Letter to the Editor

$PM_{2.5}$–Metabolic Syndrome Causal Association: A Mendelian Randomization Study*

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Metabolic syndrome (MS) is a common metabolic disorder status and defined as a pathology characterized by abdominal obesity, insulin resistance, hypertension, and hyperlipidemia (atherosclerosis) status[1]. Evidence suggests that the underlying etiology of MS is multifactorial and includes environmental factors, tobacco use, high alcohol consumption, dietary risks, and low physical activity. Thus, a deeper understanding of MS and its risk factors will be beneficial for mitigating health damage and improving the quality of life in high-risk subpopulations. $PM_{2.5}$ is an important environmental pollutant, poses a serious threat to human health, and is associated with various health problems. Several epidemiological studies have shown that exposure to $PM_{2.5}$ may influence the development of diabetes[2], cardiovascular disease, obesity, and MS. Existing evidence also suggests that the components of MS are sensitive to environmental pollutants, especially $PM_{2.5}$. However, most of them were observational studies or literature analyses based on epidemiology, and the evidence for the causal association between $PM_{2.5}$, MS, and its components remains limited. Mendelian randomization (MR), as a robust tool for making causal inferences, has been widely used in causal inference and can also overcome the limitations of observational studies. It uses single nucleotide polymorphisms (SNPs) that are closely related to exposure factors as instrumental variables to avoid the effects of confounding factors and reverse causation. As gametes follow the Mendelian laws of inheritance at formation, random assignment of alleles at conception eliminates confounding bias and follows the temporal nature of causation. Motivated by the above discussion, in this study, we first performed a two-sample MR analysis to determine the association between $PM_{2.5}$, MS, and its components, using summary-level Genome-Wide Association Studies (GWAS) datasets. Multivariable MR analysis was carried out to adjust for the effect of several potential factors on the association between $PM_{2.5}$ and MS. Furthermore, mediation MR analysis was used to verify the mediators that modified the association between $PM_{2.5}$ and MS.

According to the International Diabetes Federation (IDF) criteria for the metabolic syndrome, MS consists of five components, namely waist circumference (WA, European, male: > 94 cm, female: > 80 cm), blood pressure/hypertension (HT, systolic pressure: > 130 mmHg or diastolic pressure: > 85 mmHg), diabetes mellitus (DM, FBG: > 5.6 mmol/L), triglycerides (TG, > 1.7 mmol/L) and high-density lipoprotein cholesterol (HDL-C, male: < 0.9 mmol/L, female: < 1.1 mmol/L). For the $PM_{2.5}$, pooled genetic data were obtained from the UK Biobank GWAS (GWAS ID: ukb-b-10817), which included 423,796 European participants. This study was based on the European Cohort Study of Air Pollution Effects (ESCAPE project) and used land use regression (LUR model) to assess $PM_{2.5}$ concentrations in participants' residences. Summary-level data for MS were also available from the UK Biobank GWAS[3] (GWAS ID: ebi-a-GCST009602) and consisted of 291,107 individuals (59,677 cases and 231,430 controls). These data are the result of a GWAS analysis, aimed at exploring the relationship between population SNP and metabolic syndrome at

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the genetic level and constructing a correlation dataset between SNP and disease characterization, with genotypes, effect gene frequencies, effect coefficients for outcomes, and *P* values in the dataset. A summary of the genome-wide association data used in this study is presented in Supplementary Table S1 (available in www.besjournal.com).

The MR study was developed based on the instrumental variables (IVs). Single-nucleotide polymorphisms (SNPs) from the exposure dataset were used as genetic IVs. As listed in Figure 1, an MR study was conducted based on the three basic assumptions of IVs (more details can be found in Figure 1A). We chose $P < 1 \times 10^{-5}$ as the screening criterion for IVs. In addition, the function of the extraction instruments in the two-sample MR package was used to exclude contiguous imbalance ($R^2 < 0.001$, window distance > 10,000 kb) when the exposed SNPs were included. In addition, we used the Pheno Scanner tool to identify and exclude SNP nucleotide polymorphisms significantly associated with $PM_{2.5}$ which may affect MS through other biological pathways.

A flowchart of the two-sample MR design is shown in Figure 1. The GWAS database summary-level data on $PM_{2.5}$, MS, and its components were collected from the IEU-GWAS and GWAS catalogs, respectively, and two-sample MR analyses were performed to assess the association of $PM_{2.5}$, MR, and its components based on the genetic IVs. Inverse variance weighting (IVW) is a standard method used in Mendelian randomization analysis. In the IVW method, the existence of intercept terms is not considered and the influence of each instrumental variable is assigned a weight that is the reciprocal of its variance. The MR-Egger was modified based on IVW, allowing for multi-validity detection while considering the presence of the intercept terms. The weighted median, simple mode, and weighted mode are commonly used estimation methods in Mendelian randomization. MR-Egger regression was used to analyze pleiotropy, testing for the presence of pleiotropic SNPs in the IVs (intercept close to 0 or $P > 0.05$, indicating no multiplicity[4]). The Cochran's Q statistic for MR-Egger and IVW was used to test for heterogeneity between IVs.

In the multivariate MR analysis, we accounted for some potential life habits for consolidation and adjustment of the causal relationship between $PM_{2.5}$ and MS, including smoking, alcohol intake frequency, and depressed mood[5-7], and used the IVW method for multivariate MR analysis. Multiple comparisons were corrected using the Bonferroni correction

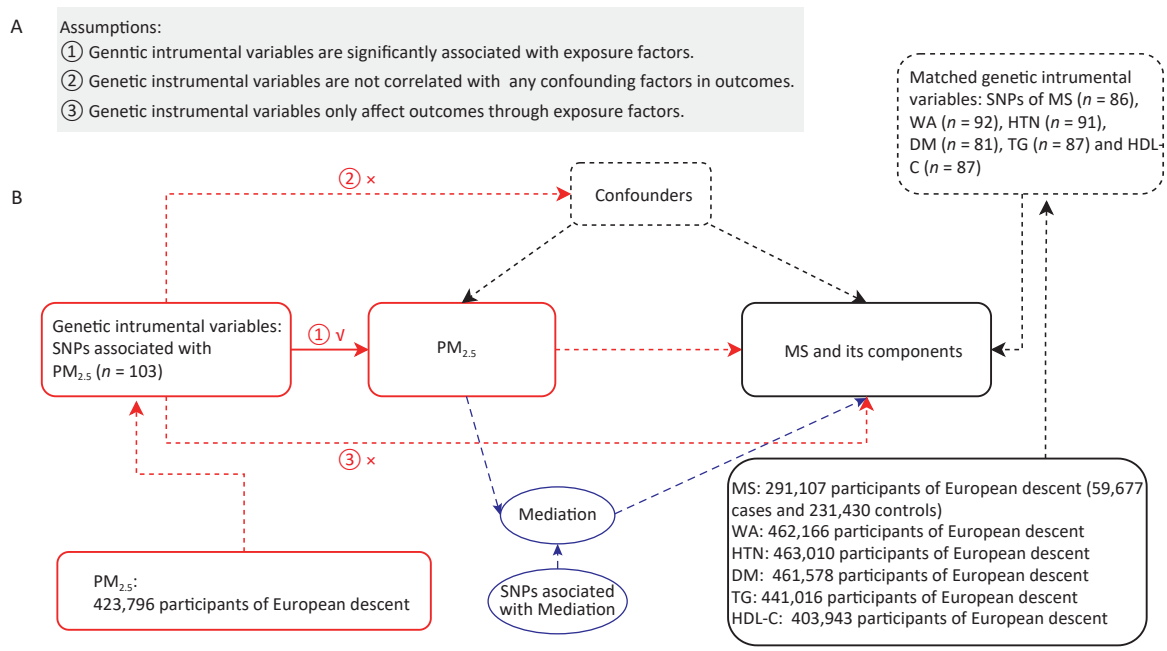

Figure 1. Mendelian randomization study design for $PM_{2.5}$ and MS. (A) Mendelian randomization analysis based on three basic assumptions. (B) Flowchart of study design. MS, metabolic syndrome; WA, waist circumference; HT, hypertension; DM, diabetes mellitus; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol.

method, and the critical *P*-value was determined by the number of confounding exposures *e* as follows: $P = 0.05/e$. Furthermore, we also performed a mediation MR analysis (two-step MR) to validate and analyze the mediators that indirectly affected the association between $PM_{2.5}$ and MS.

After excluding linkage disequilibrium (R^2 = 0.001, kb = 10,000), we extracted 103 SNPs corresponding to $PM_{2.5}$ from the GWAS dataset based on the criterion of $P < 1 \times 10^{-5}$. At the same time, 11 SNPs were associated with possible mechanistic pathways to MS (such as BMI, adiposity, and coronary heart disease); thus, these 11 SNPs were excluded from validation using the Pheno Scanner tool (Supplementary Figure S1 and Supplementary Table S2, available in www.besjournal.com) according to the basic assumptions of IVs (iii). Finally, 92 SNPs were identified for further MR analyses (Supplementary Table S3 and Supplementary Table S4, available in www.besjournal.com).

The MR-Egger intercept analysis showed that there was no pleiotropy in the MR analysis of MS and its components (Supplementary Table S5, available in www.besjournal.com). The Cochran's Q statistic for MR-Egger and IVW in the heterogeneity test suggested possible heterogeneity due to individual variations (P_{Q1} = 0.047, P_{Q2} = 0.054).

The results of the two-sample MR analysis of the associations between $PM_{2.5}$, MS, and their components are shown in Figure 2A–B, respectively. In the random-effects IVW model, MS may increase by 23.5% when the concentration of $PM_{2.5}$ increases by one standard deviation (*OR* = 1.235, 95% *CI*: 1.061–1.437, *P* = 0.006). The MR-Egger, Simple mode, and weighted mode models also validated the direction of the causal association, which is consistent with the IVW model. Previous studies

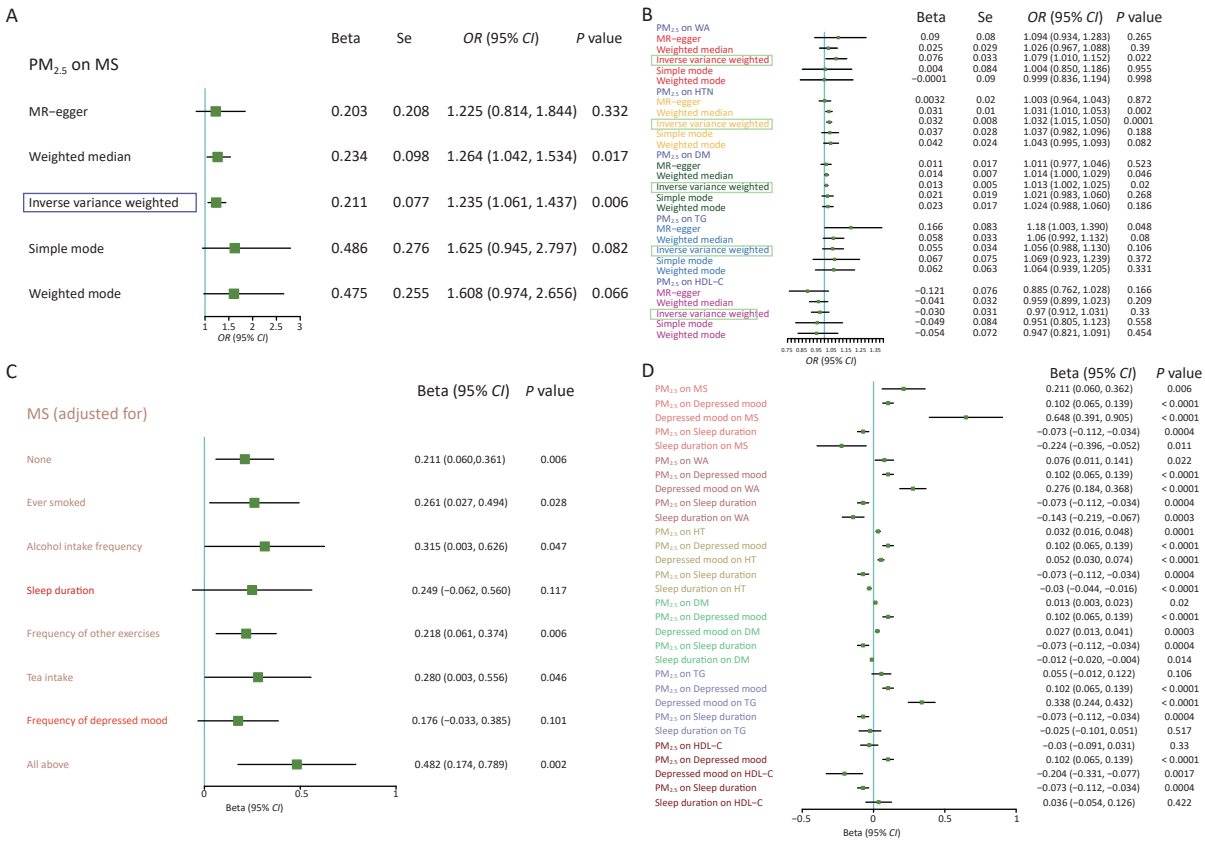

Figure 2. MR analysis results of $PM_{2.5}$ on metabolic syndrome and components. (A) MR analysis results of the effects of $PM_{2.5}$ on metabolic syndrome. (B) MR analysis results of $PM_{2.5}$ on the components of MS. (C) Multivariate analysis of the impact of $PM_{2.5}$ on metabolic syndrome. (D) Intermediary analysis of $PM_{2.5}$ on metabolic syndrome and its components. MS, metabolic syndrome; WA, waist circumference; HT, hypertension; DM, diabetes mellitus; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; MR, Mendelian randomization.

have shown that atmospheric pollutants such as $PM_{2.5}$ have an impact on chronic diseases such as metabolic syndrome, and our results have confirmed this, further demonstrating the potential threat of $PM_{2.5}$ to public health. For the MS components, these SNPs (genetic predictors of $PM_{2.5}$) were positively correlated with WA, HT, and DM (all $P < 0.05$). More precisely, the random-effects IVW models indicated that the associated effects of $PM_{2.5}$ on WA were *OR* = 1.079 (95% *CI*: 1.010–1.152, *P* = 0.022), 1.032 (95% *CI*: 1.015–1.050, *P* = 0.0001), and 1.013 (95% *CI*, 1.002–1.025; *P* = 0.020), respectively. This indicates that $PM_{2.5}$, mainly reflected in obesity, blood sugar, and blood pressure, may affect multiple aspects of metabolic processes. There was no statistical correlation between the impact of $PM_{2.5}$ on blood lipids, which may be due to the smaller impact of $PM_{2.5}$ on these specific indicators or the need for larger sample sizes and more accurate methods for validation (Supplementary Table S6, available in www.besjournal.com). Supplementary Figure S2 (available in www.besjournal.com) shows the influence of individual SNPs on outcomes and their combined effect. As shown in Supplementary Figure S3 (available in www.besjournal.com), the leave-one-out tests also validated that the association between $PM_{2.5}$, MS, and its components (WA, HT, and DM) was effective and robust. The funnel plot of Supplementary Figure S4 (available in www.besjournal.com) shows that the scatter is symmetrical, and the SNPs scatter plot of exposure and outcome is shown in Supplementary Figure S5 (available in www.besjournal.com).

We performed multivariate MR analysis to further evaluate the relationship between $PM_{2.5}$ and MS after adjusting for several potential confounding factors (Figure 2C). After adjusting for these confounding factors such as ever smoked, alcohol intake frequency, sleep duration, frequency of exercise, tea intake, and depressed mood, the relationship between $PM_{2.5}$ and MS still has significance (β = 0.482, *P* = 0.002). However, after adjusting for sleep duration and frequency of depressed mood, the relationship between $PM_{2.5}$ and MS was not significant.

To further estimate the association between $PM_{2.5}$ and MS based on the results of multivariate MR analysis, we also performed the Mediation analysis to assess the mediating effect of the sleep duration and frequency of depressed mood on the relationship between $PM_{2.5}$ and MS. As given in Figure 2D, the result suggested that the causal effect of $PM_{2.5}$ on MS may be partially mediated by frequency of depressed mood (approximately mediated by 31.32%, 95% *CI*: 13.06%–106.22%) and sleep duration (approximately mediated by 7.74%, 95% *CI*: 1.27%–31.60%), respectively. Furthermore, for the components of MS, the frequency of depressed mood mediated approximately 37.04% (95% *CI*: 14.52%–167.42%), 16.57% (95% *CI*: 7.30%–36.91%) and 21.18% (95% *CI*: 6.98%–80.25%) of the relationship between $PM_{2.5}$ and WA, HT and DM, and the sleep duration mediated approximately 13.73% (95% *CI*: 3.69%–64.07%), 6.84% (95% *CI*: 2.51%–16.68%) and 6.73% (95% *CI*: 1.42%–28.49%) of the relationship between $PM_{2.5}$ and WA, HT and DM. Interestingly, the relationship between $PM_{2.5}$, TG, and HDL-C was completely mediated by the frequency of depressed mood, which may be one reason for the insignificant relationship between $PM_{2.5}$, TG, and HDL-C levels.

As both $PM_{2.5}$ and MS are risk factors for cardiovascular diseases, their relationship has attracted extensive attention in recent years. Previous epidemiological studies have shown that $PM_{2.5}$ may be significantly and positively associated with the risk of developing metabolic syndrome. However, evidence for a causal association between $PM_{2.5}$, MS, and its components remains limited. In this study, we first used a two-sample MR to analyze the causal association between $PM_{2.5}$, MS, and its five components. Two-sample MR analysis results indicated that MS may increase by 23.5% excess risk when the concentration of $PM_{2.5}$ increases by one standard deviation (*OR* = 1.235, 95% *CI*: 1.061–1.437), these results were consistent with some existing studies across different subpopulation and regions.

As for the five components of MS, Chen et al.[8] indicated that every 10-$\mu g/m^3$ increase in annual $PM_{2.5}$ concentration was associated with an increased risk of abdominal obesity, hypertriglyceridemia, low HDL-C, hypertension, and elevated fasting blood glucose by using a longitudinal cohort in Taiwan, China. Rachel et al.[9] used a Cox Proportional Risk model and found that each 1 $\mu g/m^3$ increase in annual mean $PM_{2.5}$ concentration was associated with an HR of 1.27 (95% *CI*: 1.06–1.52) for the risk of MS, and also influenced elevated fasting glucose and hypertriglyceridemia. Moreover, some studies have suggested that an increase in $PM_{2.5}$ may be associated with increased waist circumference, HT, and the onset of diabetes. Consistent with this evidence, our two-sample MR analysis proposed that the associated effects of $PM_{2.5}$ on WA were *OR* =

1.079 (95% *CI*: 1.010–1.152), 1.032 (95% *CI*: 1.015–1.050), and 1.013 (95% *CI*: 1.002–1.025), respectively.

Furthermore, mediation analysis of MR suggested that depression and sleep duration may play a mediating role in the association between $PM_{2.5}$ and MS. The causal effect of $PM_{2.5}$ on MS may be partially mediated by the frequency of depressed mood (approximately mediated by 31.32%) and sleep duration (approximately mediated by 7.74%). $PM_{2.5}$ exposure may affect inflammation *via* the NLRP3 single pathway, which plays an important role in inducing depression. Sympathetic nervous system nerve excitation and release of pro-inflammatory factors caused by insufficient sleep lead to insulin resistance. By extension, public health strategies such as developing more rational emotional regulation and increasing sleep duration may be beneficial for mitigating the adverse effects of $PM_{2.5}$.

The biological mechanisms linking $PM_{2.5}$, MS, and its components are still limited, and some reasons may explain the relationship between $PM_{2.5}$, MS, and its five components. First, the effect of $PM_{2.5}$ on obesity may occur through systemic inflammation, altered energy metabolism, and other pathways. Secondly, abnormal energy metabolism is one of the causes[10]. Organisms exposed to $PM_{2.5}$ may affect their own and their offspring's energy balance by inducing brown adipose tissue (BAT) bleaching and regulating food intake, causing fat accumulation. Thirdly, many studies have shown that $PM_{2.5}$ can affect blood sugar and blood pressure, including but not limited to inducing insulin resistance, promoting catecholamine release, and the Notch signaling pathway.

Our study has the following limitations. First, the GWAS data used for the study were from Europe; further studies on other national populations are needed to improve the generalizability of the results. The fact that the data for different components were obtained from different studies may also have led to bias in the results. Second, due to a lack of sufficient SNPs after linkage disequilibrium, we relaxed the *P*-value threshold ($P < 1 \times 10^{-5}$) of SNPs of $PM_{2.5}$ in accordance with previous studies, which might have led to weak instrumental variables. To address this issue, we calculated the *F*-statistics to measure the power of each SNP. All SNPs used in the study with an *F*-statistic greater than 10 indicated the absence of weak instrument bias. Third, although the hypothesis of exclusivity was passed by the multiplicity test with the Phenoscanner search, we were unable to eliminate the potential multiplicity problem that may have arisen from the low number of instrumental variables. Based on the results of multivariate MR, possible mediating factors were selected; therefore, we did not conduct a separate multivariate MR analysis of the components of MS.

Our findings suggest that there may be a relationship between $PM_{2.5}$ and MS. For the MS component, $PM_{2.5}$ was more likely to affect the metabolic syndrome through waist circumference, hypertension, and hyperglycemia. Mediation MR analysis found that the association of $PM_{2.5}$ with MS may be partially mediated by the frequency of depressed mood (approximately 31.32%) and sleep duration (approximately 7.74%). Our findings recommended that the prevention and management of $PM_{2.5}$ might be enhanced for MS and its components prevention. Although these findings provide strong evidence, they should be interpreted cautiously. First, these studies were based on the genetic prediction of $PM_{2.5}$, rather than directly measuring exposure levels. Therefore, these results may be influenced by the accuracy of genetic predictions. Second, these studies were based on specific population samples and may not fully represent the entire population. Therefore, these results must be validated in larger populations.

Author Contribution Fan Ding: Analyze data and write articles, and revise subsequent articles. Ning Ma: English polishing. Shi Zhao: English polishing and article revision. Qingan Wang: Edit article pictures. Zhanbing Ma: Provide Mendelian randomization analysis ideas. Yuhong Zhang: Review of article revision. Yi Zhao: Provide guidance of physiological professional knowledge. Yu Zhao: Article writing guidance and fund support.

Ethics Approval and Consent to Participate Not applicable.

Conflict of Interest Not applicable.

Availability of Data and Materials Data may be made available by contacting the first author or corresponding author.

Competing Interests The authors declare no conflicts of interest relevant to this study.

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REFERENCES

1. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet, 2005; 365, 1415−28.
2. Li YZ, Xu L, Shan ZP, et al. Association between air pollution and type 2 diabetes: an updated review of the literature. Ther Adv Endocrinol Metab, 2019; 10, 1906149674.
3. Lind L. Genome-wide association study of the metabolic syndrome in UK biobank. Metab Syndr Relat Disord, 2019; 17, 505−11.
4. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. Eur J Epidemiol, 2017; 32, 377−89.
5. Chu C, Zhang HY, Cui SJ, et al. Ambient $PM_{2.5}$ caused depressive-like responses through Nrf2/NLRP3 signaling pathway modulating inflammation. J Hazard Mater, 2019; 369, 180−90.
6. Choi HI, Lee SJ, Kang JG, et al. Association of environmental tobacco smoke exposure with metabolic syndrome: a longitudinal Cohort Study of 71, 055 never smokers. Nutr Metab Cardiovasc Dis, 2022; 3, 2534−43.
7. Sun K, Ren M, Liu D, et al. Alcohol consumption and risk of metabolic syndrome: a meta-analysis of prospective studies. Clin Nutr, 2014; 33, 596−602.
8. Chen YC, Chin WS, Pan SC, et al. Long-term exposure to air pollution and the occurrence of metabolic syndrome and its components in Taiwan. Environ Health Perspect, 2023; 131, 17001.
9. Wallwork RS, Colicino E, Zhong J, et al. Ambient fine particulate matter, outdoor temperature, and risk of metabolic syndrome. Am J Epidemiol, 2017; 185, 30−9.
10. Chen MJ, Liang S, Zhou HF, et al. Prenatal and postnatal mothering by diesel exhaust $PM_{2.5}$-exposed dams differentially program mouse energy metabolism. Part Fibre Toxicol, 2017; 14, 3.