Environmental Pollution 289 (2021) 117913

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Urinary metabolites of multiple volatile organic compounds among general population in Wuhan, central China: Inter-day reproducibility, seasonal difference, and their associations with oxidative stress biomarkers☆

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ARTICLE INFO

Keywords:
Urine
Volatile organic compound metabolites
Oxidative stress biomarkers
Variability
Weighted quantile sum regression
General population

ABSTRACT

General population are concurrently and extensively exposed to many volatile organic compounds (VOCs), including some Group 1 human carcinogens, such as 1,3-butadiene. However, only a few studies assessed internal exposure levels of VOCs; particularly, very limited studies have examined associations between the urinary concentrations of multiple VOC metabolites (mVOCs) and oxidative stress biomarkers (OSBs) among the general population. In this study, 21 mVOCs and three OSBs including 8-hydroxy-2′-deoxyguanosine (8-OHdG; for DNA), 8-hydroxyguanosine (8-OHG; for RNA), and 4-hydroxy nonenal mercapturic acid (HNEMA; for lipid) were measured in 406 urine samples collected from 128 healthy adults during autumn and winter of 2018 in Wuhan, central China, including repeated samples taken in 3 d from 75 volunteers. Inter-day reproducibility for most mVOCs was good to excellent; urinary concentrations of mVOCs in winter were generally higher than those in autumn. Risk assessment was conducted by calculating hazard quotients for the parent compounds, and the results suggested that acrolein, 1,3-butadiene, and cyanide should be considered as high-priority hazardous ones for management. After false-discovery adjustment, 16 of the studied mVOCs were positively associated with 8-OHdG and 8-OHG (β values ranged from 0.04 to 0.48), and four mVOCs were positively associated with HNEMA (β values ranged from 0.21 to 0.78). Weighted quantile sum regression analyses were used to assess associations of mVOC mixture and OSBs, and we found significantly positive associations between the mixture index and OSBs, among which the strongest mVOC contributors for the associations were 2-methylhippuric acid for both DNA (20%) and RNA (17%) oxidative damage, and *trans,trans*-muconic acid (50%) for lipid peroxidation. This study firstly reported good to excellent short-term reproducibility, seasonal difference in autumn and winter, and possible health risk in urinary concentrations of multiple mVOCs among the general population.

1. Introduction

General population could be concurrently exposed to multiple volatile organic compounds (VOCs). Some of the VOCs could be only absorbed by inhalation, such as acrylonitrile (USEPA, 2016d), 1-bromopropane (OSHA/NIOSH, 2013; IARC, 2018a), and 1,3-butadiene (USEPA, 2016a); while some other VOCs could be absorbed by both dietary and inhalation pathways, such as acrylamide (Mojska et al., 2016), acrolein (Stevens and Maier, 2008), and crotonaldehyde (Bagchi et al., 2018) (details listed in Table S1).

Many of the VOCs are classified as human carcinogens, such as 1,3-butadiene (IARC, 2018b), ethylene oxide (IARC, 2018c), and vinyl chloride (IARC, 2008), but some of them are not, such as carbon disulfide (ATSDR, 1996) and cyanide (ATSDR, 2006) (details listed in Table S1). Some of the VOCs have low reference doses (RfDs), such as 0.5 μg/kg-body weight [bw]/day for acrolein (USEPA, 2016b), 0.6

☆ This paper has been recommended for acceptance by Wen Chen.

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https://doi.org/10.1016/j.envpol.2021.117913
Received 17 May 2021; Received in revised form 17 July 2021; Accepted 3 August 2021
Available online 5 August 2021

μg/kg-bw/day for hydrogen cyanide (USEPA, 2010), and 2 μg/kg-bw/day for acrylamide (USEPA, 2016c), while some of them have relatively higher RfDs as 100 μg/kg-bw/day [for carbon disulfide (USEPA, 2016e), *N*,*N*-dimethylformamide (USEPA, 2016g), and ethylbenzene (USEPA, 2016f)] or 200 μg/kg-bw/day [for styrene (USEPA, 2020) and xylene (Bayil et al., 2008)] (details listed in Table S1). Given the fact that the general population are widely exposed to VOCs which may possess different degrees of toxicities, identification of high-priority hazardous ones is important and can provide a basis for regulation and management. Although previous studies have conducted risk assessment of exposure to VOCs based on ambient concentration (Hu et al., 2018; Kumar et al., 2018; Zheng et al., 2020), few studies have focused on biomonitoring-based risk assessment which directly measure individual exposures and integrate exposures via multiple pathways and sources (Aylward et al., 2013).

Previous studies have suggested that urinary metabolites of VOCs (mVOCs) are useful biomarkers for assessing VOC exposure with the longer physiological half-lives of metabolites compared with the parent compounds, and the specificity of most mercapturic acid metabolites (Alwis et al., 2012). On the basis of an integrated analytical method (Alwis et al., 2012), several studies have assessed multiple urinary mVOCs among infants (El-Metwally et al., 2018), children (Jain, 2015; Kuang et al., 2019; Kuang et al., 2021), pregnant women (Boyle et al., 2016), adolescents (Jain, 2016), adults (Alwis et al., 2012), and marijuana users (Wei et al., 2016). However, the temporal variability of mVOCs in urine is still unclear (Li et al., 2021). No biomonitoring study has assessed the inter-day reproducibility and seasonal difference of the mVOCs, with repeated measurements. In addition, previous studies among occupational workers indicated that some mVOCs may cause oxidative stress (Kim et al., 2011; Sisto et al., 2020b), which is considered to be an important component of various diseases including cancer (Sosa et al., 2013). Nevertheless, very limited studies were conducted to assess the associations between urinary concentrations of multiple mVOCs and oxidative stress biomarkers (OSBs) among the general population (Kuang et al., 2019; Kuang et al., 2021); particularly, no study assessed the associations between multiple mVOCs and the OSBs of RNA (Weimann et al., 2002) and lipid (Wu et al., 2016) among the general population.

Since human beings are exposed to multiple VOCs concurrently and some mVOCs have relatively high correlations with each other, the effect of the mVOC mixture on the OSBs and the issue of collinearity has not been addressed. The newly developed method of weighted quantile sum (WQS) regression analysis (Czarnota et al., 2015) which derives a mixture index (represents chemicals scored into quantiles, and each factor's weight is calculated based on its contribution to the cumulative association with the outcome) can reduce dimensionality and address issues that arise with collinearity, then assess the mixture effect of co-exposure as well as identify the individual chemicals most strongly associated with the outcome.

Based on previous publications (Alwis et al., 2012; Kuang et al., 2019), this study was conducted to assess inter-day reproducibility and seasonal difference in multiple mVOCs, and health risks by comparing estimated daily intakes (EDIs, based on urinary mVOCs) against RfDs for the first time. This study also assessed the individual associations between the mVOCs and the select OSBs in urine, and further examined the mixture effect of multiple mVOCs on OSBs and individual contributions of each mVOC by WQS regression analyses.

2. Materials and methods

2.1. Standards and reagents

The details about the standards of target analytes and their isotope-labeled internal standards are listed in Table S2. We measured 21 mVOCs, including *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine (AAMA), *N*-acetyl-*S*-(N-methylcarbamoyl)-L-cysteine (AMCC), 2-aminothiazoline-4-carboxylic acid (ATCA), *N*-acetyl-*S*-(benzyl)-L-cysteine (BMA), *N*-acetyl-*S*-(n-propyl)-L-cysteine (BPMA), *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine (CEMA), *N*-acetyl-*S*-(2-cyanoethyl)-L-cysteine (CYMA), *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine (DHBMA), *N*-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA), *N*-acetyl-*S*-(2-hydroxyethyl)-L-cysteine (HEMA), *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine (2HPMA), *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine (3HPMA), *N*-acetyl-*S*-(3-hydroxypropyl-1-methyl)-L-cysteine (HPMMA), 2-methylhippuric acid (2MHA), 3-methylhippuric acid + 4-methylhippuric acid (3MHA + 4MHA), *N*-acetyl-*S*-(4-hydroxy-2-buten-1-yl)-L-cysteine (MHBMA3), *trans,trans*-muconic acid (MU), phenylglyoxylic acid (PGA), *N*-acetyl-*S*-(phenyl)-L-cysteine (PMA), thiodiglycolic acid (TGA), and 2-thioxothiazolidine-4-carboxylic acid (TTCA).

Besides, 8-hydroxy-2′-deoxyguanosine (8-OHdG), 8-hydroxyguanosine (8-OHG), and 4-hydroxy nonenal mercapturic acid (HNEMA) were measured as the OSBs of DNA (Guo et al., 2014; Asimakopoulos et al., 2016; Lee et al., 2019; Martinez-Moral and Kannan, 2019; Yu et al., 2021), RNA (Weimann et al., 2002), and lipid (Wu et al., 2016), respectively. We also measured urinary cotinine to evaluate the smoking status of participants. Milli-Q water, formic acid, acetonitrile, and methanol used were described elsewhere (Wang et al., 2019).

2.2. Sample collection and preparation

The present study was based on archived urine samples from a previous project which was designed to investigate the environment exposure of the general population and assess the health risk in non-occupational healthy adults, lived in areas 5 km away from the factories, in Wuhan, central China (Fig. S1). Details about the sample collection were described elsewhere (Wang et al., 2020a). Briefly, a total of 406 first-morning-void urine samples were collected in autumn (September and October 2018, $n = 278$; contained 75 repeated urine samples on three consecutive days, i.e., 128, 75, and 75 samples on the three days, respectively) and winter (December 2018, and January 2019; $n = 128$) from the general population (age $\geq$ 20 y) in Wuhan ($n = 128$), China, from three urban communities and three rural villages (Fig. S1) to reveal the seasonal and spatial distributions of mVOCs.

Midstream urine samples were collected directly using 50 mL polypropylene (PP) tubes. Samples were stored at −80 °C until analysis. This study was approved by the ethics committee of Tongji Medical College, Huazhong University of Science and Technology. Each participant provided written informed consent.

Each 0.1 mL of urine sample was spiked with 1 (for cotinine, 8-OHdG, and 8-OHG) or 10 ng (for HNEMA and mVOCs) of each isotope-labeled internal standard, then the sample was diluted to 0.5 mL with formic acid in water (*v*/*v*, 5/10,000), vortexed, transferred into an Amicon Ultra-0.5 Centrifugal Filter Unit (UFC5003BK, Merck Millipore Ltd., Tullagreen, Carrigtwohill, County Cork, Ireland), and centrifuged at 11,000 *g* for 30 min (Multifuge X1R, Thermo Scientific, Am Kalkberg, Osterode am Harz, Germany). The filtered sample was transferred into an amber LC vial for determination of the target analytes.

2.3. Instrumental analysis

Samples were analyzed by multiple reaction monitoring using an electrospray ionization triple quadrupole mass spectrometer (SCIEX 6500+, Framingham, MA, USA) coupled with a liquid chromatography (ExionLC, SCIEX, Framingham, MA, USA) system. The analytes were separated on an ACQUITY HPLC® HSS T3 column (2.1 mm × 150 mm column, 1.8 μm particle size, Waters, Milford, MA, USA) kept at 40 °C. The mobile phases for the target analytes and the programmed gradient are listed in Table S3. A 10 μL of the sample was injected; mVOCs and HNEMA were determined in negative mode; cotinine, 8-OHdG, and 8-OHG were determined in positive mode (Table S2); they were determined simultaneously.

To correct the variations in urine dilution, the concentrations of

target analytes were standardized using urinary specific gravity (SG) (Wang et al., 2020a).

2.4. Quality control and assurance

Calibration curves, transport blanks, procedural blanks, duplicates, and matrix spikes spikes were conducted (Wang et al., 2020a). Mean recoveries of the target analytes (range: 86%–115%) were determined by matrix spike samples (1 or 10 ng for each of the targets, consistent with the abovementioned internal standards, except for those compounds with extremely high concentrations, which were spiked with 100 ng of native standards). No target analyte was found in any blanks. The instrumental limit of quantifications (LOQs, defined as the signal to noise = 10 at least), and the method detection limits (MDLs), based on instrumental LOQs, dilution factor (1: 5), and matrix effects (no significance), are listed in Table S2.

2.5. Statistical analysis

Descriptive statistics were used to describe the demographic characteristics in this study with the expression of a number (%) or mean ± standard deviation (SD). The MDL, detection frequency (DF), geometric mean (GM), mean ± SD, median, and range of SG-adjusted and unadjusted concentrations of urinary mVOCs, cotinine, 8-OHdG, 8-OHG, and HNEMA were calculated for exposure assessment. Metabolite concentrations lower than the MDLs were replaced as the MDLs divided by the square root of 2 (Hornung and Reed, 1990). The intraclass correlation coefficients (ICCs) were calculated to evaluate the inter-day variabilities in urinary concentrations of the target analytes on three consecutive days in autumn, categorized as excellent (ICC ≥ 0.75), fair to good (0.75 > ICC ≥ 0.40), and poor (ICC < 0.40) reproducibility (Rosner, 2015). Wilcoxon signed-rank tests were used to compare the difference of all analytes' concentrations in autumn and winter. The concentrations of all analytes were log10-transformed because of the right-skewed distributions.

Based on the urinary concentrations of mVOCs, estimated daily intake (EDI), hazard quotients (HQs) and hazard indexs (HIs) were calculated to assess health risks of exposure to the individual and cumulative VOCs using the following equations (1)–(4) (Koch et al., 2007; Dewalque et al., 2014):

$$EDI(\mu g/kg-bw/day)=\frac{C_m(\mu g/L)\times V(L/day)\times MW_p(g/mol)}{F_{UE}\times BW(kg)\times MW_m(g/mol)} \quad (1)$$

$$HQ=\frac{EDI(\mu g/kg-bw/day)}{RfD(\mu g/kg-bw/day)} \quad (2)$$

$$HI_{(\sum 1IARC1)}=HQ_{1,3-butadiene} \quad (3)$$

$$HI_{(\sum 3IARC2A)}=HQ_{acrylamide}+HQ_{N,N-dimethylformamide}+HQ_{acrolein} \quad (4)$$

where EDI was the estimated daily intake of the relevant VOC per day; C_m was the SG-adjusted urinary concentration of the relevant mVOC; V was excretion rate of urine with 1.6 L/d and 1.2 L/d for males and females, respectively (ICRP, 2002); MW_P and MW_m was molecular weight of the parent compound and corresponding metabolite, respectively; BW was the body weight of each participant; F_{UE} was the excretion fraction of VOC in urine after intake (Table S1). Since some VOC exposures (e.g., acrolein, acrylamide, and xylene) were tested through two metabolites' concentrations (e.g., CEMA and 3HPMA for acrolein), we multiplied the sum of metabolites' molar concentrations and the molecular weight of corresponding parent compound as C_m when estimating the EDIs for these VOCs, F_{UE} of which was calculated by summing the individual metabolite F_{UE}s, and MW_m of which was the F_{UE}-weighted average molecular weight of each metabolite. RfD was the reference dose of VOC extracted from the United States Environmental Protection Agency (USEPA) (Table S1).

Generally, we only calculated EDI and HQ for the VOC which has specific metabolite and established F_{UE} and RfD (including acrolein, 1,3-butadiene, cyanide, acrylamide, acrylonitrile, *N*,*N*-dimethylformamide, and xylene) (Table S4); 1,3-butadiene was included because it is a recognized human carcinogen (its RfD was estimated from its RfC) (Table S1). $HI_{(\sum 1IARC1)}$ and $HI_{(\sum 3IARC2A)}$ were calculated to assess cumulative risk of the VOCs classified by International Agency for Research on Cancer (IARC) as group 1 (carcinogenic to humans) or group 2A (probably carcinogenic to humans), respectively, considering their possibly higher toxicities to humans.

We used linear mixed models (LMMs) to evaluate the associations between log10-transformed SG-adjusted concentrations of mVOCs and the OSBs (8-OHdG, 8-OHG and HNEMA) with adjustment for age (20–45, 45–60, ≥60 years), gender (male/female), body mass index (BMI) as linear variables, education (≤9, 10–12, ≥13 years), annual per capita income (< 30,000, 30,000–50,000, ≥50,000 yuan), smoking status (smokers/nonsmokers, based on SG-adjusted urinary concentration of cotinine ≥ 50 or < 50 ng/mL) (Benowitz et al., 2002), and drinking status (drinkers/nondrinkers). Each participant was treated as a random effect in the models. The false discovery rate was used to control for multiple comparisons (Glickman et al., 2014). Further stratified analyses between log10-transformed $HI_{(\sum 1IARC1)}$ and $HI_{(\sum 3IARC2A)}$ and OSBs were conducted by major characteristics, including age, sex, BMI, and smoking and drinking status. Interaction term was added into the LMMs to estimate modification by each stratified characteristic. Season-specific associations were also evaluated by using generalized estimating equation (GEE) models with a multiplicative interaction term between season and mVOCs.

In addition, we used WQS regression analyses which derived a mVOC mixture index (represents tertiled mVOCs) incorporated with multivariable linear regression models to assess the mixture effect of exposure to multiple VOCs on OSBs and estimate the contributing effect of each mVOC. Averaged concentrations of all analytes in autumn and winter were used in WQS analyses with covariates adjusted. The final weights assigned to individual mVOCs were estimated as the mean of estimated values across 1000 bootstrap samples.

All of the analyses were conducted with SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA) and R, version 4.0.4 (R Development Core Team, Vienna, Austria).

3. Results and discussion

3.1. Participant characteristics

Table 1 shows the demographic characteristics of the study population (n = 128). Approximately half of the participants (52.3%) were males. The mean (±SD) age was 51.4 ± 16.2 years. The mean (±SD) BMI was 22.6 ± 3.0 kg/m^2 and most of the participants had normal to low BMI (70.3%). Almost half of the population received at least a high school education (41.4%) and made annual per capita income greater than 30,000 yuan (48.4%). Half of the participants resided in urban area (50.8%).

3.2. Urinary concentrations of VOC metabolites and oxidative stress biomarkers, inter-day reproducibility, seasonal difference, and risk assessment

Table 2 presents the distributions of urinary SG-adjusted concentrations of mVOCs, cotinine, 8-OHdG, 8-OHG, and HNEMA in autumn and winter of 2018. All the target analytes were frequently detected in 83.6–100 % of the samples, except for PMA and MHBMA3 (14.8% and 9.4%, respectively). Among all the mVOCs, HPMMA had the highest GM concentration in both autumn (1420 ng/mL) and winter (1887 ng/mL), followed by 3HPMA, DHBMA, TGA, and CEMA; PMA had the lowest GM concentration (< MDL in both autumn and winter). This was similar to

Table 1
Demographic characteristics of the samples ($n = 128$).

Characteristics	n	Mean ± SD or percentage
Sex		
Male	67	52.34%
Female	61	47.66%
Age (y)	128	51.4 ± 16.2
20–45	46	33.9 ± 7.3 (35.94%)
45–60	42	52.5 ± 4.3 (32.81%)
≥60	40	70.2 ± 6.8 (31.25%)
Height (m)	128	1.6 ± 0.1
Weight (kg)	128	61.4 ± 10.1
BMI (kg/m^2)	128	22.6 ± 3.0
<24	90	21.0 ± 1.8 (70.31%)
≥24	38	26.3 ± 1.9 (29.69%)
Education (y)		
≤9 (less than high school)	75	58.59%
10–12 (high school)	28	21.88%
≥13 (more than high school)	25	19.53%
Annual per capita income (RMB, yuan)		
<30,000	66	51.56%
30,000–50,000	27	21.09%
≥50,000	35	27.34%
Place of residence		
Urban	65	50.78%
Rural	63	49.22%

Abbreviation: SD, standard deviation.

the results reported by Kuang et al. (2019) who focused on children aged 6–12 years in China and the results reported by Boyle et al. (2016) who focused on pregnant women in the U.S.. Kuang et al. (2019) found low urinary DF of PMA (58%) and MHBMA3 (< MDL in all samples) and high concentrations of TGA, ATCA, 3HPMA, and HPMMA. Boyle et al. (2016) reported low DF of urinary PMA (52%) and high concentrations of HPMMA, DHBMA, MU, and 3HPMA, although they also found a high DF of MHBMA3 (94%). Most of the mVOC concentrations in the present study were about 1–4 folds higher than those of children in Kuang et al.'s study (Kuang et al., 2019) and were in the same order of magnitude with that of the population in Boyle et al.'s study, except for HEMA and HPMMA, the concentrations of which in the present study were 1–2 orders of magnitude higher than those in the two other studies, indicating the probably higher exposure levels of acrylonitrile/vinyl chloride/ethylene oxide (the parent compounds of HEMA; Table S1) and crotonaldehyde/methyl vinyl ketone/methacrolein (the parent compounds of HPMMA and its isomers; Table S1) among the general population in Wuhan, central China. Distributions of urinary unadjusted concentrations of all analytes in autumn and winter are listed in Table S5.

SG-adjusted concentrations of most mVOCs, 8-OHdG, and 8-OHG in the winter were relatively higher than those in the autumn ($p < 0.05$) (Table 2). This was generally consistent with the report in Schlink et al.'s study (Schlink et al., 2010) which found lower indoor air concentrations of VOCs in autumn in comparison to those in winter in Leipzig, Germany. The seasonal difference was also consistent with the difference of other contaminants observed in air of Wuhan, China (Li et al., 2018; Mao et al., 2020; Wang et al., 2020c). Increasing time spent indoors and reduced ventilation indoors in winter, different emission rates of VOCs in different seasons (Lyu et al., 2016; Hui et al., 2018), and diffusion conditions for pollutants (the temperature, the atmospheric pressure et al.) (Ho et al., 2004; Paciência et al., 2016; Mao et al., 2020) might explain the seasonal difference. However, for HEMA, the concentration of which in the winter (71 ng/mL) was one order of magnitude higher than that in the autumn (2.71 ng/mL) (Table 2). To our knowledge, three parent compounds shared the metabolite HEMA (acrylonitrile, vinyl chloride, and ethylene oxide) (Table S1). The significantly higher concentration of HEMA in the winter cannot be explained by higher level of acrylonitrile or vinyl chloride in air, since urinary concentrations of CYMA (another metabolite of acrylonitrile besides HEMA) and TGA (another metabolite of vinyl chloride besides HEMA) in the winter were less than two times higher than those in the autumn (Table 2). Thus, the high concentration of HEMA in winter was more likely due to elevated level of ethylene oxide or other unknown parent compounds. With the development of petrochemical industry in Wuhan these years, several industrial chains have been established including one for production of ethylene oxide (WBS, 2018). The high urinary level of HEMA

Table 2
Urinary SG-adjusted concentrations of VOC metabolites, cotinine, 8-hydroxy-2′-deoxyguanosine (8-OHdG; for DNA), 8-hydroxyguanosine (8-OHG; for RNA), and 4-hydroxy nonenal mercapturic (HNEMA; for lipid) in the general population who donated urine samples in both autumn and winter in Wuhan (ng/mL).

Analytes	MDL	Autumn ($n = 128$)					Winter ($n = 128$)					P[a]
		DF (%)	GM	Mean ± SD	Median	Range	DF (%)	GM	Mean ± SD	Median	Range	
AAMA	0.50	100	40.2	52.5 ± 49.6	39.9	5.65–456	100	53.2	68.6 ± 51.2	52.0	6.14–278	<0.01
AMCC	2.50	94.5	39.9	73.2 ± 89.4	34.8	< MDL-433	96.1	52.3	83.0 ± 91.5	44.8	< MDL-517	<0.01
ATCA	0.50	100	85.3	123 ± 115	85.9	6.16–673	100	155	200 ± 151	159	24.8–833	<0.01
BMA	0.10	100	2.78	4.68 ± 10.5	2.53	0.49–112	100	3.11	4.41 ± 4.88	3.14	0.35–43.7	0.07
BPMA	0.50	92.2	3.94	9.69 ± 16.3	3.97	< MDL-96.2	83.6	3.50	13.3 ± 64.9	3.08	< MDL-733	0.98
CEMA	0.98	100	110	150 ± 148	112	10.5–1053	100	137	182 ± 146	136	10.0–865	<0.01
CYMA	0.09	100	3.42	24.1 ± 60.0	1.72	0.28–377	99.2	4.71	28.2 ± 71.1	2.38	0.32–493	<0.01
DHBMA	2.50	100	154	180 ± 114	154	25.1–969	99.2	181	212 ± 117	181	14.1–601	<0.01
GAMA	0.29	100	3.34	4.08 ± 2.96	3.32	< MDL-22.2	99.2	4.59	5.76 ± 4.00	4.58	0.49–17.3	<0.01
HEMA	0.27	97.7	2.71	4.99 ± 6.67	2.22	0.41–39.4	100	71.0	113 ± 87.2	95.0	1.61–417	<0.01
2HPMA	0.27	100	24.6	31.9 ± 26.7	23.8	5.55–151	100	22.4	30.3 ± 29.9	20.8	3.76–226	0.08
3HPMA	0.55	100	462	759 ± 937	410	34.6–5174	100	572	920 ± 1043	538	16.1–5056	0.02
HPMMA	1.41	100	1420	2550 ± 3814	1116	138–23825	100	1887	3189 ± 4478	1644	113–28155	<0.01
2MHA	1.00	100	26.4	38.0 ± 40.6	23.6	3.00–209	100	34.1	47.9 ± 50.6	29.9	3.70–326	<0.01
3MHA + 4MHA	1.00	100	49.8	109 ± 191	40.8	< MDL-1233	100	56.3	98.1 ± 154	47.7	8.08–970	0.31
MHBMA3	1.00	9.4	< MDL	1.13 ± 1.38	< MDL	< MDL-9.61	9.4	< MDL	1.27 ± 1.57	< MDL	< MDL-11.1	0.31
MU	5.00	99.2	57.5	86.9 ± 84.6	51.8	6.50–452	99.2	86.1	129 ± 135	85.8	7.07–953	<0.01
PGA	2.50	86.7	35.1	69.7 ± 71.3	51.6	< MDL-402	85.2	49.8	92.3 ± 73.6	82.6	< MDL-355	<0.01
PMA	0.25	18.0	< MDL	< MDL ± 0.16	< MDL	< MDL-1.15	14.8	< MDL	0.26 ± 0.20	< MDL	< MDL-1.41	0.50
TGA	10.00	100	133	163 ± 124	127	26.5–887	100	147	295 ± 1290	140	30.8–14443	0.29
TTCA	2.50	91.4	29.6	94.2 ± 162	42.5	< MDL-960	90.6	29.3	73.4 ± 144	31.2	< MDL-1088	0.35
Cotinine	0.50	91.4	8.62	285 ± 686	3.27	< MDL-3260	84.4	7.19	221 ± 518	2.39	< MDL-3130	<0.01
8-OHdG	1.00	96.1	4.77	5.79 ± 4.33	4.86	< MDL-27.9	94.5	5.50	6.44 ± 3.47	5.86	1.03–21.0	<0.01
8-OHG	0.50	100	7.95	8.82 ± 4.42	7.95	1.78–27.1	99.2	9.01	10.1 ± 4.83	9.40	0.71–27.8	<0.01
HNEMA	0.50	97.7	33.0	80.1 ± 115	33.0	< MDL-805	94.5	32.6	91.1 ± 140	29.2	< MDL-986	0.61

Abbreviations: SG, specific gravity; MDL, method detection limit; DF, detection frequency; GM, geometric mean; SD, standard deviation.
[a] Wilcoxon signed-rank tests were used to compare the difference of all analytes' concentrations in autumn and winter.

in Wuhan in December 2018 and January 2019 was more likely to be explained by an accident [e.g., the leakage of ethylene oxide from the petrochemical plant located in Wuhan (Fig. S1)], which warranted further exposure assessment of VOCs among the population in Wuhan.

Urinary concentrations of ATCA, CYMA, 3HPMA, MU, and TTCA in participants living in rural area were relatively higher than those in urban area ($p < 0.05$), in both the autumn and winter (Table S6), except for BPMA, the concentration of which in rural area (2.05 ng/mL) were relatively lower than that in urban area (5.88 ng/mL) in the winter ($p < 0.05$) (Table S6). The difference between rural area and urban area might be due to different life style of residents, such as more burning of coal and wood and less frequent use of kitchen ventilator in rural area, and more frequent use of dry-cleaning (source for parent compound of BPMA; Table S1) in urban area.

In addition, we found good to excellent inter-day reproducibility of 18 out of 19 frequently detected mVOCs on three consecutive days in the autumn (Fig. 1; Table S7), which was similar to that of Mentese et al.'s study (Mentese et al., 2012) which reported no marked intra- and inter-day variability of indoor VOC levels during five subsequent days in both winter and summer in Ankara, Turkey. The biological half-lives of most urinary VOC metabolites are less than two days (roughly 2.1–34 h) (Boyle et al., 2016). The exposure level of VOCs among the general population primarily depends on the indoor and outdoor air or dietary ingestion, and the participants in our studies were the local residents with regular behavior in a relatively stable environment. This may explain the good to excellent inter-day reproducibility for most (18 out of 19 frequently detected) mVOCs in urine we observed. Poor inter-day reproducibility of PGA (ICC: 0.35, Table S7), the biological half-life of which is 10–12 h (ATSDR, 2010), may be due to that both ethylbenzene and styrene (the parent compounds of PGA) exist naturally in food such as fruits (IARC, 2000; IARC, 2019), and a person's fruit consumption can change from day to day to certain extent.

The correlation coefficients (CEs) between the averaged concentrations of mVOCs in autumn and winter ranged from −0.08 to 0.87 (Fig. S2). Generally, the correlations between mVOCs did not change too much in the autumn (range: −0.02 to 0.87, Fig. S3) and the winter (−0.14 to 0.88, Fig. S4). For instance, the mVOCs from the same parent compounds or isomers were highly correlated with each other in both autumn and winter, such as AAMA and GAMA (metabolites of acrylamide; CE was 0.77 in autumn and 0.82 in winter), CEMA and 3HPMA (metabolites of acrolein; CE was 0.83 in autumn and 0.88 in winter), and 2MHA and 3MHA + 4MHA (metabolites of xylenes; CE was 0.87 in autumn and 0.84 in winter). Besides, DHBMA was highly correlated with AAMA, CEMA, 2HPMA, 3HPMA, and HPMMA (CEs ranged from 0.54 to 0.77 in autumn, and 0.70 to 0.73 in winter).

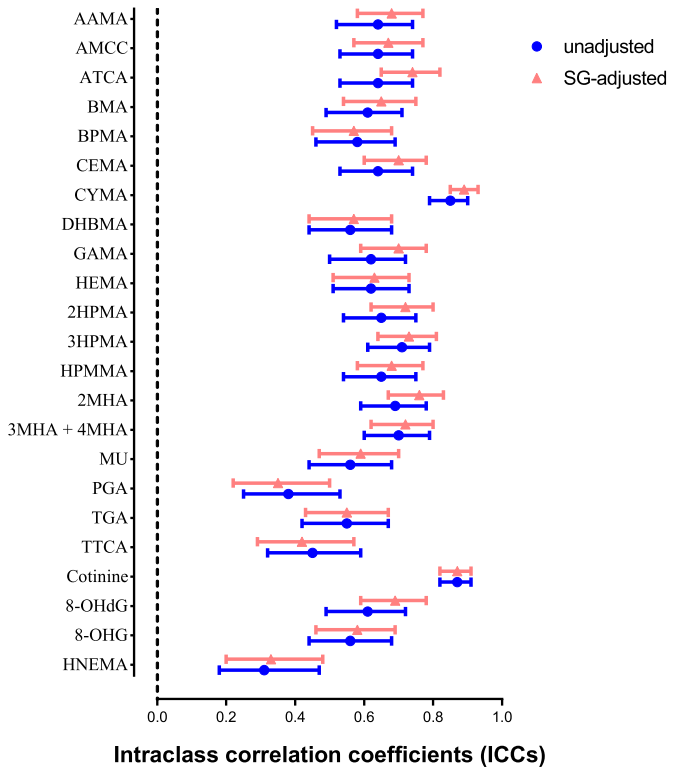

Fig. 1. Intraclass correlation coefficients (ICCs) for inter-day variations of unadjusted and specific gravity (SG)-adjusted urinary concentrations of target analytes on the three consecutive days in autumn ($n = 75$).

Table 3 shows the distribution of estimated HQs of seven parental VOCs and HIs of IARC group 1 and 2A in the autumn and the winter. Specifically, HQs of acrolein, 1,3-butadiene, cyanide, and acrylonitrile in 126 (98.4%), 125 (97.7%), 114 (89.1%), and 20 (15.6%) participants exceeded one in the autumn, respectively. $HI_{(\sum 1IARC1)}$ and $HI_{(\sum 3IARC2A)}$ in 125 (97.7%) and 126 (98.4%) participants, respectively, exceeded one in the autumn. HQs and HIs of VOCs in the winter exceeded one in a similar proportion as those in the autumn. The data in the present study implied that residents in the whole city of Wuhan suffered high risks of them. Zheng et al. (2020) also found that acrolein and 1,3-butadiene had the highest non-carcinogenic and carcinogenic risk, respectively, in the ambient air of a petrochemical industrial park in Wuhan, which was close to the residential area.

3.3. Associations of individual VOC metabolites and oxidative stress biomarkers

After adjustment for false discovery rate and potential confounders, the LMMs considering log10-transformed SG-adjusted urinary concentrations of mVOCs in the autumn and winter showed that most of the mVOCs were significantly associated with the increase of 8-OHdG (β value ranged from 0.04 to 0.48) and 8-OHG (β value ranged from 0.04 to 0.40), except that BPMA, TTCA and ATCA showed positive but not significant associations (Fig. 2 and Table S8). In addition, MU, ATCA, 3HPMA, and TTCA were found to be positively associated with HNEMA (β value ranged from 0.21 to 0.78). However, significantly negative associations were observed of AMCC and PGA with HNEMA.

Most previous studies involved in urinary concentrations of mVOCs and OSBs focused on populations with occupational exposure (Kim et al., 2011; Zhang et al., 2013; Sisto et al., 2020b), while a few studies focused on a single mVOC or a small number of mVOCs among the general (non-occupational) population (Yoon et al., 2010; Kwon et al., 2018; Wang et al., 2020b). Only two studies by Kuang et al. investigated the associations of multiple mVOCs with 8-OHdG (Kuang et al., 2019) and oxidative DNA damage-mediated childhood asthma (Kuang et al., 2021), and both of the two studies used single spot urine measurement which might lead to misclassification of exposure assessment and confounding bias.

In the present study, we found that most of the studied mVOCs were significantly associated with increasing 8-OHdG and 8-OHG, which was generally consistent with the results reported by Kuang et al. (2019) who found significant positive correlations between all studied mVOCs and 8-OHdG in urine samples from Chinese children (without SG adjustment and repeated measurements) using Spearman correlation analysis. Another study by Kuang et al. (2021) also observed significant relationships between most mVOCs and increasing 8-OHdG with multiple linear regression models.

Few epidemiological studies have investigated the effect of exposure to multiple VOCs on lipid peroxidation. One study related to the effect of volatile organic solvents on antioxidant enzyme system found that blood concentration of malondialdehyde (MDA, one of the biomarkers of lipid peroxidation) was significantly higher in textile workers than in controls (Bayil et al., 2008). Another experimental study observed exposure to benzene (parent compound of MU and PMA) was significantly

Table 3
Hazard quotients (HQs) and hazard indexs (HIs) based on reference dose values ($n = 128$).

Analytes	Autumn						Winter					
	Min	25th	50th	95th	Max	>1 *N* (%)	Min	25th	50th	95th	Max	>1 *N* (%)
Acrolein	0.48	**3.58**	**5.66**	39.84	75.19	**126 (98.44)**	0.30	**4.63**	**7.80**	**52.20**	69.91	**126 (98.44)**
1,3-Butadiene	0.54	**2.60**	**3.80**	10.28	26.91	**125 (97.66)**	0.33	**3.19**	**4.45**	**13.15**	18.82	**125 (97.66)**
Cyanide	0.20	**1.71**	**2.83**	12.47	29.07	**114 (89.06)**	1.07	**3.56**	**5.53**	**20.28**	29.22	**128 (100.00)**
Acrylamide	0.01	0.05	0.08	0.24	0.99	0 (0.00)	0.01	0.07	0.11	0.38	0.70	0 (0.00)
Acrylonitrile	0.01	0.03	0.04	4.08	11.18	**20 (15.63)**	0.01	0.03	0.06	**4.90**	15.45	**23 (17.97)**
N,*N*-Dimethylformamide	<0.001	0.01	0.02	0.17	0.31	0 (0.00)	0.001	0.02	0.03	0.18	0.38	0 (0.00)
Xylene	<0.001	0.002	0.003	0.03	0.08	0 (0.00)	<0.001	0.002	0.003	0.02	0.07	0 (0.00)
$HI_{(\sum 1IARC1)}$	0.54	**2.60**	**3.80**	10.28	26.91	**125 (97.66)**	0.33	**3.19**	**4.45**	**13.15**	18.82	**125 (97.66)**
$HI_{(\sum 3IARC2A)}$	0.50	**3.65**	**5.83**	40.16	75.44	**126 (98.44)**	0.33	**4.75**	**8.02**	**52.83**	70.15	**126 (98.44)**

Abbreviations: HQ, hazard quotient; HI, hazard index; IARC, International Agency for Research on Cancer.
$HI_{(\sum 1IARC1)}$ represents sum of HQs of VOCs which were classified as IARC group 1 (carcinogenic to humans, including 1,3-butadiene); $HI_{(\sum 3IARC2A)}$ represents sum of HQs of VOCs which were classified as IARC group 2A (probably carcinogenic to humans, including acrylamide, *N*,*N*-dimethylformamide, and acrolein).

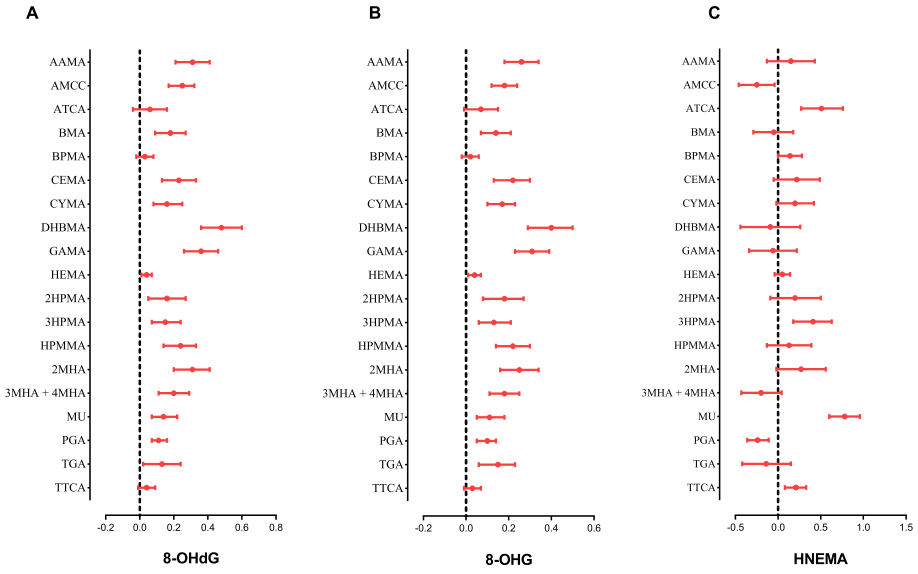

Fig. 2. Associations of individual metabolites of volatile organic compounds with oxidative stress biomarkers: linear mixed models were used to evaluate associations of log10-transformed specific gravity (SG)-adjusted urinary mVOCs levels with 8-hydroxy-2′-deoxyguanosine (8-OHdG; for DNA), 8-hydroxyguanosine (8-OHG; for RNA), and 4-hydroxy nonenal mercapturic (HNEMA; for lipid) in autumn and winter ($n = 128$).

associated with increasing MDA in fish (Otitoloju and Olagoke, 2011). However, endogenous lipid peroxidation can be one of the exposure sources of some aldehyde VOCs, such as acrolein (the parent compound of CEMA and 3HPMA) (Stevens and Maier, 2008) and crotonaldehyde (the parent compound of HPMMA) (Bagchi et al., 2018), which added uncertainty to the causal inference. Results in this study added evidence to the associations between lipid peroxidation effect and some mVOCs.

In season-specific analyses (Fig. S5), significantly positive associations were consistently observed between most of the mVOCs and 8-OHdG and 8-OHG in both the autumn and winter. Nevertheless, BPMA was found to be only positively associated with 8-OHdG ($\beta = 0.10$; 95% CI: 0.03, 0.17) and 8-OHG ($\beta = 0.07$; 95 %CI: 0.01, 0.13) in winter, while TGA was found to be positively associated with 8-OHdG ($\beta = 0.26$; 95 %CI: 0.11, 0.42) and 8-OHG ($\beta = 0.27$; 95 %CI: 0.14, 0.40) in autumn. MU and ATCA were positively associated with HNEMA in both the autumn and winter, whereas AMCC and PGA were negatively associated with HNEMA in winter only. To our knowledge, this is the first time to investigate the season-specific associations between mVOCs and biomarkers of DNA and RNA oxidative damage, and the results indicated that the adverse health effects existed persistently in both autumn and winter.

Further stratified analyses (Table S9) showed that the significantly positive associations of $HI_{(\sum 1IARC1)}$ with 8-OHdG and 8-OHG were persistent in all subgroups ($p < 0.05$), and significantly positive associations of $HI_{(\sum 3IARC2A)}$ with OSBs were persistent in subgroups of male/female, age < 45 years, BMI < 24 kg/m^2, nonsmokers and nondrinkers. All of the associations were not modified by the stratified demographic characteristics.

3.4. Associations of VOC metabolite mixture and oxidative stress biomarkers

In the WQS analyses, we observed that increasing levels of 8-OHdG ($\beta = 0.27$; 95% CI: 0.17, 0.36) and 8-OHG ($\beta = 0.23$; 95% CI: 0.17, 0.29) for every unit increase in the derived mVOC mixture index with adjustment for relevant covariates (Fig. 3). Approximately 78% of the mixture effect on 8-OHdG was attributed to 2MHA (20%), AAMA (18%), HEMA (13%), TGA (10%), AMCC (9%), and DHBMA (8%). Approximately 87% of the mixture effect on 8-OHG was attributed to 2MHA (17%), TGA (16%), AMCC (15%), AAMA (13%), GAMA (9%), HEMA (9%), and TTCA (8%). Additionally, a mVOC mixture index predominated by MU (50%), ATCA (26%), and HEMA (10%) was significantly associated with increasing HNEMA ($\beta = 0.44$; 95% CI: 0.28, 0.60).

We found that 2MHA, a metabolite of xylene which was classified as IARC group 3 (not classifiable because of inadequate evidence for its carcinogenicity in humans; Table S1), was the most important

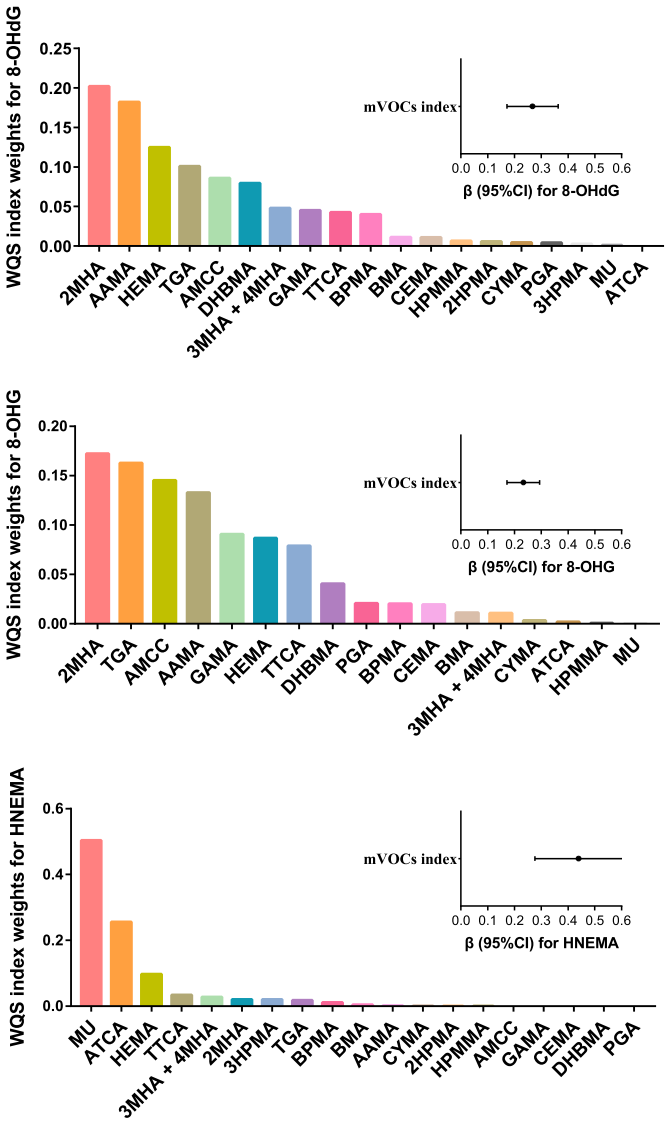

Fig. 3. Associations of multiple metabolites of volatile organic compounds with oxidative stress biomarkers: weighted quantile sum (WQS) regression models were used to evaluate associations of mVOC mixture with 8-hydroxy-2′-deoxyguanosine (8-OHdG; for DNA), 8-hydroxyguanosine (8-OHG; for RNA), and 4-hydroxy nonenal mercapturic (HNEMA; for lipid) based on log10-transformed averaged specific gravity (SG)-adjusted urinary concentrations in autumn and winter ($n = 128$). Three separate WQS indices were generated and modeled in the positive direction with respect to 8-OHdG, 8-OHG, and HNEMA, respectively.

contributor to the increase of both 8-OHdG and 8-OHG (Fig. 3). While other studies have shown the DNA and RNA oxidative stress effects of exposure to xylene (Xiong et al., 2016; Sisto et al., 2020a; Sisto et al., 2020b; Kuang et al., 2021), this study suggested the predominant effect of 2MHA after considering the co-existence of multiple correlated mVOCs. Additionally, we found that MU had the highest contribution to the increase of HNEMA (Fig. 3). MU was a metabolite of benzene, which was classified as IARC group1 (Table S1) and proved to be associated with lipid peroxidation in animal experiment study (Otitoloju and Olagoke, 2011); it is also a metabolite of sorbic acid, a preservative widely used in food and personal care products, and sorbic acid was also able to induce lipid peroxidation (Tsuchiya and Yamaha, 1983) or disrupt hepatic lipid metabolism (Chen et al., 2020a). ATCA was also one of the predominant contributors to the increase of HNEMA. While cyanide (the parent compound of ATCA; Table S1) was not classified by IARC, increasing evidence supported the oxidative stress effect of cyanide in lipid (Hariharakrishnan et al., 2009; Satpute et al., 2019). Correspondingly, risk assessment abovementioned showed that EDI of cyanide exceeded the RfD in most of the participants in this study, implying its potentially harmful effect which needed more attention in future studies. Nevertheless, we cannot deny the possibility of dietary ingestion of 4-hydroxy nonenal and sorbic acid or cyanide simultaneously, which might increase the associations. Besides, we found that WQS analyses placed non-negligible weights on HEMA (Fig. 3) that was weakly associated or not significantly associated with increasing OSBs in individual mVOCs analyses, which might be due to a lack of power in individual analyses (Czarnota et al., 2015).

This study possesses several strengths. This is the first study to characterize the inter-day reproducibility and seasonal difference in urinary concentrations of multiple mVOCs among the general population, to observe urban-rural variations in the concentrations of mVOCs, to explore the associations between mVOCs and OSBs of DNA, RNA, and lipid based on the above measurements; thus, the exposure assessment of which could be more reliable. Second, risk assessment can help better identify specific high-priority hazardous VOCs for management in the studied population. Third, the use of WQS regression model can investigate the mixture effect of multiple mVOCs on OSBs, and identify the contribution of each mVOC.

This study has several limitations. The major limitation is the lack of data on ambient VOCs which are helpful to understand the variations in mVOCs. Future studies should analyze ambient air samples to determine the VOCs in the environment with methods like U.S. EPA TO-15 and try to establish a cause-and-effect relationship. Besides, some isomers were not well separated in the present study [such as the two isomers of GAMA: *N*-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA) and *N*-acetyl-*S*-(1-carbamoyl-2-hydroxyethyl)-L-cysteine (iso-GAMA) (Zhang et al., 2015), and the three isomers of HPMMA: *N*-acetyl--*S*-(3-hydroxypropyl-1-methyl)-L-cysteine (HPMMA-1), *N*-acetyl--*S*-(3-hydroxypropyl-2-methyl)-L-cysteine (HPMMA-2), and *N*-acetyl-*S*-(3-hydroxypropyl-3-methyl)-L-cysteine, (HPMMA-3)], which might derive from different parent compounds (Chen et al., 2020b); the separation of them could be considered in future studies. Exposure characteristics during spring and summer and larger sample size are also needed to assess the seasonal variations of mVOCs in further studies. Furthermore, we only evaluated the exposure among adults but children may be more vulnerable to the exposure of hazardous air pollutants than adults; more studies on sensitive populations such as pregnant women and children should also be included.

4. Conclusions

Inter-day reproducibility and seasonal difference in urinary concentrations of multiple mVOCs among general population were characterized for the first time. In view of risk assessment, acrolein, 1,3-butadiene, and cyanide should be considered as high-priority hazardous ones for management among the corresponding VOCs in Wuhan, central China. This study also found significantly positive associations of individual mVOCs and mVOC mixture with OSBs, respectively, and the strongest mVOC contributors for the associations involved 2MHA for both DNA and RNA, and MU for lipid. Considering the important role of oxidative stress in various diseases, further researches are warranted to corroborate the detrimental health effect of exposure to multiple VOCs and illuminate the potential mechanisms in corresponding diseases.

Credit roles

Xi Qian (Investigation, Writing – Original Draft); Yanjian Wan (Conceptualization, Investigation, Writing – Original Draft – Review & Editing); Aizhen Wang (Investigation – Review & Editing); Zong Yang (Resources, Review & Editing); Zhenyu He (Conceptualization, Resources, Review & Editing); Shunqing Xu (Resources, Review & Editing);

Wei Xia (Conceptualization, Resources, Writing – Review & Editing).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was funded by the National Key Research and Development Plan of China (2019YFC1804502), the Health commission of Hubei Province scientific research project (grant numbers WJ2019H307, WJ2019H303, and WJ2019Q004), the Wuhan Preventive Medicine Research Project (MY19M01), the Project of the Chinese Ministry of Environmental Protection (2111101-04), and Program for HUST Academic Frontier Youth Team (2018QYTD06). We thank Chenhui Yang, Meng Wang, and all the participants, co-workers, and friends for their assistance in sampling. Yanjian Wan is grateful to Dr. Kurunthachalam Kannan for his mentoring. The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the department.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.117913.

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