to the manuscript, but the authors should make note of this for future studies.</p> <p>Figure 1 has been improved.</p> <p>The pattern of shading that appears in the Table 3 is confusing. It seems that Time interval 7 should also be shaded if the shading is intended to indicate periods of CPF application.</p>
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<b>REVIEWER</b>	Peter Egeghy National Exposure Research Laboratory U.S. Environmental Protection Agency
<b>REVIEW RETURNED</b>	23-Dec-2013

<b>GENERAL COMMENTS</b>	The authors have done a good, comprehensive job of addressing previous comments. I believe the paper is of good quality and merits publication in BMJ Open.
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