OPEN PEER REVIEW

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Peer Review

Health Scope
Article DOI: https://doi.org/10.5812/jhealthscope.82962
Published in: Health Scope: 8(1) ; e82962

Peer Review Report for "Quantification of Urinary Metabolites of Toluene and Xylene Isomers as Biological Indices of Occupational Exposure in Printing Industry Workers"

Author(s): Maryam Hormozi, Ramazan Mirzaei, Ramazan Mirzaei, Alireza Nakhaee, Abolfazl Payandeh, Shahrokh Izadi, Javid - Dehghan Haghighi, Razzagh Rahimpoor

Review Timeline:

Submit Date:	5 Aug 2018
Revised Date:	14 Nov 2018
Accepted Date:	28 Nov 2018

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Monireh Khadem : Reviewer — 20 Aug 2018

Dear EIC:
The manuscript entitled "Quantification of urinary metabolites of toluene and xylene isomers as biological indices of occupational exposure in printing industry workers" was studied. According to the comments below, some corrections are required to decide on the publication of the manuscript.

Hossein Ansari : Reviewer — 12 Sep 2018

Dear Authors:
1- The chi-square test is not used to compare the means between groups. The statistical analysis section should be revised
2- In the first line of the results section, the authors have written that" there was no significant difference in the mean age, gender, smoking habit...".The mean of gender or smoking habit?!!! I do not understand.
3- It is unknown how many samples have been taken for the evaluation of exposure levels (I mean table 2)
4- In Table 3, there is no mean and variation for o-MHA and M-&p-MHA in the non-exposed group. How did the researchers examine and report the P-values?
5- It would be better if the results of multiple regression were reported.

Raheleh Hashemi Habybabady : Associate Editor 16 Sep 2018

Dear author, Please revise the following comments made by referees:
Reviewer1:
This article describes the evaluation of occupational exposure to toluene and xylene as well as biomarkers of exposure in workers of the printing industries. The present study has worked on environmental and biological monitoring for workers exposed to toluene and xylene. It found that the printing workers' occupational exposure to toluene was higher than the recommended value by ACGIH, and there was a positive correlation between the concentration of this compound in the air and biological samples.
I feel that studies on this topic, especially biological monitoring, can be valuable in industries. Since this manuscript, derived from a thesis project, is aimed to be published in an ISI journal, it is required to consider some comments and reply to some questions as follows:

I feel that studies on this topic, especially biological monitoring, can be valuable in industries. Since this manuscript, derived from a thesis project, is aimed to be published in an ISI journal, it is required to consider some comments and reply to some questions as follows:

1. In the objective section, you have said that " To the best of our knowledge, so far no study has been conducted in Iran on printing workers to consider occupational exposure to toluene and xylene and their relationship with exposure biomarkers". I think there is much work on environmental and biological monitoring of BTEX in workers of different industries, and this issue is a routine test in some workplaces around the world.

2. In the method section:

- Why did you apply the NIOSH method 2549? Usually, method 1501 is used to measure the exposure to BTEX.

- What was the length of the GC column? How could you separate xylene isomers (O-, P-, M-) in GC?

- You have said "C18 column and UV detector were used for the simultaneous determination of hippuric acid (HA) and methyl hippuric acids". It is recommended that a chromatogram related to this simultaneous determination be added to the manuscript.

- According to some literature, Toluene in alveolar air and o-cresol (o-CR) in urine can be used to monitor exposure to low levels of Toluene, whereas urinary hippuric acid (HA) cannot be used to monitor low exposure to this compound. Why did you select HA for monitoring?

3. In the result section:
- You have said: "Toluene exposure also correlated positively with levels of urinary biomarkers (m-, & p-MHA), while no significant correlation with the urinary o-MHA levels was observed". Why did you test the correlation between toluene concentration and biomarkers of xylene? Is it required to test this correlation?

- In your opinion, what is the reason for the higher amount of p-xylene than the other isomers?

- It is recommended to add some information about method validation, such as LOD, LOQ, RSD, and the Linear range of the calibration curve.

4. In the discussion section:
- Again, you explained about urinary HA and MHA as "It has been proposed that unmetabolized toluene in urine and blood samples, as the biological indicators of low-level exposure to toluene (e.g., in the range less than 50 ppm), could be a more specific biomarker than HA" and " This fact can be suggested as the antagonistic activity of solvents which is related to ….". As it was mentioned above, you can add some explanation about urinary o-cresol and the reasons for your selection (HA).

- Personal protective equipment (PPE) can considerably affect the amount of biomarkers. Usually, there is no correlation between the airborne concentration of a compound and its biomarker concentration when workers use PPE. It seems that you did not accurately consider the pattern of using PPE by tested workers. It is an important parameter, and I cannot see any information about that in demographic data or other part of the manuscript.

Reviewer2:
1- The chi-square test is not used to compare the means between groups. The statistical analysis section should be revised
2- In the first line of the results section, the authors have written that” there was no

significant difference in the mean age, gender, smoking habit...".The mean of gender or smoking habit?!!! I do not understand.
3- It is unknown how many samples have been taken for the evaluation of exposure levels (I mean table 2(
4- In Table 3, there is no mean and variation for o-MHA and M-&p-MHA in the non-exposed group. How did the researchers examine and report the p-values?
5- It would be better if the results of multiple regressions were reported

Ramazan Mirzaei : EIC

16 Sep 2018

Dear author, Please revise the following comments made by referees:
Reviewer1:
This article describes the evaluation of occupational exposure to toluene and xylene as well as biomarkers of exposure in workers of the printing industries. The present study has worked on environmental and biological monitoring for workers exposed to toluene and xylene. It found that the printing workers' occupational exposure to toluene was higher than the recommended value by ACGIH, and there was a positive correlation between the concentration of this compound in the air and biological samples.
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I feel that studies on this topic, especially biological monitoring, can be valuable in industries. Since this manuscript, derived from a thesis project, is aimed to be published in an ISI journal, it is required to consider some comments and reply to some questions as follows:

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2. In the method section:

- Why did you apply the NIOSH method 2549? Usually, method 1501 is used to measure the exposure to BTEX.

- What was the length of the GC column? How could you separate xylene isomers (O-, P-, M-) in GC?

- You have said "C18 column and UV detector were used for the simultaneous determination of hippuric acid (HA) and methyl hippuric acids". It is recommended

that a chromatogram related to this simultaneous determination be added to the manuscript.

- According to some literature, Toluene in alveolar air and o-cresol (o-CR) in urine can be used to monitor exposure to low levels of Toluene, whereas urinary hippuric acid (HA) cannot be used to monitor low exposure to this compound. Why did you select HA for monitoring?

3. In the result section:
- You have said: "Toluene exposure also correlated positively with levels of urinary biomarkers (m-, & p-MHA), while no significant correlation with the urinary o-MHA levels was observed". Why did you test the correlation between toluene concentration and biomarkers of xylene? Is it required to test this correlation?

- In your opinion, what is the reason for the higher amount of p-xylene than the other isomers?

- It is recommended to add some information about method validation, such as LOD, LOQ, RSD, and the Linear range of the calibration curve.

4. In the discussion section:
- Again, you explained about urinary HA and MHA as "It has been proposed that unmetabolized toluene in urine and blood samples, as the biological indicators of low-level exposure to toluene (e.g., in the range less than 50 ppm), could be a more specific biomarker than HA" and " This fact can be suggested as the antagonistic activity of solvents which is related to ….". As it was mentioned above, you can add some explanation about urinary o-cresol and the reasons for your selection (HA).

- Personal protective equipment (PPE) can considerably affect the amount of biomarkers. Usually, there is no correlation between the airborne concentration of a compound and its biomarker concentration when workers use PPE. It seems that you did not accurately consider the pattern of using PPE by tested workers. It is an important parameter, and I cannot see any information about that in demographic data or other part of the manuscript.

Reviewer2:
1- The chi-square test is not used to compare the means between groups. The statistical analysis section should be revised.
2- In the first line of the results section, the authors have written that" there was no significant difference in the mean age, gender, smoking habit...".The mean of gender or smoking habit?!!! I do not understand.
3- It is unknown how many samples have been taken for the evaluation of exposure levels (I mean table 2)

4- In Table 3, there is no mean and variation for o-MHA and M-&p-MHA in the non-exposed group. How did the researchers examine and report the values?

5- It would be better if the results of multiple regressions were reported.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Editor-In-Chief

“Journal of Health Scope”

Manuscript ID: 82962

The manuscript entitled "Quantification of urinary metabolites of toluene and xylene isomers as biological indices of occupational exposure in printing industry workers.

Firstly, we would like to thank the Journal reviewers for their time and valuable comments. Their comments have been highly appreciated and answered point by point and can be seen in red color in the main text of the revised article:

Reviewer 1:

- In the objective section, you have said that “To the best of our knowledge, so far no study has been conducted in Iran on printing workers to consider occupational exposure to toluene and xylene and their relationship with exposure biomarkers". I think there is much work on environmental and biological monitoring of BTEX in workers of different industries, and this issue is a routine test in some workplaces around the world.

With many thanks, as the respectable referee pointed out, several studies have been carried out at various occupational settings, but so far, no study has been conducted in Iran on workers in the printing industry.

- In the method section:

- Why did you apply the NIOSH method 2549? Usually, method 1501 is used to measure the exposure to BTEX.

Thank you very much for your comments. It was a typographical mistake, as the NIOSH method 1501 for air monitoring toluene and xylene isomers was applied; we corrected it according to page 3, line 26 in the revised manuscript.

- What was the length of the GC column? How could you separate xylene isomers (O-, P-, and M-) in GC?

A GC column capable of separating mixed xylenes from the extraction solvent. An HP-5 Agilent fused-silica capillary column (30 m × 0.32 mm i.d. × 0.25 mm film thickness) was applied for the separation of analyses.

- You have said "C18 column and UV detector were used for the simultaneous determination of hippuric acid (HA) and methyl hippuric acids". It is recommended that a chromatogram related to this simultaneous determination be added to the manuscript.

According to your invaluable comment, the chromatogram related to the simultaneous determination of hippuric acid (HA) and methyl hippuric acids was added to the revised manuscript according to Figure 4 and page 6, lines 27 to 29.

- According to some literature, Toluene in alveolar air and o-cresol (o-CR) in urine can be used to monitor exposure to low levels of Toluene, whereas urinary hippuric acid (HA) cannot be used to monitor low exposure to this compound. Why did you select HA for monitoring?

Appreciating your insightful comment, hippuric acid is the principal metabolite of toluene. According to some studies (e.g., Ikeda et al., 2008), the validity of o-CR and HA in urine as markers to monitor TWA toluene exposure will be reduced when the toluene exposure is low (e.g., in the range less than 50 ppm). However, it has been pointed out that HA and o-CR are among the markers of choice to monitor occupational toluene exposure at high levels, and that only metabolized toluene in urine or in blood is recommended when toluene exposure level is low (e.g., 10 ppm or less). In our study, the mean of toluene exposure was at high levels (about 38 ppm). Therefore, we selected urinary hippuric acid (HA) for monitoring.

• In the result section:

- You have said: "Toluene exposure also correlated positively with levels of urinary biomarkers (m-, & p-MHA), while no significant correlation with the urinary o-MHA levels was observed". Why did you test the correlation between toluene concentration and biomarkers of xylene? Is it required to test this correlation?

Thanks again for your comment. Previous studies have shown the correlation coefficient between the concentration of different solvents, such as toluene and xylene isomers, in urine. Given that, in the printing industries studied, no information on solvent components was available, and many hazardous compounds were undisclosed in the labels of the containers. Thus, our results may suggest using at least one type of solvent-containing material (i.e., toluene with xylene) for solvent component analysis in the printing industry.

- In your opinion, what is the reason for the higher amount of p-xylene than the other isomers?

Appreciating your comment, it is mentioned that individual xylene isomers can be present in the workplace in any combination and level. In agreement with the previous studies (Reference No. 29), the results of this study showed that the level of p-xylene was slightly higher than o-xylene, which may concern as a major combination of xylene isomers.

- It is recommended to add some information about method validation, such as LOD, LOQ, RSD, and the linear range of the calibration curve.

In this study, the evaluation of LOD was performed according to the method standardized and validated by the National Institute for Occupational Safety and Health (NIOSH, 8301) and can be seen in the main text of the manuscript (page 4, lines 19 to 20).

Following your useful comment, information about method RSD, and the Linear range of the calibration curve was added to the revised article according to page 4, lines 22 to 27 and page 5, lines 1 to 6.

• In the discussion section:

- Again, you explained about urinary HA and MHA as "It has been proposed that metabolized toluene in urine and blood samples, as the biological indicators of low-level exposure to toluene (e.g., in the range less than 50 ppm), could be a more specific biomarker than HA" and " This fact can be suggested as the antagonistic activity of solvents which is related to ….". As it was mentioned above, you can add some explanation about urinary o-cresol and the reasons for your selection (HA).

As we explained in the above comment, according to some studies (reference No.22), although the validity of o-CR and HA in urine as markers to monitor TWA toluene exposure will be reduced when the toluene exposure is low (e.g., in the range less than 50 ppm). However, it has been pointed out that HA and o-CR are among the markers of choice to monitor occupational toluene exposure at high levels, and that only metabolized toluene in urine or in blood is recommended when toluene exposure level is low (e.g., 10 ppm or less).

In addition, toluene is eliminated from the human body partly by expiration of metabolized toluene (15 %-20 %) and partly as the urinary metabolites hippuric acid and cresols. The major metabolic pathway is oxidation of the side chain to benzoic acid, followed by conjugation with glycine to hippuric acid (about 80 %). However, only a very small proportion of the retained toluene is excreted as o-cresol (about 1 %), which raises the question whether this metabolite really is suitable for biological monitoring at low exposures.

Appreciating your insightful comment, and also the explanations given in the above comment, we corrected this sentence by replacing reference No.22 and also, in the main text of the revised article (page 7, lines 29 to 30 and page 8, lines 1 to 3).

- Personal protective equipment (PPE) can considerably affect the amount of biomarkers. Usually, there is no correlation between the airborne concentration of a compound and its biomarker concentration when workers use PPE. It seems that you did not accurately consider the pattern of using PPE by tested workers. It is an important parameter, and I cannot see any information about that in demographic data or other part of the manuscript.

With many thanks, as none of the printing workers in this study used gloves, glasses, and/or respiratory protective equipment, and ventilation and climate control were generally not sufficient. So, we couldn't analyses the correlation between airborne concentration of solvents and their biomarker levels and usage of PPE. Also, we have mentioned in the “Discussion section” on page 9, lines 11 to 12.

Reviewer 2:

1- The chi-square test is not used to compare the means between groups. The statistical analysis section should be revised

Thanks a lot for your comment. We have employed the chi-square test to compare proportions between exposed and non-exposed groups. It was a typographical mistake in reporting of results in Table 1. Hence, we corrected the “Statistical Analysis” section and Table 1, and may be seen in red color on page 5, line 13 in the revised article.

2- In the first line of the results section, the authors have written that" there was no significant difference in the mean age, gender, smoking habit…".The mean of gender or smoking habit?!!! I do not understand.

With many thanks, for the clarity of the sentence, the "Results section" was edited according to page 5, lines 22 to 23.

3- It is unknown how many samples have been taken for the evaluation of exposure levels (I mean table 2)

We have mentioned in Table 2 the sample size for the Exposed group (n=44)

4- In Table 3, there is no mean and variation for o-MHA and M-&p-MHA in the non-exposed group. How did the researchers examine and report the P values?

Thanks a lot for your great idea. As slight concentrations of o-MHA and M-&p-MHA were found in the urine of the non-exposed group, we disregard these values. However, according to your useful comment, data was added to Table 3.

5- It would be better if the results of multiple regressions were reported

Multiple regression analysis was applied to identify factors that affect urinary solvent concentration. Regarding the fact that in the fit of multiple regression model, the effect of independent variables was not significant, which can be due to a lack of information in some category of qualitative variables? Therefore, the discussion section was edited by deleting the results of this analysis according to page 8, lines 28 to 30.

OPEN PEER REVIEW

Revision (1)

Monireh Khadem : Reviewer — 3 Nov 2018

Dear Editor of Health Scope:
The revised version of manuscript No. 82962 was reviewed. The author's questions and the corrections in the manuscript are satisfactory. In my opinion, this manuscript can be considered for publication in Health Scope. The final decision depends on the editorial section and the viewpoints of other reviewers. There is no reviewing file as an attachment.

Regards,

Hossein Ansari : Reviewer — 5 Nov 2018

Dear editor:
The authors have revised the manuscript partly based on the reviewers' comments, but in my opinion, the results of multiple regression should be reported.

Raheleh Hashemi Habybabady : Associate Editor — 12 Nov 2018

Dear Author:
Your article has been reviewed, and the manuscript has been revised based on the reviewers' comments. However, according to one of the reviewers, the following minor revision is recommended.

Reviewer 1:

In my opinion, the results of multiple regression should be reported.

Ramazan Mirzaei : EIC — 12 Nov 2018

Dear Author:
Your article has been reviewed, and the manuscript has been revised based on the reviewers' comments. However, according to one of the reviewers, the following minor revision is recommended.

Reviewer 1:

In my opinion, the results of multiple regression should be reported.

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Editor-In-Chief
" Journal of Health Scope "

The manuscript entitled **"Quantification of urinary metabolites of toluene and xylene isomers as biological indices of occupational exposure in printing industry workers.**

Firstly, we would like to thank Journal reviewers for their time and valuable comments.

Comment of Reviewer 2:

1- In my opinion the results of multiple regression should be reported

Thanks a lot for your great idea, as we explained in the revised manuscript, regarding the fact that in the fit of multiple regression model, the effect of independent variables was not significant, which can be due to a lack of information in some category of qualitative variables. Therefore, we could not report the results of multiple regression.

OPEN PEER REVIEW

Revision (2)

Raheleh Hashemi Habybabady : Associate Editor — 26 Nov 2018

Dear Author:
Given that the authors have carefully answered all the comments and confirmed by the reviewers, the article is ready for publication in this journal.

Ramazan Mirzaei : EIC — 28 Nov 2018

Dear EIC:
Given that the authors have carefully answered all the comments and confirmed by the reviewers, the article is ready for publication in this journal.