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# Characterising the exposure of Australian firefighters to polycyclic aromatic hydrocarbons generated in simulated compartment fires

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ABSTRACT

Firefighters are exposed to a wide variety of chemicals including polycyclic aromatic hydrocarbons (PAHs) while attending fire scenes. The objective of this study was to understand the exposure of firefighters to PAHs when attending simulated compartment fires that consisted of either a diesel pan or particleboard fire. Firefighters remained in the compartment fires for 15 min while using standard gear including self contained breathing apparatus (SCBA). Firefighters were able to remove firefighting clothing and shower within 10 min of leaving the burn. Air samples were collected from inside the compartment during the fire. Twenty-six (26) firefighters participated in the study providing urine and skin wipe samples collected from the wrist and neck before and after either one of the burn types. The concentrations of PAHs were measured in skin wipes and air samples, while concentrations of monohydroxy metabolites of PAHs (OH-PAHs) were measured in urine. The concentrations of all PAHs were significantly higher ($p < 0.05$) in the smoke layer of particleboard fires than in diesel pan fires. Correspondingly, the level of PAHs deposited on the wrists and necks of participants attending the particleboard fires was higher than those attending diesel pan fires. Urine samples from participants who attended diesel pan fires showed no significant difference ($p > 0.05$) in the concentration of all OH-PAHs between pre-burn and post-burn. Samples from participants who attended particleboard fires, showed no significant difference ($p > 0.05$) between 1-hydroxypyrene (1-OH-PYR) concentrations in urine pre- and post-burn. However, median concentrations of hydroxynaphthalenes (OH-NAPs), hydroxyfluorenes (OH-FLUs) and hydroxyphenanthrenes (OH-PHEs) increased significantly from 5.2, 0.44 and 0.88 $\mu g\ g^{-1}$ creatinine pre-burn to 12, 1.4 and 1.2 $\mu g\ g^{-1}$ creatinine post-burn, respectively. This suggests that in compartment burns with high concentrations of PAHs in the smoke layer, such as those created by the particleboard fires, exposure to PAHs can be observed though urinary OH-PAH metabolites. Overall, concentrations of urinary OH-PAHs were relatively low considering the potential exposure in these burns. This suggests protective equipment in combination with rapid removal of firefighting ensembles and showering are relatively effective in controlling exposure.

## 1. Introduction

Occupational exposure of firefighters to hazardous chemicals is an ongoing concern. Higher incidence rates of respiratory diseases, coronary heart disease and certain cancers have been recorded in firefighters than in the general population (Daniels et al., 2014; Edelman et al., 2003; LeMasters et al., 2006; Mustajbegovic et al., 2001; Pukkala et al., 2014). A factor contributing to higher incidence of disease may be occupational exposure to harmful chemicals when attending both real fire suppression activities and training fires. These chemicals include polycyclic aromatic hydrocarbons (PAHs), which are ubiquitous by-products of incomplete combustion. Some of which are known carcinogens, such as benzo(a)pyrene and benz(a)anthracene (IARC, 2010; Kim et al., 2013).

Firefighters are usually equipped with personal protective clothing (PPC) and self contained breathing apparatus (SCBA). However, this

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equipment may not adequately protect firefighters from chemical exposure. Firefighters can also be exposed to chemicals from inadequately cleaned clothing and equipment, and elevated chemical concentrations within fire stations (Banks et al., 2020; Kirk and Logan, 2015; Oliveira et al., 2016; Pleil et al., 2014; Shaw et al., 2013; Shen et al., 2015). Firefighters are not only exposed to PAHs through inhalation, but can also be exposed though dermal contact with PAHs from a build-up of soot and debris (Fent et al., 2017). Monohydroxy-PAHs (OH-PAHs) and conjugated OH-PAHs of four-ring or less are mainly excreted through urine and have been used as biomarkers to assess an individual's exposure to PAHs. Several urinary monohydroxy PAHs (OH-PAHs) have been used as biomarkers to assess human exposure to PAHs in the general population (Navarro et al., 2019; Thai et al., 2020), as well as in biomonitoring studies of firefighters (Oliveira et al., 2016; Wingfors et al., 2018). While 1-hydroxypyrene (1-OH-PYR) is the most common biomarker used for monitoring PAH exposure, several studies of exposure to firefighters have used multiple OH-PAHs as indicators of exposure to PAHs (Fernando et al., 2016; Wingfors et al., 2018).

Several previous studies have also highlighted the importance of the dermal pathway to the overall exposure as it appears to be a major pathway for PAHs to enter firefighters' bodies.

(Fent et al., 2014; Fernando et al., 2016; Wingfors et al., 2018). Several studies have found positive correlations between the concentration of PAHs on firefighters' skin and the concentrations of OH-PAHs measured in urine (Wingfors et al., 2018; Fernando et al., 2016). Due to potential differences in PPC and after-fire decontamination practices between Australian and international fire services, it is necessary to assess the exposure of Australian firefighters to combustion products.

This study aimed to measure firefighters' exposure to PAHs in simulated compartment fires using different fuels through the changes in their urinary OH-PAH concentrations. It also aimed to assess exposure pathways by measuring the deposition of PAHs on firefighters' skin, and the concentrations of PAHs in the smoke layers of the fires they attended.

## 2. Materials and methods

### 2.1. Participant recruitment and questionnaire

Participants for this study were recruited through the Queensland Fire and Emergency Services (QFES). Participants had to be active firefighters and non-smokers. All samples and information were collected in accordance with an ethics approval obtained from The University of Queensland (approval number: 2018000198). Informed consent was obtained from participants involved in this study. A questionnaire was used to collect information on age, height, gender and factors potentially affecting OH-PAH concentrations in urine, including eating barbequed food, exposure to smoke 24 h prior to and after attending the burns.

### 2.2. Fire characterisation and firefighter activities

Participants in this study attended one of the two distinct controlled compartment fire types: diesel pan fires and particleboard fires. Both fire types were conducted in the back of a shipping container that was 12.2 m long, 2.4 m wide and 2.6 m tall.

#### 2.2.1. Diesel pan fires

In the back of the shipping container, two 0.57 m diameter pans were filled with diesel and an accelerant to ensure ignition of the diesel fuel. This was used to provide approximately 1 MW of heat output and smoke layer with temperatures ranging between 150 and 200 °C. Participating firefighters (n = 12) entered the compartment 1 min after ignition. Firefighters carried a weight from the entrance of the container to 2 m from the fire, waited for 10 s, before returning to the entrance of the container. This was repeated until the firefighters had been in the compartment fire for 15 min in total.

#### 2.2.2. Particleboard fires

Structures were built at the back of the shipping containers with particleboard. The particleboard consisted of wood particles (>85%), bonded together with melamine/urea/formaldehyde resin (<13%), paraffin wax (<2%), and formaldehyde (0.0001%). The fire was ignited and allowed to develop before firefighters entered the container. Participating firefighters (n = 14) remained under the smoke layer and moved around the container for the duration of the burn, without prescribed instructions about movement inside the container. Firefighters remained in the compartment fire for 15 min.

#### 2.2.3. Post-burn activity

After exiting the simulated compartment fires, participating firefighters transitioned into a separate building where they removed SCBA and structural firefighting gear, and post-burn skin wipe sampling took place. Firefighters were then free to remove the remaining clothes worn into the compartment fires and shower within 10 min of leaving the fire.

### 2.3. Sampling

Sampling was undertaken between May and October 2018.

#### 2.3.1. Smoke

Samples of fire smoke were collected inside the containers from the time firefighters entered through to when they exited. A sampling port was located on the side of the container 6 m from the end of the container and 1.23 m above from the ground. The samples were collected by an 8 mm stainless steel sampling probe, connected to a sample collector. The latter drew the air from the container through a condenser, cooling before being drawn through glass wool and XAD-2 sandwiched between two polyurethane foam (PUF) disks. Particulates were collected on the glass wool and first layer of PUF, while vapor phase chemicals were collected in the PUFs and XAD-2. The smoke layer was drawn though the sampling train at approximately 15 L $min^{-1}$ using a vacuum pump (Model:VTE 6, Werner Rentschler, Switzerland). The glass wool, XAD-2 and PUF disks were removed from the sampling train and placed into an amber glass jar. The sampling train was rinsed with 200 mL of 1:1 acetone:*n*-hexane into the same amber glass jar to remove any deposited particulate or PAHs that may have condensed onto the sampling train before the PUFs and XAD-2 trap. After collection, samples were stored at −20 °C until analysis.

#### 2.3.2. Urine

Pathology collection containers and biohazard zip lock bags were provided to participants in cooler boxes. Participants were asked to collect their own urine samples. Pre-burn urine samples were collected less than an hour before each burn. Post-burn urine samples were collected at each bladder void for four bladder voids after attending the compartment fires. Samples were stored at −20 °C prior to analysis.

#### 2.3.3. Skin wipe

Skin wipe samples were collected using 70% isopropanol wipes that are commercially available. This is similar to the sampling techniques for PAHs used previously in skin deposition and surface wipe studies (Keir et al., 2017; Engelsman et al., 2019). Prior to entering a compartment fire, a 20 $cm^2$ wipe sample was taken using a template by a laboratory technician from the left wrist and left side of the neck from each participant. Post-burn wipe sampling was repeated on participants on their right side immediately after removing firefighting ensembles. This was done to ensure the pre- and post-burn sampling areas did not overlap. Wipe samples were stored in polypropylene containers at −20 °C until analysis.

### 2.4. Sample extraction, analysis and quantity control

The details for sample extraction, analysis and quality control are presented in the supplementary information (SI). In brief, skin wipe samples were spiked with internal standards, solvent extracted in 3:1 dichloromethane:toluene before being concentrated using a gentle stream of nitrogen to 1 mL and transferred into a vial for analysis. Smoke layer samples were extracted in acetone:*n*-hexane solution particulates allowed to settle overnight before a fraction of the extract solution was aliquoted into a vial along with internal standards. Urine samples were spiked with internal standards. β-Glucuronidase (HP-2) enzyme was used to hydrolyse urinary conjugates of OH-PAHs. The sample was then liquid:liquid extracted with toluene:*n*-pentane. The toluene:*n*-pentane mixture was taken to near dryness before OH-PAHs were silylated with N,O-bis(trimethylsilyl) trifluoroacetamide + trimethylchlorosilane prior to analysis. All samples were run on a TRACE GC Ultra coupled to a TSQ Quantum XLS triple quadrupole mass spectrometer. The recoveries of internal standards ranged from 74 to 130% for skin wipe samples, 66–128% for smoke layer samples and from 69 to 105% for urine samples.

### 2.5. Statistical analysis

Statistical analysis was performed using GraphPad Prism (version 8.00, GraphPad Software Inc). The Shapiro–Wilk test indicated that concentrations of OH-PAHs in urine samples and PAHs from smoke and skin wipe samples did not have a normal distribution. The Shapiro–Wilk test was repeated once data had been log10 transformed, and indicated normal distribution of data. Log10 transformed data were used for statistical analysis. Differences in concentration of OH-PAHs in urine and PAHs from skin wipes were determined using paired t-tests. Bivariate correlations (Pearson correlation test) were used to investigate the correlations between the concentration of PAHs in smoke, on the skin and of OH-PAHs in urine. Single sample t-tests were used to compare the mean concentration of OH-PAHs in firefighters' urine with that of the general population. Statistical significance was set at $p < 0.05$. For analysis purposes, half the method detection limit (MDL/2) was used when concentrations of <50% of an analyte were below the MDL in a particular sample set. If >50% of the values for a particular sample set were below the MDL, this sample set was excluded from statistical analysis.

## 3. Results and discussion

### 3.1. Participant characteristics

Responses from the participant questionnaire are presented in Table S6. In summary, among the 26 participants in this study, 85% (22) were above 40 years of age, 81% (21) were over 175 cm high and only one participant was female. All participants in this study self-reported not eating barbequed food, smoking or being exposed to smoke 24 h prior to and after attending the simulated compartment fires.

### 3.2. Smoke layer

Table 1 provides the results from the 15 PAHs measured from the smoke layers inside the simulated compartment fires. In total, smoke samples were collected from five diesel pan fires and three particleboard fires. Concentrations of all PAHs were above the MDLs in all the samples. The $\sum_{15}$ PAHs concentrations ranged from 3400 to 180,000 μg $m^{-3}$. In all samples, naphthalene had the highest concentration followed by phenanthrene. Naphthalene and phenanthrene contributed on average 49% and 15% respectively to the $\sum_{15}$ PAHs concentrations measured in smoke layer samples.

The concentrations of all individual PAHs and $\sum_{15}$ PAHs were significantly higher ($p < 0.05$) in particleboard fires than in diesel pan fires. For example, smoke from diesel pan fires had an arithmetic mean concentration of 3700 μg $m^{-3}$ for $\sum_{15}$ PAHs, whereas the corresponding value from the particleboard fires was 120,000 μg $m^{-3}$. Such difference is reasonable to observe as the liquid diesel fuel burns more completely than the solid particleboard, producing fewer PAHs. Similar levels of PAHs were also reported in compartment fires with wood and chipboard fuels (Wingfors et al., 2018; Fent et al., 2019).

### 3.3. Skin wipes

Data from the skin wipes are presented in Fig. 1 and S1 and, Table S7. Naphthalene concentrations in skin wipes were below the MDL in all the samples both pre- and post-burn and thus not included in Fig. S1. As the levels of PAHs are significantly different between the smoke from diesel pan fire and the smoke from particleboard fire, data for skin deposition of PAHs on participants attending the two fires are presented separately.

As expected, the level of $\sum$PAHs deposited on skin of participants attending the diesel pan fires was lower than those attending the particleboard fires. This was also the case with pyrene. As shown in Fig. S1, the most prominent changes in concentration of PAHs on skin after the diesel pan fires were observed for phenanthrene and pyrene on the neck. The median concentrations increased by 4.5 and 8.5 times, respectively. Participants who had attended particleboard fires saw a significant increase in the concentrations on skin wipes collected post-burn compared to pre-burn. Median concentrations of various PAHs increased on skin wipes from wrists (7–10 times for fluorene, phenanthrene and pyrene) and from the neck (12–15 times for phenanthrene and pyrene).

The levels of PAHs deposition on firefighters measured in this study are comparable to the data reported in other studies on firefighters in

**Table 1**
Concentrations (μg.$m^{-3}$) of PAHs in the smoke layer of simulated compartment fires.

| | Diesel Pan Fires | | | | | Particleboard Fires | | |
|---|---|---|---|---|---|---|---|---|
| | Burn 1 | Burn 2 | Burn 3 | Burn 4 | Burn 5 | Burn 6 | Burn 7 | Burn 8 |
| Naphthalene | 1800 | 1300 | 2200 | 2400 | 2800 | 54000 | 32000 | 31000 |
| Fluorene | 120 | 74 | 120 | 170 | 180 | 15000 | 7900 | 7300 |
| Phenanthrene | 430 | 230 | 440 | 580 | 520 | 32000 | 20000 | 18000 |
| Anthracene | 74 | 41 | 65 | 73 | 86 | 19000 | 7200 | 5100 |
| Fluoranthene | 290 | 130 | 280 | 330 | 270 | 16000 | 7100 | 4300 |
| Pyrene | 370 | 180 | 350 | 390 | 360 | 15000 | 6700 | 4000 |
| Chrysene +Benz[a]anthracene | 150 | 77 | 140 | 170 | 120 | 8400 | 3700 | 2000 |
| Benzo[b]fluoranthene +Benzo[k]fluoranthene | 0.17 | 18 | 0.10 | 0.17 | 35 | 1500 | 600 | 350 |
| Benzo[e]pyrene +Benzo[a]pyrene | 130 | 51 | 120 | 110 | 110 | 5200 | 2200 | 1300 |
| Indeno[1,2,3-c,d]pyrene | 31 | 15 | 35 | 45 | 47 | 2000 | 990 | 520 |
| Dibenzo[a,h]anthracene | 17 | 13 | 13 | 29 | 22 | 390 | 180 | 91 |
| Benzo[ghi]perylene | 1.4 | 29 | 71 | 68 | 1.7 | 2400 | 1200 | 620 |
| **$\sum_{15}$ PAHs** | 3400 | 2200 | 3800 | 4300 | 4700 | 180000 | 91000 | 75000 |

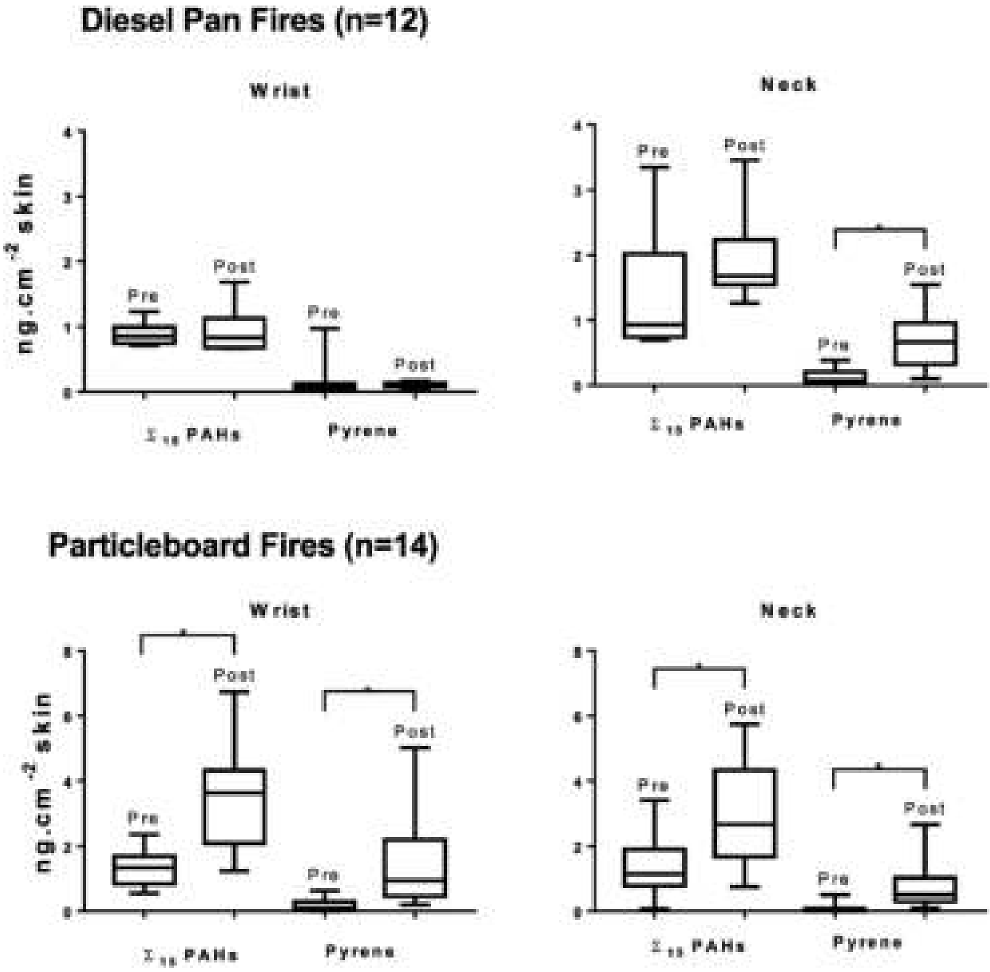


**Fig. 1.** Concentrations of $\sum_{15}$PAHs and pyrene from skin wipes. *significantly different with $p < 0.05$ (paired *t*-test).

Canada (Keir et al., 2020), Sweden (Wingfors et al., 2018) and the United States (Fent et al., 2017). In summary, all the deposition data so far indicated that despite the use of PPC, firefighters are still exposed to toxic chemicals, as demonstrated by the concentration of PAHs on the skin post-burn.

### 3.4. Urine

The results for OH-PAHs in urine are presented in Fig. 2 and Table S8. OH-PAHs have been summed by their parent PAH and reported as hydroxynaphthalenes (OH-NAPs = Σ1+2-hydroxynaphthalene), hydroxyfluorenes (OH-FLUs = Σ2+3+9-hydroxyfluorene), hydroxyphenanthrenes (OH-PHEs = Σ1+2 + 3+4-hydroxyphenanthrene) and 1-hydroxypyrene (1-OH-PYR). All OH-PAHs were above the MDLs in all urine samples.

Samples from participants who attended diesel pan fires showed, no significant difference (paired *t*-test, $p > 0.05$) in the concentrations of OH-PAHs between pre-burn and post-burn urine void samples. Samples from participants who attended particleboard fires also showed no significant difference ($p > 0.05$) between 1-OH-PYR concentrations in pre-burn and post-burn samples. However, the concentrations of OH-FLUs were significantly ($p < 0.05$) higher than pre-burn urine for the first, second and third urine void post-burn. OH-NAPs and OH-PHEs concentrations were significantly higher in the second and third post-burn urine void samples. The concentrations of OH-NAPs and OH-FLUs were highest in the second post-burn urine void samples. The median concentration more than doubled from 5.2 to 0.44 μg $g^{-1}$ creatinine for OH-NAPs and OH-FLUs respectively, to 13 and 1.4 μg $g^{-1}$ creatinine. Meanwhile, the median concentration of OH-PHEs peaked in the third post-burn urine void samples, increasing from the pre-burn concentration of 0.80–1.2 μg $g^{-1}$ creatinine.

It is noted that the lack of increase in the urinary concentration of 1-OH-PYR between pre- and post-burn urine samples in our study is contrary to many previous studies on firefighter exposure, which measured significant increases of 1-PYR in urine samples of firefighters after fire events (Andersen et al., 2018b; Andersen et al., 2017; Caux et al., 2002; Fent et al., 2019; Fernando et al., 2016; Feunekes et al., 1997; Keir et al., 2017; Laitinen et al., 2010).

In this study, the median concentrations of OH-NAPs, OH-FLUs, OH-PHEs and 1-OH-PYR in firefighters' urine prior to attending the compartment fires were 4.7, 0.32, 0.81 and 0.15 μg $g^{-1}$ creatinine respectively. By comparison, the average concentrations of those biomarkers from an Australian cohort >15 year old in 2017 were 31, 0.66, 0.25 and 0.11 μg $g^{-1}$ creatinine respectively (Thai et al., 2020). It was expected that concentrations of urinary OH-PAHs in the Australian general population would be higher than that of firefighters, as 13.8% of Australians in 2017 were smokers (ABS, 2019). This assumption was true for OH-NAPs and OH-FLUs, but not for OH-PHEs and 1-OH-PYR. It is possible that the 26 firefighters in this study have had occupational exposure that would increase the regular intake of those two PAHs (Oliveira et al., 2017; Banks et al., 2020). The occupational exposure to firefighters may include exposure through fire suppression activities, air and dust in fire stations, from PPC and from firefighting equipment (Alexander and Baxter, 2014; Mayer et al., 2019). PAHs occur in vapor and particle phases, are associated with dust, and can sorb onto materials. The higher the vapor pressure, the more likely they are to be

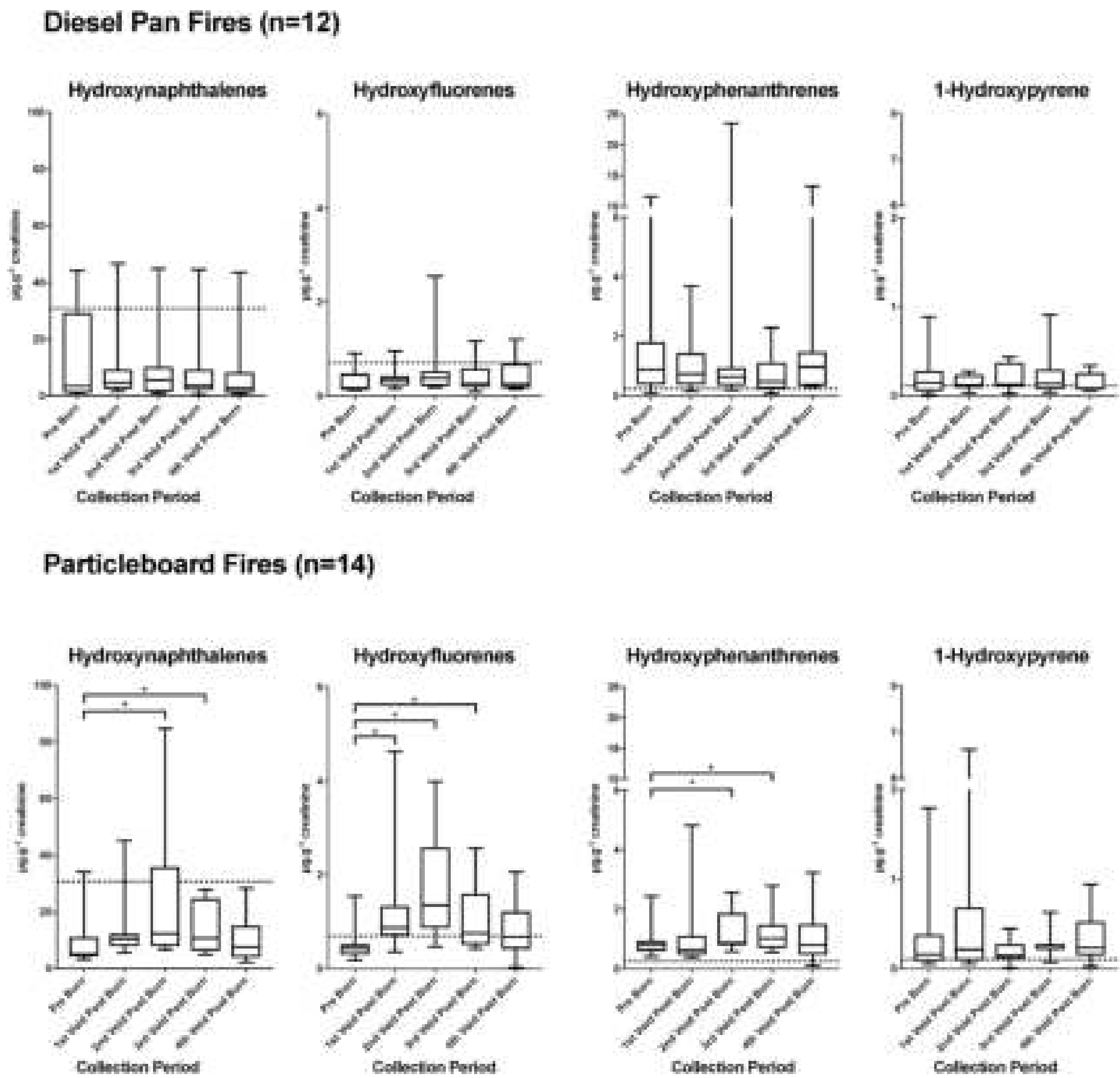


**Fig. 2.** Concentrations of OH-PAHs in firefighter urine pre- and post-burn. Dashed lines are the average concentrations for each chemical group in Australian adults (>15 years old) from 2017 (Thai et al., 2020). The Australian average results were converted to μg.g$^{-1}$ creatinine using a creatinine concentration of 1304 mg.L$^{-1}$ (Barr et al., 2005). *significantly different from pre-burn urine (paired *t*-test).

**Table 2**
Urinary concentrations of OH-PAHs in non-exposed urban firefighters and selected representative populations (mean, geometric mean or median).

| Study | Country | n | Smokers (%) | OH-PAHs (μg.g$^{-1}$ creatinine) | | | |
|---|---|---|---|---|---|---|---|
| | | | | ∑OH-NAPs | ∑OH-FLUs | ∑OH-PHEs | 1-OH-PYR |
| Non-Exposed Firefighters | | | | | | | |
| This study | Australia | 26 | 0 | 4.7 | 0.32 | 0.81 | 0.15 |
| Caux et al. (2002) | Canada | 43 | 4.6 | | | | 0.21 |
| Keir et al. (2017) | Canada | 27 | 0 | 5.6 | 0.48 | 0.35 | 0.10 |
| Cherry et al. (2019) | Canada | 68 | 2.7 | | | | 0.065 |
| Andersen et al. (2018a) | Denmark | 53 | 0 | | | | 0.52 |
| Andersen et al. (2018b) | Denmark | 22 | 18 | | | | 1.0 |
| Fent et al. (2019) | United States | 24 | 0 | 3.7–5.4 | 2.6–3.6 | 0.11–0.14 | 0.32–0.35 |
| Oliveira et al. (2017) | Portugal | 75 | 0 | | | | 0.026–0.28 |
| Wingfors et al. (2018) | Sweden | 20 | 0 | 3.3 | 1.4 | | 0.27 |
| General Population | | | | | | | |
| Thai et al. (2020) | Australia | 1600[a] | 13.8 | 31 | 0.66 | 0.25 | 0.11 |
| Health Canada, 2017 | Canada | 2500 | 10.8 | 4.3 | 0.48 | 0.30 | 0.088 |
| CDC (2019a) | United States | 2640 | 13.7 | 4.7 | 0.25 | 0.25 | 0.10 |

[a] Urine pooled from Australians 15 years old and over. Results from the general population studies Thai et al. (2020) and CDC (2019a) were converted to μg.g$^{-1}$ creatinine using the creatinine concentration of 1304 mg.L$^{-1}$ (Barr et al., 2005). Prevalence of smoking for general population data were taken from ABS (2019), Canadian Tabacco (2018) and CDC (2019b).

associated with the vapor phase. The increased concentrations of OH-PHEs and 1-OH-PYR, whose parent PAHs have lower vapor pressures may indicate occupational exposure though particles, dust or materials.

The higher concentrations of OH-PHEs and 1-OH-PYR measured in firefighters compared to the general population is consistent across this study and several international studies, in which groups of non-exposed urban firefighters were measured for OH-PAHs (Table 2). The concentration of OH-PHEs measured in firefighter urine was significantly different (single sample *t*-test, $p < 0.05$) from the Australian average concentration, while the concentration of 1-OH-PYR measured in firefighters' urine was not statistically different ($p > 0.05$) from the Australian average concentration.

### 3.5. Factors that could influence the level of exposure

The different concentrations of PAHs between the diesel pan fire and the particleboard fire affected the exposure levels of firefighters. Weak correlations were found between the concentrations of phenanthrene and pyrene in the smoke layer, and in the change in concentrations of phenanthrene and pyrene on the neck (Pearson correlation, r: 0.68, 0.51, $p < 0.05$). Weak correlations were also found between the concentration of fluorene in the smoke layer and the concentration of OH-FLUs in the second urine post-burn (r: 0.54, $p < 0.05$). Correlations were found between the concentration of naphthalene and phenanthrene in the smoke layer and the concentrations of OH-NAPs and OH-PHEs in the third urine post-burn (r: 0.57, 0.89, $p < 0.05$). The correlations between the change in dermal concentration of phenanthrene and pyrene as well as concentrations of OH-NAPs, OH-FLUs and OH-PHEs in urine with smoke layer concentrations shows that firefighters participating in fires with higher PAHs levels have higher exposure than those attending fires with lower PAHs levels. In Kirk and Logan (2015), there were correlations between the concentration of PAHs from the air inside and outside firefighting ensembles when attending a compartment fire. This supports the association of higher PAH concentration in the smoke layer and higher exposure described above.

In this study, there were no significant correlations ($p > 0.05$) between PAHs measured from the neck and wrist wipes and their OH-PAH metabolites measured in urine. This is contrary to other studies such as Fernando et al. (2016) and Wingfors et al. (2018), in which a significant correlation was found between an increase in the concentration of an individual PAH on skin and its urinary metabolite in firefighters after burn events. The probable reason is that in our study, firefighters were able to remove firefighting ensembles and shower within 10 min of finishing the burn, resulting in a total exposed time of less than 30 min for each event. The ability to shower quickly might have removed most of the deposited PAHs on the skin, thus limiting the exposure (exposure = concentration × time). It has been shown that quick decontamination practices such as cleansing wipes have reduced PAH contamination on the skin of firefighters (Fent et al., 2017). Therefore, it is expected that showering would have a stronger decontamination effect in removing PAHs on the skin of firefighters, resulting in further reducing their exposure to PAHs and other potentially toxic chemicals.

### 3.6. Limitations

We acknowledge several limitations of this study. Firstly, the simulated compartment fires and the activities firefighters undertook in these fires may not be representative of real-world firefighting scenarios. Secondly, the comparison of urinary OH-PAH concentrations between the firefighters and the general population may be affected by seasonal or other potential longitudinal variations in OH-PAH concentration as well as differences between the population demographics. In addition to this, the general population OH-PAH concentrations from Thai et al. (2020) and CDC (2019a) were creatinine normalised using a single average creatinine value. This average creatinine value is derived from a large and diverse population (Barr et al., 2005), and while we considered it as reasonable to apply this to population average values for creatinine normalisation purposes, there is still the potential that difference in the population demographics, locations and season would vary this value. Finally, this study does not address individual characteristics of PAH metabolism, including but not limited to the case of 1-hydroxynapthalene, which is not only a metabolite of naphthalene but also a metabolite of the insecticide carbaryl.

## 4. Conclusions

Our study found that firefighters who attended the diesel pan fires did not show any significant change in urinary OH-PAH concentration. Samples from participants who attended the particleboard fires, which had a higher concentration of PAHs in the smoke layer, showed an increase in the concentrations of OH-NAPs, OH-FLUs and OH-PHEs. Further study is warranted regarding practices, including handling of gear and post-burn decontamination that may reduce firefighter exposure.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2020.113637.

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# Daily changes in household water access and quality in urban slums undermine global safe water monitoring programmes

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ABSTRACT

Global drinking water monitoring programmes and studies on water quality in urban slums often overlook short-term temporal changes in water quality and health risks. The aim of this study was to quantify daily changes in household water access and quality in an urban slum in Malawi using a mixed-method approach. Household drinking water samples ($n$ = 371) were collected and monitored for *E. coli* in tandem with a water access questionnaire ($n$ = 481). *E. coli* concentrations in household drinking water changed daily, and no household had drinking water that was completely safe to drink every day. Seasonal changes in drinking water availability, intermittent supply, limited opening hours, and frequent breakdown of public water points contributed to poor access. Households relied on multiple water sources and regularly switched between sources to meet daily water needs. There were generally similar *E. coli* levels in water samples considered safe and unsafe by residents. This study provides the first empirical evidence that water quality, water access, and related health risks in urban slums change at much finer (daily) temporal scales than is conventionally monitored and reported globally. Our findings underscore that to advance progress towards Sustainable Development Goal (SDG) Target 6.1, it is necessary for global water monitoring initiatives to consider short-term changes in access and quality.

## 1. Introduction

Globally, 785 million people lack access to even the most basic drinking water services (UNICEF and WHO, 2019), which can significantly affect their health and wellbeing. For example, in 2016, there were an estimated 485,000 deaths from diarrhea attributable to inadequate water access (Prüss-Ustün et al., 2019). On a global scale, access to safely managed drinking water services has improved over recent years (Fuller et al., 2016). However, the burden of unsafe water is still disproportionately higher in low and middle-income countries, particularly countries in sub-Saharan Africa (SSA) where, in 2015, the number of deaths attributable to water pollution was higher than any other region in the world (Forouzanfar et al., 2016; Landrigan et al., 2018).

In SSA and elsewhere in the Global South, population growth and rapid urbanization have created 'slums' where most of the urban population lives. While we acknowledge the negative connotations of the term 'slum,' we use it here broadly to capture a diversity of settlements that lack access to basic services, while recognising that other terms (e.g. informal settlement) have their own drawbacks (Ezeh et al., 2017; Gilbert, 2007). An estimated 881 million people lived in slums in 2014 (UN-Habitat, 2016), and this number is predicted to grow to at least 3 billion by 2030 (UN-Habitat, 2014). Most residents in slums are poor, water insecure, and regularly deal with overcrowded and risky environmental conditions (Adams et al., 2020). There are often pervasive deficiencies in the water supply (and issues of access) within slums because they lie outside of centralised urban water infrastructure (Ezeh et al., 2017). Many slum dwellers do not own private taps, so they depend on costly but often unsafe water from private water vendors (Adams and Vasquez, 2019).

Target 6.1 of the SDGs calls for "universal and equitable access to safe and affordable drinking water for all" by 2030 (UN General Assembly, 2015). Achieving this target requires improved monitoring of safe water over both short and long time scales, particularly in slums where access to safe water may vary with multiple

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temporally-dependent factors, including income, season, and availability, as well as being shaped by social relations and poverty (Adams et al., 2020; Price et al., 2019). In order to measure progress towards Target 6.1, a 'drinking water ladder' was developed and has become an integral part of the WHO & UNICEF's Joint Monitoring Programme (JMP) (WHO and UNICEF, 2017). The JMP drinking water ladder benchmarks service levels across countries and provides an aspirational global target of a 'top rung,' which in practice means that everyone in that country has access to *safely managed water*; i.e., "drinking water from an improved water source which is located on premises, available when needed, and free from faecal and priority contamination". This definition represents a significant step forward from the Millennium Development Goals (2000–2015), where water quality was often inferred from the water source (Bain et al., 2014).

Currently, the WHO (World Health Organisation) recommends the enumeration of *Escherichia coli* (*E. coli*) in drinking water as the best faecal indicator organism for monitoring recent faecal contamination (Kostyla et al., 2015; UNICEF and WHO, 2019). The majority of drinking water quality studies measuring *E. coli* in slums have been cross-sectional (e.g., Blanton et al., 2015; Debela et al., 2018) or seasonal (e.g., Kostyla et al., 2015) in design. A major limitation of these studies is that they overlook the potential for household drinking water quality to change over much shorter timescales (e.g., day-to-day) in response to changes in source contamination, availability, reliability, and affordability (Price et al., 2019). For example, in SSA residents often have a secondary (or 'back up') drinking water source for when their preferred source is unavailable (Okotto et al., 2015; Tutu and Stoler, 2016), e.g. due to breakdown of the source or limited supply.

Understanding short-term changes in water quality and access is vitally important for rapid progress towards target SDG6.1 since one-off measurements by month or season can mask other temporal changes in access, quality, and associated health risks experienced by residents of slums. However, monitoring changes in drinking water quality at such high temporal resolution is challenging because of the increased resource requirements of such monitoring and the lack of standardised study designs that take this approach. Therefore, our aim was to assess how the key factors that underpin access to water in slums (i.e., accessibility, reliability, affordability) change over time and to quantify whether access to safe drinking water changes from day-to-day. To achieve this aim, we undertook daily water quality (*E. coli*) monitoring of drinking water at the household level in parallel with a community-wide questionnaire and examined issues of drinking water access in Bangwe, an urban slum in Blantyre, Malawi.

## 2. Methods

### 2.1. Study area

This study was undertaken in Bangwe, a slum to the east of Blantyre, Malawi's second-largest city and commercial capital (Fig. 1). Between 2008 and 2018, Blantyre City's population grew by 2% per annum, and its population in 2018 was 800,264, with nearly 65% of the population living in urban low-income, informal, and unplanned settlements (Malawi Government, 2019). Bangwe was chosen as the study area because of its high population density and the low-income status of its residents. It has a particularly hilly topography, which means that there is a reliance on a variety of tap, ground, and surface water sources, and many residents use communal water points rather than relying on household taps (Magoya, 2018).

Blantyre's geographic location, in the Shire-Zambezi river basin, means that freshwater is relatively abundant in comparison to some parts of southern Africa (Tchuwa, 2018). In Blantyre City, tap water is abstracted, treated, and distributed by the local water board (Kalulu and Hoko, 2010). However, the city faces many water management challenges, including a lack of tap water infrastructure into low-income communities, water losses through the piped system, the poor water supply and quality, and lack of reliable electricity supply (Adams and Zulu, 2015).

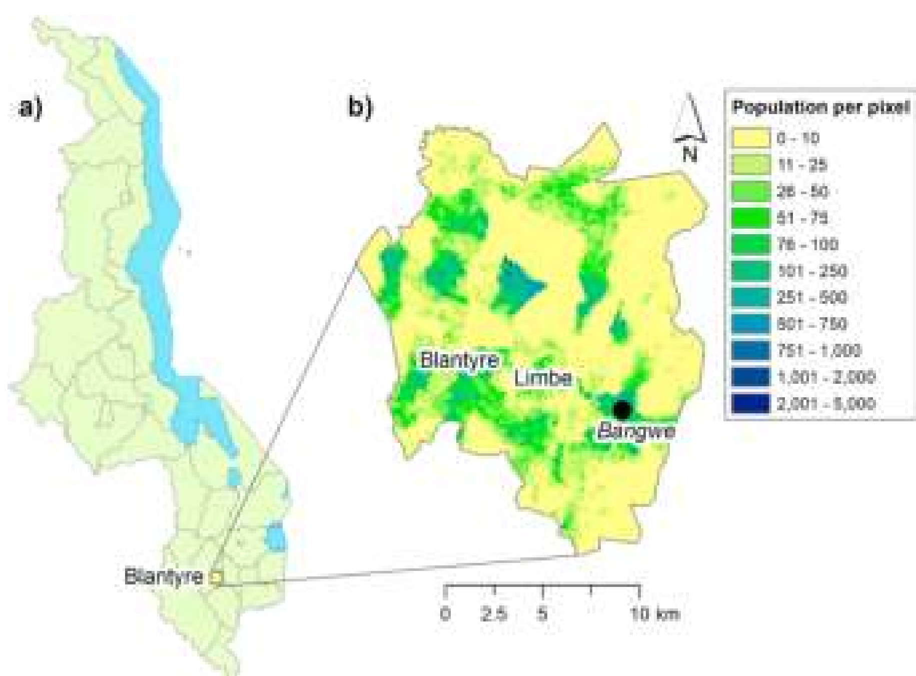


**Fig. 1.** Study site locations: (a) Malawi and (b) the Blantyre district, including the study site Bangwe. Blantyre district has been shaded by population numbers per pixel (WorldPop, 2017).

### 2.2. Data collection and analysis

To determine the temporal dynamics of water access and water quality, a multi-level mixed methods approach was used. In-depth questionnaires (section 2.2.1) were used to explore community-level views on the temporal dynamics of water access and quality. A subsample of these households were also recruited for a household drinking water quality study, which combined daily monitoring of their household water quality with a daily water practices questionnaire designed to explore factors that could temporally affect household water quality (section 2.2.2).

#### 2.2.1. In-depth water access questionnaires

Questionnaires were used to gather information about household residents' socio-economic status, water access and use, water consumption, storage and treatment practices, sanitation facilities, hygiene practices, and future community water recommendations from adult residents. The questionnaire generated both quantitative (from multiple choice and yes/no questions) and qualitative (from open-ended questions) data. Questionnaires were designed by the research team, with some questions building on standard question designs (e.g., those used in the WHO & UNICEF JMP (UNICEF and WHO, 2018)) and others developed *de novo*. The study protocol was approved by the University of Stirling General University Ethics Panel (reference: GUEP169). The questionnaires were piloted by the research team and adapted as necessary.

A stratified random sampling approach was used during the two field missions in July/August 2017 (the dry season) and January 2018 (the rainy season). In brief, communities were geographically split into near-equal sized parcels using Google Maps satellite imagery, and each parcel was targeted for questionnaire sampling on a different day with the aim of covering the whole of Bangwe between the two sampling campaigns. In this way, each household included in the sample participated only once. Within each parcel, a research assistant (RA) was allocated a walking transect, designed to ensure that coverage within the parcel was maximised. Along each walking transect, RAs stopped at every *n* house (where *n* was determined by rolling a dice) to ask if they would participate. The number of households that declined to participate was not systematically recorded by RAs, but anecdotally was below approximately 10%. The household head or, if the household head was unavailable, another adult (over 18 years of age) in the household was recruited. Community members were asked to read an information sheet (or have this read to them) before giving their consent to participate. The survey questions were asked in the local language, Chichewa, and the responses ($n = 481$ [314 in July/August 2017 and 167 in January 2018]) recorded in English on paper questionnaires. Data was subsequently input to SPSS (version 23) by an independent researcher, and quality assurance and quality control checks were undertaken to reduce data input errors.

#### 2.2.2. Daily drinking water quality testing study

A subset of 30 households that had participated in the in-depth questionnaire (section 2.2.1) were selected to participate in the second phase of the study. The 30 participating households were selected based upon their geographic location, their primary source of water, and their willingness to participate in both the baseline and the follow-up sampling campaign. During the two sampling campaigns, these households were visited every day for seven consecutive days, and an RA collected a sample of their household drinking water, which was linked to a daily questionnaire exploring some of the key water practices that may affect household drinking water quality. The decision to focus on daily drinking water monitoring (rather than weekly or hourly, for example) represented a balance between being able to capture the variation in *E. coli* that we theorised in (Price et al., 2019) (resulting from changes in factors including water access and water practices) and the practicality of collecting and analysing the drinking water samples.

A total of 371 drinking water samples were collected from the 30 households during the maximum of seven consecutive days in both July 2017 and January 2018 by trained RAs. Concurrently, a daily water quality practices questionnaire was undertaken with residents. The questionnaire was purposefully short and was used to collect information on the source of the water, perceptions of safety, transport and storage, water treatment, and household cases of diarrhea in the previous 24 h.

For some households, it was not possible to collect a drinking water sample every day because there was no one at home, e.g., residents were at work or had gone to church, while some residents moved house between the two sampling campaigns. The transiency of slum populations over short and long timescales is a major challenge in undertaking this type of research, and this needs to be fully considered in future research designs.

All drinking water samples were collected in sterile Whirl-Pak bags (Whirl-Pak®, Nasco, USA), stored in a coolbox, and processed within 6 h of collection. Each water sample was briefly shaken and 100 mL vacuum-filtrated through a 0.45 μm cellulose acetate membrane (Sartorius Stedim Biotech., Gottingen, Germany). The membrane was aseptically transferred to the surface of a plate containing *E. coli* selective membrane lactose glucuronide agar (MLGA) (CM1031, Oxoid, Basingstoke, UK). The plate was inverted, incubated at 37 °C, and enumerated 18–24 h later. Based on the concentration of *E. coli* 100 $ml^{-1}$, each water sample was classified into a health risk category, i.e., safe (zero *E. coli* 100 $ml^{-1}$), low risk (1–10 *E. coli* 100 $ml^{-1}$), medium risk (11–100 *E. coli* 100 $ml^{-1}$) and high risk (>100 *E. coli* 100 $ml^{-1}$) (Rocha-Melogno et al., 2019).

Statistical testing was undertaken to explore whether the two samples (the households for which drinking water quality data was collected ($n = 30$) and those for which we did questionnaires ($n = 451$) were from the same population. This included the Mann Whitney *U* test for the age of the respondent and the number of people living in the household and Fisher's exact test for gender, education, employment status, house ownership status, and main water source. For Fisher's exact test, where multinomial probability distributions between those households for which we collected water quality data and those for which we did not were statistically significantly different, we undertook a post hoc analysis involving pairwise comparisons using multiple Fisher's exact tests ($2 \times 2$) with a Bonferroni correction. Statistical significance was accepted at $p < 0.01$. For all other statistical testing, the significance level was set at 0.05.

## 3. Results

A total of 481 residents from 481 individual Bangwe households completed the in-depth water access questionnaire (Table 1), of which 30 households also took part in the daily drinking water quality testing study (Table 2). The mean household size and age of respondents were 5 and 34, respectively. The households for which drinking water quality data were collected were not significantly different from other households in the community in terms of the age of the respondent and household size (Mann Whitney *U* test), and the gender, education level, employment status and household ownership (Fisher's exact test) (Table 2). Furthermore, there was no difference in the choice of the main water source of those households where water samples were collected and other households (Fisher's exact test where post hoc testing identified that all pairwise comparisons were not statistically significant). Therefore, households for which we collected water quality data were broadly similar to those for which we did not collect water quality data, suggesting that our sampling was representative of the general population.

### 3.1. Daily household drinking water quality

Based on *E. coli* concentrations, none of the 30 households had water

**Table 1**
Summary data for all the households that took part in the in-depth water access questionnaire study ($n$ = 481).

| Variable | | |
|---|---|---|
| | Mean | |
| **Number of people in the household** | 5.0 | |
| **Age of respondent** | 34 | |
| | ***n*** | **%** |
| **Gender of respondent** | 389 | 80.9 |
| Female | 92 | 19.1 |
| Male | | |
| **Completed education level of respondent** | 21 | 4.4 |
| No education | 207 | 43.0 |
| Primary | 229 | 47.6 |
| Secondary | 24 | 5.0 |
| College or higher | | |
| **Employment status of respondent** | 65 | 13.5 |
| Employed for wages | 196 | 40.7 |
| Self-employed | 177 | 36.8 |
| Unemployed | 26 | 5.4 |
| Student | 11 | 2.3 |
| Retired | 6 | 1.2 |
| Other | | |
| **Ownership status of home** | 122 | 25.4 |
| Owners (with property title) | 100 | 20.8 |
| Owners (without property title) | 251 | 52.2 |
| Renters | 8 | 1.6 |
| Other | | |
| **Main water source used** | 94 | 19.5 |
| *Improved sources* | 201 | 41.8 |
| Piped to dwelling, yard, plot or/neighbour | 85 | 17.7 |
| Piped to public tap | 47 | 9.8 |
| Borehole | 38 | 7.9 |
| Protected well | 4 | 0.8 |
| Protected spring | 5 | 1.0 |
| *Unimproved sources* | 7 | 1.5 |
| Unprotected well | | |
| Surface water | | |
| Other | | |

**Table 2**
Summary data for the households that took part in the in-depth water access questionnaire study and the daily drinking water quality study ($n$ = 30).

| Variable | | |
|---|---|---|
| | Mean | |
| **Number of people in the household** | 4.5 | |
| **Age of respondent*** | 33 | |
| | ***n*** | **%** |
| **Gender of respondent** | 25 | 83.3 |
| Female | 5 | 16.7 |
| Male | | |
| **Completed education level of respondent** | 1 | 3.3 |
| No education | 14 | 46.7 |
| Primary | 15 | 50.0 |
| Secondary | 0 | 0.0 |
| College or higher | | |
| **Employment status of respondent** | 2 | 6.7 |
| Employed for wages | 17 | 56.7 |
| Self-employed | 9 | 30.0 |
| Unemployed | 1 | 3.3 |
| Student | 1 | 3.3 |
| Retired | 0 | 0.0 |
| Other | | |
| **Ownership status of home** | 7 | 23.3 |
| Owners (with property title) | 6 | 20.0 |
| Owners (without property title) | 16 | 53.3 |
| Renters | 1 | 3.3 |
| Other | | |
| **Main water source used** | 0 | 0.0 |
| *Improved sources* | 13 | 43.3 |
| Piped to dwelling, yard, plot or/neighbour | 11 | 36.7 |
| Piped to public tap | 5 | 16.7 |
| Borehole | 1 | 3.3 |
| Protected well | 0 | 0.0 |
| Protected spring | 0 | 0.0 |
| *Unimproved sources* | 0 | 0.0 |
| Unprotected well | | |
| Surface water | | |
| Other | | |

that was safe to drink every day (Fig. 2), although there were no obvious trends in *E. coli* concentrations related to specific days of the week. Out of the matched households between the two sampling seasons ($n$ = 23), 52% had water in the 'high risk' category (>100 *E. coli* 100 $ml^{-1}$) on at least one day of the week. A slightly higher proportion of all drinking water samples ($n$ = 371) were in the 'high risk' category in the rainy season (11% of 187 samples) compared to the dry season (8% of 184 samples), although slightly more drinking water samples were classified as 'safe' in the rainy season (46%) compared to the dry season (44%).

Although it was rare for respondents in the 30 sample households to consider their drinking water unsafe for drinking (only 5% of drinking water samples, $n$ = 17), a comparison of drinking water samples considered "safe" and "unsafe" by residents showed generally similar *E. coli* levels (Fig. 2). The majority (97%) of water samples were from covered household storage containers; however, the quality of the cover varied and included plastic or metal lids, plastic or metal plates and dishes, plastic buckets, and weaved baskets. Drinking water was more likely to be stored in the household for over 24 h during the dry season (29%) than in the rainy season (25%). There was no obvious difference in *E. coli* concentrations in water samples stored for under 24 h and more than 24 h.

Considering only matched households between the July 2017 and January 2018 sampling campaigns, 43% of households switched their drinking water from one source category to another (e.g., tap to borehole) for one day or more (Fig. 2). While household drinking water samples from tap water (public and private taps) were more likely to be perceived by households as 'safe' compared to other sources, the water they provided was often found to be unsafe for consumption; 8% ($n$ = 153) of drinking water samples from public taps and 12% ($n$ = 25) of drinking water samples from private taps were in the 'high risk' category for *E. coli* (Fig. 3).

### 3.2. Household water access

Most residents (85%) utilised a secondary source of water when their main source of water was unavailable (Fig. 4), with only 18% of people switching to an alternate water point within the same source category (e.g., borehole to borehole). Those using improved drinking water sources generally transferred to another improved drinking water source (e.g., public tap to borehole), rather than switching to an unimproved source. Nearly all respondents (90%) considered their main drinking water source to be safer than their secondary drinking water source, while 59% considered their secondary source safe.

Most drinking water sources were not available for 24 h per day (Table 3). On average, public taps, boreholes, and protected springs were only available for 12 h per day, with the most common reasons for restricted availability being limited opening hours and irregular supply. There was a clear difference in availability for piped water from different sources; drinking water from public taps was available for 12 h per day and drinking water from a private tap (i.e., located in the dwelling, yard, plot, or at neighbour's house) was available for 21 h per day, on average.

From the sample population of 481 residents, 81% were frustrated with some aspect of safe drinking water access in their community (Table 4). Key frustrations included intermittent supply (including seasonal changes in the quantity of water available, fixed or limited water point opening hours, or breakdown (25%)), affordability concerns related to the cost of water per bucket or the billing system (20%), and the lack of water points (20%).

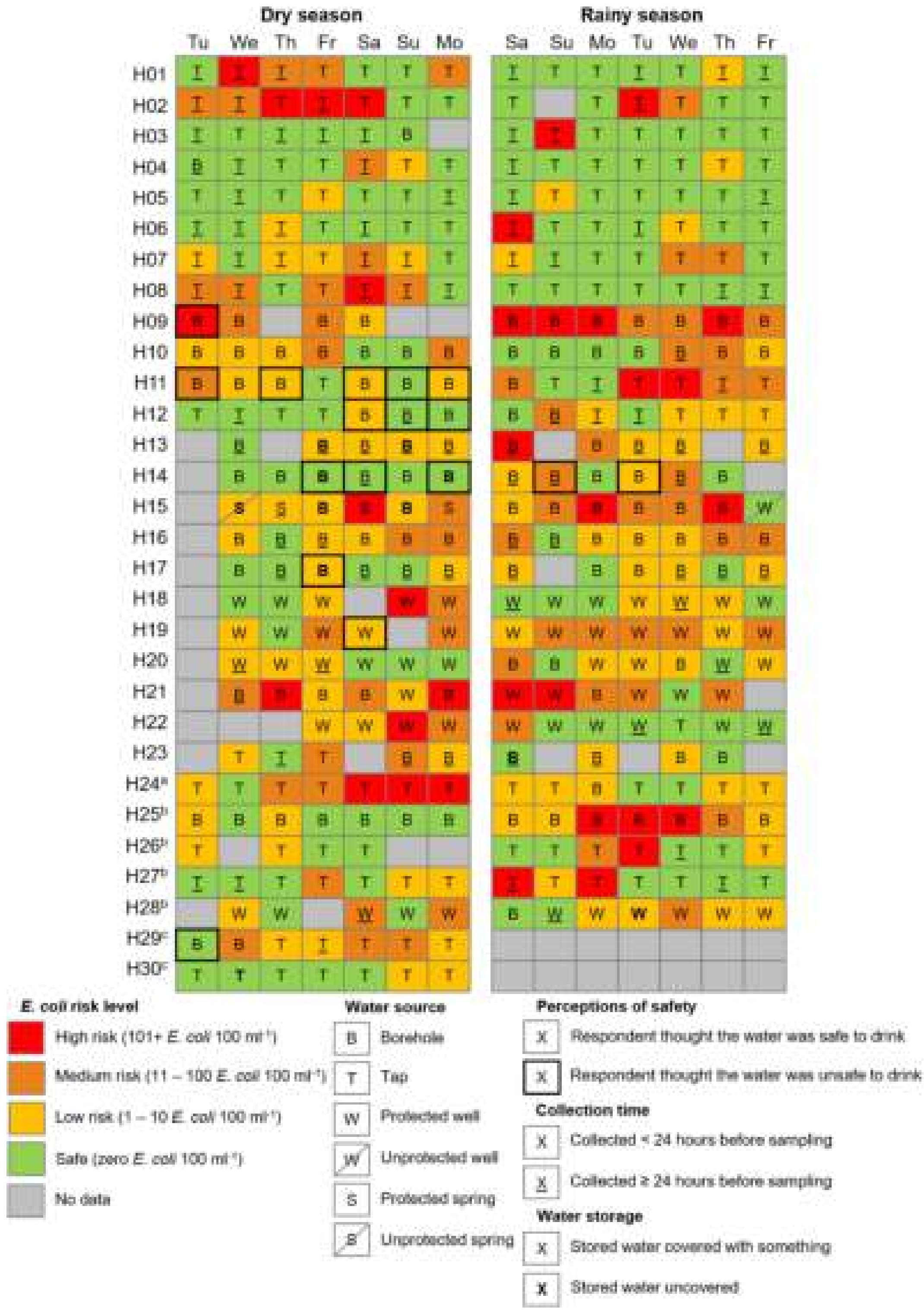


**Fig. 2.** Daily *E. coli* concentrations for the 30 households (H01 – H30) across two seasons. Households were matched between the two seasons, except for households H$n^a$ (where the residents moved out of the house after the first sampling campaign into a neighbouring house where they then participated in the study), H$n^b$ (where residents moved out of the house after the first sampling campaign and replacement respondents in the same household were identified for inclusion), and H$n^c$ (where residents moved out of the house after the first sampling campaign and no replacements were identifiable).

## 4. Discussion

### 4.1. Daily changes in household drinking water quality

This study is the first to demonstrate empirically that water access and quality in slums vary over shorter timescales than traditionally measured in global monitoring programmes. All 30 of the households in this study used drinking water with evidence of faecal contamination on at least one day during the sampling periods. These daily changes in water quality underpin changing waterborne disease risks that are not captured by the standard “one-off” or “seasonal” approaches to monitoring drinking water quality in research studies and national

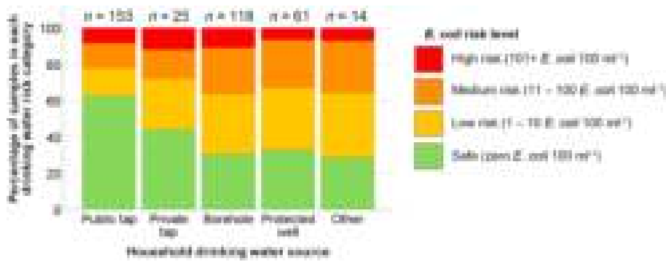


**Fig. 3.** *E. coli* contaimination in household drinking water samples collected from various water sources. The 'other' category includes water collected from protected or unprotected springs, unprotected wells, and surface water.

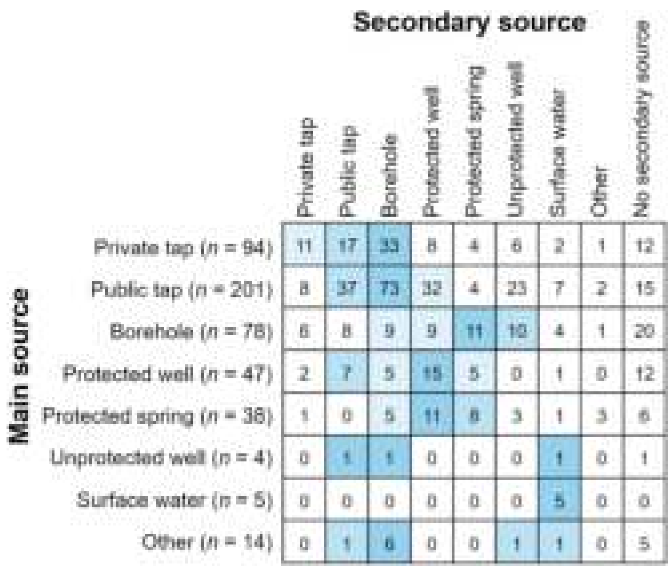


**Fig. 4.** Cross-tabulation comparing the main drinking water source of 481 residents with the source they switch to if their main water source is unavailable (e.g., due to season, breakdown, or opening hours). The most popular switches from each main source are highlighted in blue (darker colours = a greater number of people switching). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 3**
Availability of drinking water from most common sources.

| Drinking water source | *n* | Average availability per day (hrs) | % water points available < 24 h/day | Most common reason(s) for availability < 24 h/day* |
|---|---|---|---|---|
| Piped to dwelling | 94 | 21 | 24 | A, B |
| Piped to public tap | 201 | 12 | 95 | A, B |
| Borehole | 85 | 12 | 94 | A |
| Protected well | 47 | 15 | 74 | A, B |
| Protected spring | 38 | 12 | 97 | A |
| Unprotected well | 4 | 24 | 0 | n/a |
| Other | 7 | 20 | 43 | A |
| Missing data | 5 | n/a | n/a | n/a |

interventions (Debela et al., 2018; K'oreje et al., 2016; Okotto-Okotto et al., 2015).

While the WHO & UNICEF JMP highlight that water quality testing should ideally be undertaken regularly across the year, they also include in their calculations one-off water quality measurements, although they do acknowledge that this only provides a "snapshot" of reality (WHO and UNICEF, 2018). That drinking water is "free from faecal and priority contamination" is a key requirement for *safely managed water* on the JMP drinking water ladder (UNICEF and WHO, 2019). Therefore, such water quality measurements feed into calculations of what percentage of the population resides on each "rung" of the water ladder and ultimately assesses a country's progress towards SDG6.1. However, our findings provide evidence that the approach currently used by the JMP fails to capture the changing waterborne disease risk to urban slum residents from short-term temporal changes in water quality.

There were no clear differences in *E. coli* concentrations in drinking water samples collected in the dry season compared to the rainy season (based on visual inspection of the data in Fig. 2). The lack of seasonal differences in *E. coli* concentrations may reflect the fact that the majority of the population uses publicly shared rather than household taps, and therefore transportation, storage, and handling may be more important determinants of *E. coli* contamination than season. Water collection, transport, handling, and household storage are key entry points for contamination even for water sources that may be clean at source (Wright et al., 2004; Rufener et al., 2010; Boateng et al., 2013). Household observations indicated several contamination risk factors from water collection and handling. For example, drinking water was commonly transported uncovered, and it was routinely carried on the head with fingers resting in the water during transport. Some residents complained that they found dead insects and other foreign objects in their stored water. Storage covers for drinking water were often not designed for that purpose. For example, dishes, other buckets, and baskets were frequently used as ad hoc covers. This practice, combined with other household drinking water management practices, e.g., unclean storage containers (Meierhofer et al., 2019), the method of extracting drinking water from storage containers (Harris et al., 2013), and the extent of mixing of water from different sources (Adams et al., 2020), can compromise the quality of stored drinking water.

**Table 4**
Summary of people's frustrations in accessing drinking water in Bangwe ($n$ = 481).

| Frustration with drinking water | *n* | % | Typical comments |
|---|---|---|---|
| Affordability | 98 | 20 | *Water is very expensive to access; Billing system is not good* |
| Distance/terrain | 35 | 7 | *Long distances to fetch water; Terrain is bad and situation becomes worse when it rains; We cross the main road to get water so it is dangerous* |
| Impact on people's time and energy | 17 | 4 | *A lot of energy needed to fetch water; Very long queue at source; Quarrelling at water source* |
| Lack of water points | 73 | 15 | *Very few water sources; We are always trying to find water; Few boreholes and public taps* |
| Intermittency of supply | 119 | 25 | *When water stops we find it difficult to source alternative water; During dry season most water sources dry up; Pipeline breaks disturb daily life* |
| Water quality and health | 20 | 4 | *Salty water; Insects found in water* |
| Other reason | 27 | 6 | *Misuse of water; Not enough storage containers* |
| No frustrations | 92 | 19 | *We have no problem with the water supply* |

### 4.2. Daily changes in household drinking water access

Our findings reaffirm our recent proposition that a household's ability to access sufficient, safe drinking water changes over time in response to multiple factors, including availability, reliability, and perceptions of water safety (Price et al., 2019). We have shown that most drinking water sources were not available for 24 h per day. Intermittent supply, whether that be predictable (e.g., opening hours), irregular, or unreliable, is a common problem in developing countries (Galaitsi et al., 2016). Even within the 'tap water' category, there was a large difference in terms of the mean number of hours per day the source was available between public taps/public water kiosks (12 h per day) and private taps (21 h per day) since community water sources are generally closed at night (Adams, 2018). Given the same level of reliability between publicly shared sources and privately owned or onsite household taps, the latter offers more guarantee of availability compared to publicly shared taps that are limited by many factors. Most residents (85%) relied on a secondary drinking water source when their main source was unavailable, consistent with previous studies (e.g. Kumpel and Nelson, 2015; Adams, 2018). This was a vital coping strategy for water insecurity as it buffered against water shortages from intermittent supply or limited opening hours at the primary source. In addition, access to water was constrained by its high cost (20% noted that water was too expensive). Recent work shows that households with higher water expenditures are more likely to be water insecure than those with lower expenditures (Stoler et al., 2019). Decision making about choice of water source is largely influenced by cost, availability, and intermittency, all of which can ultimately influence daily variations in household exposure to *E. coli*.

A residents' perception about the safety of their drinking water may pose additional risks for water contamination. Although most residents were confident in the quality of their drinking water and considered it safe to drink, only 45% of the drinking water samples we monitored met the requirements for safe drinking water as determined by *E. coli* contamination (WHO, 2017). No household included in the drinking water quality study perceived their water to be unsafe to drink on every day that a drinking water sample was collected. Instead, perceptions changed on a day-to-day basis based on the source of the water and sensory observations of water quality (e.g., taste and smell) (Subbaraman et al., 2015). However, residents overwhelmingly put their faith in the safety of piped water (both piped onto premises and public taps or kiosks), by responding that water that was unsafe to drink never came from a tap.

## 5. Conclusion

In this paper, we provide the first empirical evidence that the quantities of the indicator bacteria *E. coli* (a proxy for the presence of hazardous faecal contamination) changed from day-to-day in household drinking water in a Malawian slum. This day-to-day variability in contamination needs to be considered by policy makers when pursuing 'universal and equitable access to safe and affordable drinking water for all' (SDG6.1). These findings also have important implications for monitoring progress towards SDG6.1 and suggest that one-off or infrequent monitoring will not capture the changing contamination risk that people experience especially in urban households. Further work to determine the most appropriate sampling interval (e.g. sub-daily or weekly) to fully characterise the changing contamination risk needs to be undertaken as well as an exploration of how these day-to-day changes in contamination impact public health. While water quality testing at finer temporal resolution will take significant time and resources, our findings highlight that alternative, low-cost approaches to regular water quality testing at the household level are necessary to better characterise changing levels of contamination. This could include utilising citizen science approaches to data collection and analysis in slum contexts, although careful consideration needs to be given to this to ensure that the research benefits both the researchers and the citizens and the time and energy burden placed upon vulnerable populations is not too high. In addition to the monitoring of drinking water within the household, further research should also explore the temporal dynamics of drinking water contamination risk at the source and during transit to help inform intervention design aimed at reducing contamination risk, e.g. whether to pursue interventions based on improving water infrastructure or hygiene-based interventions.

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# Human experimental exposure to glyphosate and biomonitoring of young Swedish adults

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A B S T R A C T

Glyphosate (GLY), N-(phosphonomethyl) glycine, is the most widely used herbicide in the world. It is a broad-spectrum herbicide, also used in crop desiccation. Agricultural workers may be occupationally exposed and general populations may be exposed to GLY mainly through diet. We studied the kinetics of GLY by measuring the parent compound and its metabolite aminomethylphosphonic acid (AMPA) in urine samples of three volunteers after an experimental oral exposure. We further examined GLY exposure by measuring GLY and AMPA in spot urine samples of 197 young adults in the general population in Scania, southern Sweden. Urine samples were analyzed using LC-MS/MS. In the experimental exposure, three healthy volunteers received an oral dose equivalent to 50% of the ADI for GLY. Urinary samples were collected up to 100 h after the exposure. The excretion of GLY to urine seemed to follow first-order kinetics and a two-phase excretion. The excretion half-life of GLY (density adjusted) was 6–9 h in the rapid phase and 18–33 h in the slower phase. The total dose recovered as unchanged GLY in the urine samples of volunteers was 1–6%. The metabolite AMPA was found to be 0.01–0.04% of the total dose of GLY. In the population of young adults, the median concentration was below 0.1 μg/L and a maximum concentration being 3.39 μg/L (density adjusted). AMPA was generally detected in lower concentrations (maximum = 0.99 μg/L). A moderate correlation (Spearman's $\rho = 0.56$) was observed between GLY and AMPA concentrations. Overall, the results may suggest that GLY and AMPA partly originate from separate exposures and that unchanged GLY is a more suitable biomarker of exposure.

## 1. Introduction

Although glyphosate is currently the most widely used herbicide (Connolly et al., 2020) and an important topic of discussion worldwide, little is known about the human toxicology, metabolism, and population exposure. Glyphosate (GLY), N-(phosphonomethyl) glycine, a broad-spectrum herbicide also used for crop desiccation, is the active ingredient in several commercial products, such as Roundup®. Since GLY is extensively used in agricultural food production, populations may be directly exposed in occupational settings or environmentally exposed through residues in food. GLY has previously been considered to pose a relatively low risk both for the environment and for the health of non-target species such as mammals (Williams et al., 2000). However, some recent studies report indications of associations with adverse outcomes in soil ecosystems, animals, and humans (Myers et al., 2016). Possible adverse health effects include carcinogenic, endocrine-disruptive, and microbiome-disruptive effects (Davoren et al., 2018; Kogenivas, 2019; Zhang et al., 2019). Furthermore, some studies have raised concerns over the possible health effects of other ingredients and/or mixture effects in commercial formulations of GLY-based herbicides and over the toxicity of the GLY metabolite aminomethylphosphonic acid (AMPA) (Davoren et al., 2018). The current risk classifications and regulations are contradictory, with WHO—International Agency for Research on Cancer (WHO—IARC) classifying GLY as "probably carcinogenic to humans", whereas the European Food Safety Authority (EFSA), US Environmental Protection Agency (EPA), and European Chemicals Agency (ECHA) conclude that the scientific evidence regarding GLY does not indicate carcinogenic potential for humans (Kogenivas, 2019). Further, a re-evaluation for a renewed approval of glyphosate as an active substance in plant protection products after December 2022 is being assessed within the EU (European Commission, 2020).

EFSA has previously established an acute reference dose (ARfD) and an acceptable daily intake (ADI) of 0.5 mg per kg of body weight for GLY (EFSA, 2015). Data from animal studies suggest that GLY is poorly bio-transformed and rapidly excreted unchanged, mainly in feces, but around 10–30% in urine (JMPR, 2016). Small amounts of the metabolite AMPA were reported in rats, from the microbial degradation of GLY after oral administration (Anadon et al., 2009).

The excretion of GLY in animals is reported to be two-phased, with a

*Abbreviations:* ADI, acceptable daily intake; AMPA, aminomethyl phosphonic acid; LC-MS/MS, liquid chromatography triple quadrupole mass spectrometry; LOD, limit of detection; GLY, N-(phosphonomethyl) glycine; QC, quality control; UER, urinary excretion rate.

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half-life of around 6 h for the shorter phase and 79–106 h for the longer phase (Williams et al., 2000). Information about the elimination kinetics of GLY in humans is very limited, but a recent oral exposure study reported a half-life of 9 h for GLY (Zoller et al., 2020) and another study of horticulturists reported the half-life in the range of 5.5–10 h (Connolly et al., 2019). Some biomonitoring studies assessing GLY exposure are available in the literature (Acquavella et al., 2004; Connolly et al., 2017; Conrad et al., 2017; Knudsen et al., 2017; Kongtip et al., 2017; Nova et al., 2020; Zhang et al., 2020), but human data on glyphosate are surprisingly scarce. Available European biomonitoring studies on GLY in non-occupationally exposed populations have mainly reported low concentrations, with median concentrations below the limit of detection (LOD) (Conrad et al., 2017; Connolly et al., 2018a,b; Soukup 2020). Occupationally exposed groups in several countries have reported higher concentrations ranging from a geometric mean at 0.7 μg/L in Irish horticulturists (Connolly et al., 2017) and up to 292 μg/L (Zhang et al., 2020) in Chinese workers within GLY production. With such few studies available, there is insufficient evidence to indicate that GLY is metabolized to AMPA in humans, or whether AMPA concentrations measured in human samples mainly derive from AMPA residues in cultivated food products (Connolly et al., 2020; Soukup et al., 2020). More information on the kinetics and fate of GLY in humans is required to support the interpretation of biomonitoring data, to relate exposure concentrations to currently existing health-based guidance values and to perform robust risk assessments (Connolly et al., 2020).

We aimed to study the excretion of GLY and AMPA in urine by conducting an experimental oral exposure in humans. Furthermore, we aimed to determine the concentrations of GLY and AMPA in urine samples of 197 young adults from a general population in southern Sweden.

## 2. Materials and methods

### 2.1. Experimental oral exposure

GLY was dissolved in 10 mL of Milli-Q water to a concentration of 10 mg/mL, which was used as a stock solution. Three healthy volunteers (one female and two males) received a single oral dose of GLY, equivalent to 50% of the ADI diluted in 250 mL of Milli-Q water. The volunteers fasted for 2 h before and after the oral dose and avoided conventionally grown commodities during the experiment. A pre-exposure urine sample was collected, and all voided urine was collected *ad libitum* up to 100 h after the oral dose. The times of voiding and the total volume of each sample were registered. All the samples were stored at −20 °C until analysis.

### 2.2. Analysis

The urine samples were analyzed using a method presented by Jensen et al. (2016), with minor modifications. The urine samples, calibration standards, quality controls (QCs), and chemical blanks (prepared in Milli-Q water) were added with 2% formic acid and internal standard. The samples were prepared in 96-well plates and analyzed using liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS; QTRAP 5500, AB Sciex, Foster City, CA, USA). The limit of detection (LOD) was determined as three times the standard deviation of the GLY concentration in blank urine samples as 0.1 μg/L for both GLY and AMPA. A detailed method description including validation data is presented in the supplementary material. The laboratory participates in the German External Quality Assurance Scheme (G-EQUAS) for GLY analysis coordinated by the University of Erlangen-Nuremberg, Germany, with good results (Supplementary material).

### 2.3. Adjustment for urinary dilution

Creatinine concentrations in the urine samples were determined using an enzymatic method (Mazzachi et al., 2000) and were analyzed at the Department of Clinical Chemistry, Skåne University Hospital, Lund, Sweden. The laboratory is accredited for creatinine analysis. The concentrations of GLY were then divided by the creatinine concentrations in the samples to adjust for differences in urinary dilution. Additionally, the density of the urine samples was measured in our laboratory using a hand-held refractometer. The adjustment for urinary density, $C_d$, was calculated according to $C_d = C_{observed} \times (1 - \rho_{mean})/(1 - \rho_{sample\ density})$, where $C_{(observed)}$ is the concentration of the analyte in the urine sample, $\rho_{mean}$ is the mean urine density for the study participants, and $\rho_{sample\ density}$ is the density of the urine sample. The mean urine density of the volunteers (1.015) was used for density adjustment in the oral exposure experiment, and the mean urine density of the population (1.019) was used for adjustment in the population biomonitoring. Furthermore, full void volume was measured for each sample in the oral exposure experiment and was used to estimate the urinary excretion rate (UER).

### 2.4. Estimation of elimination half-life

The elimination half-life was estimated using the quantified concentrations of GLY in the urine samples obtained from the volunteers in the oral experimental exposure. The excretion half-lives were calculated from the slope of the curve obtained from the plot of the natural log-linear concentrations versus mid-time points. Estimations were made using unadjusted data and for concentrations adjusted for density, creatinine, and urinary excretion rate (UER).

### 2.5. Biomonitoring of GLY in young adults

A spot urine sample was collected from each of 197 young adults (109 females and 88 males) in 2017 to assess environmental exposure to GLY through analyses of GLY and AMPA. The recruitment was performed at three schools in Scania, southern Sweden. The participants were aged 18–19 years and were in their last year of secondary school. They were informed and recruited at their schools, where we also collected the samples. The recruitment was originally performed for a national biomonitoring program organized by the Swedish Environmental Protection Agency (EPA), to assess exposure to environmental pollutants in the population (Noren et al., 2020).

### 2.6. Ethical approval

The human experimental exposure studies and the biomonitoring of the exposure to GLY in the studied population in southern Sweden were ethically approved by the Regional Ethical Review Board, Lund University, Sweden (Dnr 463/2005, Dnr 2010/41, Dnr 2010/465, and Dnr 2013/6). The volunteers and the participants in the population study gave written informed consent to participate.

## 3. Results

### 3.1. Experimental oral exposure

After oral administration of GLY, the urinary concentrations of GLY increased rapidly and $C_{max}$ was reached within 2 h (Table 1). The measured GLY concentrations were 3–1400 μg/L. The dose recovery was 1–4% within the first 24 h, and the total dose recovery up to 100 h after exposure was 1–6% for the different volunteers. The $C_{max}$ of AMPA was reached 5–6 h after exposure (Table 1). The measured AMPA concentrations were low, between the LOD and 6.4 μg/L, and the total dose recovery was 0.01–0.04% of the administered GLY dose. The concentrations of GLY and AMPA in the pre-exposure samples were below the LOD of 0.1 in all volunteers. The freshly made solution of 200 μg/L of the

**Table 1**
Descriptive characteristics of the study volunteers, the oral doses of GLY and the urinary concentrations before and after the exposure (density adjusted). Recovery of the dose as unchanged GLY and the metabolite AMPA is presented in percentages after 24 h and total dose recovery. $C_{max}$ is the maximum concentration (density adjusted) excreted in the urine, and estimated $T_{max}$ is the mid-time point of maximum excretion.

| | | | | | Pre-exposure | | GLY | | | | AMPA | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Volunteers | Age (years) | Weight (kg) | BMI | Oral dose[a] (mg) | GLY (μg/L) | AMPA (μg/L) | Recovery (%) 24 h | total | Cmax (μg/L) | Tmax (h) | Recovery | total (%) | Cmax (μg/L) | Tmax (h) |
| Female | 73 | 57 | 23 | 14.3 | <LOD | <LOD | 1.2 | 1.9 | 234 | 2 | 0.02 | 1.7 | | 6 |
| Male 1 | 48 | 75 | 22 | 18.8 | <LOD | <LOD | 0.9 | 1.1 | 197 | 2 | 0.01 | 0.8 | | 5 |
| Male 2 | 42 | 59 | 20 | 14.8 | <LOD | <LOD | 4.2 | 6.3 | 1014 | 2 | 0.04 | 6.4 | | 6 |

[a] The oral dose of GLY was 50% of the ADI and was calculated according to the body weight of each participant.

GLY formula administered to the volunteers was analyzed for AMPA residues, which were found to be below LOD.

The urinary elimination of GLY seemed to follow first-order kinetics and a two-phase excretion, with an initial rapid phase between 6 and 9 h followed by a slower phase between 18 and 33 h. Fig. 1 shows the elimination curves of GLY after the exposure, with unadjusted concentrations and concentrations adjusted for creatinine, density, and UER. The normal scale unadjusted excretion plot of GLY is available in the supplementary material (Fig. S2). The urine concentrations did not return to the pre-exposure values even after 80–100 h (Fig. S2). AMPA elimination seemed to follow a similar two-phase elimination curve but was more difficult to determine because of the low concentrations and great variability between the volunteers. The density-adjusted, creatinine-adjusted, and unadjusted kinetic plots for AMPA are shown in the supplementary material (Fig. S1A–C). The estimated excretion half-lives of GLY and AMPA are shown in Table 2.

### 3.2. Biomonitoring of GLY in young adults

In this population, GLY was found in detectable concentrations above the LOD of 0.1 μg/L in 20% of the urine samples, and AMPA was found in concentrations above the LOD of 0.1 μg/L in 29% of the samples. The median and mean concentrations of GLY were both below LOD. The maximum concentration (density adjusted) was 3.39 μg/L for

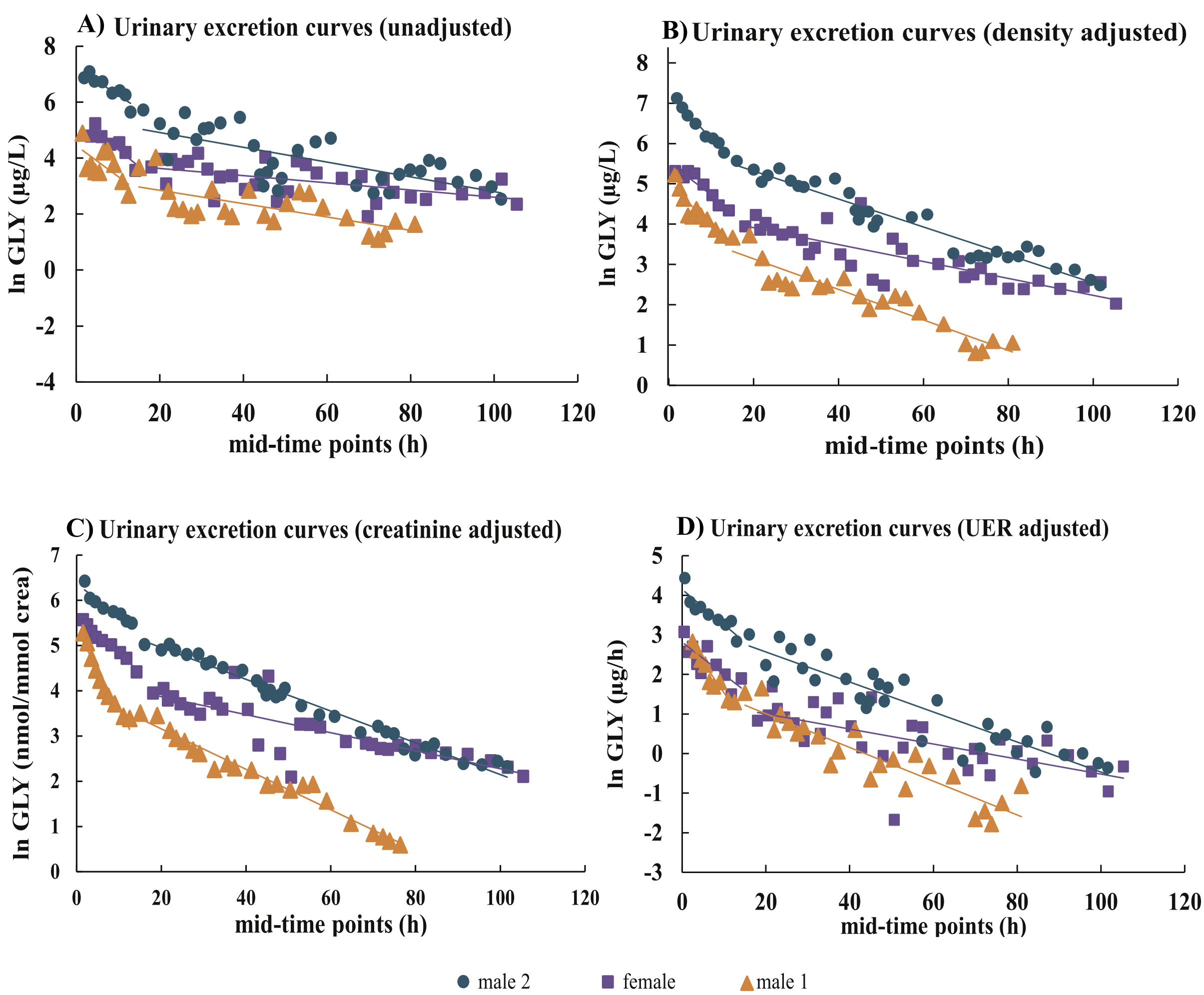


**Fig. 1.** Graphs of urinary excretion curves for GLY (parent compound) for the three volunteers after a single oral dose of GLY. The graphs show ln-transformed: A) unadjusted concentrations (μg/L), B) density-adjusted concentrations (μg/L), C) creatinine-adjusted concentrations (nmol/mmol creatinine), and D) UER-adjusted concentrations (μg/h) plotted against the mid-time points of sample collection.

**Table 2**
Estimated excretion half-lives of GLY in urine samples of study volunteers. Data on two-phase excretion half-lives (h) in urine are presented as unadjusted, density-adjusted, creatinine-adjusted, and UER-adjusted data with the correlation coefficients ($r$) for the short and long excretion phases, separately.

| Compound | Volunteer | Excretion phase | $t_{1/2}$, unadjusted (h) | $r$ | $t_{1/2}$, density adjusted (h) | $r$ | $t_{1/2}$, creatinine adjusted (h) | $r$ | $t_{1/2}$, UER adjusted (h) | $r$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **GLY** | **Female** | short | 5.7 | 0.82 | 8.6 | 0.89 | 9.4 | 0.98 | 8.5 | 0.65 |
| | | long | 54 | 0.33 | 33 | 0.70 | 35 | 0.67 | 37 | 0.45 |
| | **Male 1** | short | 6.3 | 0.42 | 6.0 | 0.83 | 3.9 | 0.94 | 4.5 | 0.92 |
| | | long | 29 | 0.45 | 18 | 0.88 | 16 | 0.97 | 16 | 0.82 |
| | **Male 2** | short | 7.0 | 0.83 | 6.2 | 0.98 | 10 | 0.98 | 7.8 | 0.82 |
| | | long | 26 | 0.51 | 20 | 0.57 | 20 | 0.57 | 18 | 0.83 |
| **AMPA** | **Female** | short | 4.6 | 0.88 | 4.4 | 0.99 | 4.8 | 0.94 | – | – |
| | | long | 173 | 0.02 | 56 | 0.26 | 62 | 0.22 | – | – |
| | **Male 1** | short | 61 | 0.02 | 14 | 0.77 | 10 | 0.63 | – | – |
| | | long[a] | – | – | – | – | – | – | – | – |
| | **Male 2** | short | 3.3 | 0.92 | 3.6 | 0.97 | 3.8 | 0.99 | – | – |
| | | long | 50 | 0.26 | 29 | 0.88 | 27 | 0.83 | – | – |

[a] **Concentrations** for the long excretion phase of Male 1 were <LOD.

GLY in urine and 0.99 μg/L for AMPA. Urinary concentrations are summarized in Table 3. The Spearman rank correlation indicated a significant, but only moderate, correlation ($\rho = 0.56$) between GLY and AMPA in the population.

## 4. Discussion

GLY is the most widely applied herbicide worldwide, and there is growing scientific and public interest in its effects on both human health and the environment. It is therefore noteworthy that there are not more available studies of GLY exposure, health effects, and fate in humans. The high polarity and amphoteric nature of GLY make it a difficult compound to analyze, and few publications address the issue of measurements in biological fluids.

This is one of few current studies reporting on oral exposure experiments with GLY in humans, using a known dose of 50% of ADI and observing the urinary excretion up to 100 h. The experiment was limited to three volunteers but may still be of value for the interpretation of GLY exposure when biomonitoring populations or intoxication cases. We found a low recovery of GLY in the urine of the three volunteers, in good agreement with a recent study by Zoller et al. (2020). They suggested a total recovery of 1% based on a study with 12 participants exposed through food, in which the GLY and AMPA doses were quantified in the food samples. Both results are in agreement that the elimination of GLY in urine is much lower in humans than in animals, as animal studies show that more than 20% of administered GLY concentrations were recovered in urine (Anadon et al., 2009; Brewster et al., 1991; Williams et al., 2000). Furthermore, the recovery of AMPA in our study was in line with the results of Zoller et al. (2020), who reported recovery of 0.09–0.28% of AMPA related to the administered dose of GLY. However, relative to the dose of AMPA detected in food samples, 10–33% of AMPA was recovered in urine by Zoller et al. (2020). Whether the lower urinary excretion rates of glyphosate in humans also indicate a lower absorption/resorption, and lower systemic availability, in humans warrants further investigation in future research.

In our observations, the urinary elimination appeared to be two-phased, similar to the observations reported in animal studies. One study reported an initial excretion phase at 6 h and a second phase between 79 and 106 h (Williams et al., 2000), another observed a half-life of 14 h in plasma after oral administration in rats (Anadon et al., 2009), and yet another reported a half-life of 8–11 h in the plasma of rats after oral administration by gavage (FAO/WHO 2017). Two recent studies in humans reported a half-life for GLY of 9 h after urine collection up to 48 h (Zoller et al., 2020) and an average half-life in the range of 5.5–10 h when calculations were based on different urinary adjustment metrics (Connolly et al., 2019). The results from the rapid excretion phase in our study are very similar to these findings. Further, Connolly et al. (2018a) reported that $C_{max}$ was reached in horticulturists between 1 and 3 h after the end of the work shift, and Zhang et al. (2020) reported that $C_{max}$ was reached in workers involved in GLY production within 1 h after the shift ended. This is in line with our results where $C_{max}$ was reached at 2 h after the oral dose, but the different routes of exposure should be considered in this comparison. The urine collection period and exposure dose might affect the observed excretion pattern, in which a two-phase excretion could have been missed due to a shorter follow-up or a lower exposure dose. However, only a small fraction of the recovered GLY concentration was excreted after 24 h. There seem to be some inter-individual differences in GLY excretion observable in the three volunteers, even though they were administered a GLY dose related to each person's weight. The concentrations of both GLY and AMPA were below LOD in the pre-exposure urine samples from the volunteers. Since our data is limited to three individuals, no conclusions can be drawn on why the recovery and maximum concentration were much higher in Male 2 compared to the other two participants. The excretion patterns of all volunteers, including Male 2, in Fig. 1 does not suggest any additional exposure during the experiment. The estimated half-life of AMPA appeared to be two-phased, but its concentrations were close to the LOD with great variability between the volunteers.

The urinary concentrations and detection frequency of GLY and AMPA in the population of Swedish young adults were low. Comparing the highest concentration in this population with the $C_{max}$ in the experimentally exposed volunteers shows that GLY concentrations in the population are far below the concentrations from an exposure equivalent to 50% of the ADI. Overall, the urinary concentrations are in line with previously reported results from other countries. Danish school children and mothers had detectable concentrations with means of 1.96 and 1.28 μg/L, respectively (Knudsen et al., 2017). In a time-trend study (2001–2015) of German adults, median concentrations ranged from below LOQ to 0.18 μg/L in 24-h urine samples in different years (Conrad et al., 2017). Another German study of an adult population found detectable concentrations above LOQ only in 8.3% of the population (Soukup et al., 2020). A study of adults from 18 European countries presented a mean GLY concentration of 0.21 μg/L (Hoppe et al., 2013). A recent study from Portugal of adults from the general population volunteering to participate reported an arithmetic mean concentration

**Table 3**
Urinary concentrations adjusted for density (μg/L) and creatinine (nmol/mmol creatinine), and detection frequency of GLY and AMPA in a Swedish population of young adults.

| Biomarker | LOD | %>LOD | $N$ | Median | Mean | 95th P | Max |
|---|---|---|---|---|---|---|---|
| **GLY (dens)** | 0.1 | 20 | 196 | <LOD | <LOD | 0.24 | 3.39 |
| **GLY (crea)** | | | 194[a] | <LOD | <LOD | <LOD | 1.10 |
| **AMPA (dens)** | 0.1 | 29 | 195 | <LOD | <LOD | 0.25 | 0.99 |
| **AMPA (crea)** | | | 193[a] | <LOD | <LOD | 0.16 | 0.53 |

[a] Two of the samples did not contain enough volume for analysis of creatinine.

of GLY at 0.05 μg/L and 0.1 μg/L in urine from two different test rounds, and 0.08 μg/L and 0.1 μg/L of AMPA (Nova et al., 2020). An occupationally exposed group of Irish horticulturists had a mean GLY concentration of 0.66 μg/L in post-work samples (Connolly et al., 2017). A recent study from China reported median urinary concentrations of GLY in the range of 67–3297 μg/L and AMPA 22–388 μg/L in a population working with glyphosate production, reported to be exposed through inhalation (Zhang et al., 2020). A review of population exposures to GLY observed arithmetic mean concentrations in urine of 0.16–7.6 μg/L (Gillezeau et al., 2019). Despite the differences in analytical methods for GLY analysis and sample collection procedures, the reported concentrations in Europe are in good agreement with the low concentrations and detection frequencies of GLY and/or AMPA in urine samples found in different populations. Furthermore, we observed a moderate correlation between AMPA and GLY concentrations in the samples from the general population of Swedish young adults. GLY and AMPA seemed to have similar elimination half-lives and kinetics based on observations from our oral exposure assessment with a slightly different $T_{max}$ and recovery. If GLY is metabolized to AMPA in humans, they should be detected and correlate to some extent in spot urine samples after exposure to GLY. However, possible inter-individual differences in metabolism and/or elimination should still be considered. Other studies have also found a moderate or weak correlation between GLY and AMPA concentrations (Conrad et al., 2017; EFSA, 2015; Nova et al., 2020; Soukup et al., 2020). It is plausible that GLY is metabolized to AMPA by soil microbiota before entering the body via diet, resulting in different exposure sources. Still, a small fraction of the AMPA measured in urine is attributable to the metabolism of GLY based on our observations in the oral exposure experiment. An animal study suggests that the metabolism of GLY to AMPA mainly is the result of gut microflora activity (Brewster et al., 1991).

Overall, the available results suggest that the absorption and elimination patterns of GLY after exposure differ between human and animal studies, with sufficient human data being lacking. A few animal studies have assessed distribution and uptake and compared different routes of exposure. A recent study from China assessed occupational exposure to GLY in indoor air, where the highest measured median concentration range was 3–12 mg/m$^3$ (Zhang et al., 2020). The studied workers had median concentrations of GLY in urine ranging from 292 to >3000 μg/L. In the present study, we administered an oral dose of 14–19 mg, resulting in urinary $C_{max}$ values of 200–1000 μg/L. If we assume a working day of 8 h, that the inhalation volume is 1 m$^3$/h and a similar uptake of 1%, exposure routes can be crudely compared. Based on these assumptions, the estimated exposure dose from indoor air would be 24–100 mg of GLY when the reported urinary $C_{max}$ was 2000–8000 μg/L. This comparison indicates that GLY uptake is similar for oral uptake and inhalation, with possibly a slightly greater uptake from inhalation. There is a great need for studies of GLY that assess different exposure routes in humans to explore this matter further.

It should be considered that GLY may bind to metal cations due to its chelating property (Mertens et al., 2018). We used Milli-Q water as the medium for oral administration of the GLY dose in the exposure assessment to avoid possible complex formation or GLY interactions with ions. Variations between our results and those of Zoller et al. (2020) could be due to the different administration of the oral dose of GLY in Milli-Q water or through food products containing GLY residues.

Furthermore, only a few studies present measurements of GLY in human blood samples from acute intoxication cases. One study concluded that blood concentrations correlated well with ingested doses and were more useful for clinical evaluations than were urinary concentrations (Zouaoui et al., 2013). Further exposure assessments including measurements of GLY and AMPA in blood samples would advance our understanding of the absorption, distribution, and elimination of GLY in humans. More data are needed to investigate whether different exposure routes and differences in diet might affect the uptake, bioavailability, and/or elimination of GLY in humans.

### 4.1. Adjustment for urinary dilution

All urinary GLY concentrations adjusted for density or creatinine, or presented in terms of UER, gave better correlations (*r*) in the half-life plots than did the non-adjusted concentrations. Still, both creatinine and density are affected by the body composition of the individual and by diet, age, and sex, among other factors. However, density values are suggested to be less affected by some factors than are creatinine concentrations (Suwazono et al., 2005). In biomonitoring studies, in which the population composition is usually complex due to a wide age range, a particular sex ratio, and varied health status, density may be an appropriate choice for dilution correction, especially when comparing different groups such as men and women. However, density might also be affected by several other factors, including common diseases such as diabetes or kidney disorders (Chadha et al., 2001), and the measurement itself is difficult to automate. Dilution correction with UER is only an option when timed full-void urine samples are available. Overall, applying any of the methods for adjusting the urinary dilution should be preferred to using unadjusted urine concentrations.

## 5. Conclusions

In the human oral experimental exposure, the urinary excretion of GLY seemed to follow a two-phase excretion. The elimination half-lives (density adjusted) of GLY in urine were 6–8 h for the initial short excretion phase and 18–33 h for the longer second excretion phase. The recovery of the oral dose of GLY was 1–6% in the three volunteers; AMPA was found in trace concentrations of around 0.01–0.04% of the administered dose of parent GLY. In the Swedish population of young adults, the median and maximum GLY concentrations in urine were 0.03 and 3.39 μg/L, respectively; AMPA was generally found in lower concentrations. A moderate correlation between GLY and AMPA concentrations ($\rho = 0.56$) together with concentrations of AMPA in the same range as GLY in the population, as opposed to our experimental exposure, may suggest that the detected GLY and AMPA reflect separate exposures from different sources. The concentrations of GLY found in the Swedish population were consistent with the results of other European studies. Our observations indicate that the urinary concentrations in the studied population are far below the maximum concentrations found in our human exposure study, after intake of a dose equivalent to half of ADI (0.5 mg/kg body weight per day).

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2020.113657.

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# Lead exposure and its association with cardiovascular disease and diabetic kidney disease in middle-aged and elderly diabetic patients

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Diabetic kidney disease

A B S T R A C T

*Aims:* Previous studies have suggested that a high blood lead level (BLL) is associated with cardiovascular outcomes and impaired renal function in the general population; however, studies investigating the effect of a high BLL on diabetic vascular complications have been limited. We aimed to investigate whether a higher BLL is associated with cardiovascular disease (CVD) and diabetic kidney disease (DKD).

*Methods:* We analyzed 4234 individuals out of 4813 diabetic participants enrolled from seven communities in China in 2018 in this cross-sectional study. Macrovascular measurements, including assessment of common carotid artery (CCA) plaques and their diameters, were performed with ultrasound. CVD was defined as a composite measure including a previous diagnosis of coronary heart disease, myocardial infarction, or stroke. The definition of DKD was an albumin to creatinine ratio (ACR) ≥30 mg/g or an estimated glomerular filtration rate (eGFR) < 60 ml/min per 1.73 $m^2$. Linear and logistic regression analyses were used to measure the associations.

*Results:* The median age and BLL of the participants were 67 years (interquartile range, 62–72 years) and 26 μg/L (interquartile range, 18–36 μg/L). Compared with the first quartile, the odds ratio (OR) (95% CI) of CCA plaques ranging from none to bilateral in the ordinal logistic regression analysis associated with BLL was 1.53 (1.29, 1.82) in the fourth BLL quartile (*P for trend* <0.01), and the odds of having CVD was significantly increased by 44% for participants in the fourth quartile (1.44 (1.17, 1.76)) (*P for trend* <0.01). The odds of DKD in the fourth BLL quartile increased by 36% (1.36 (1.06, 1.74)) compared with that in the first quartile (*P for trend* <0.05). These associations were adjusted for potential confounders.

*Conclusions:* A high BLL may be a potential risk factor for CVD and DKD in middle-aged and elderly diabetic adults.

## 1. Introduction

Type 2 diabetes mellitus and its complications have become highly prevalent. Among adults in 2017, there was an estimated 425 million cases of type 2 diabetes worldwide, affecting approximately 1 in 11 adults (Cho et al., 2018). It was reported that 27% with macrovascular complications (cardiovascular disease (CVD)) and half of diabetic adults present with diabetic microvascular complications (diabetic kidney disease (DKD), diabetic retinopathy and neuropathy) (Zheng et al., 2018). In China, CVD was the cause of 40% of deaths (Zhao et al., 2019) and the all-age mortality rate due to DKD increased by one-third from 4.5 in 1990 to 6.0 per 100,000 in 2016, (Liu et al., 2018). CVD and DKD,

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multifactorial conditions with both major environmental and genetic components, are the leading cause of mortality in diabetic individuals, carrying enormous financial burden (Cole and Florez, 2020; Moss, 2018; Zhou et al., 2019). Therefore, prevention and treatment of diabetic complications are the key goals in lifetime diabetic treatment.

Lead is one of the world's highest volume heavy metals in use today and remains ubiquitous in polluted soil, air, water, food, batteries, toys, house paint, and industrial sources, as well as in firsthand and secondhand smoke (Apostolou et al., 2012; Wang et al., 2015). In addition to being a neurotoxin (Lanphear et al., 2005), lead is also considered an endocrine disruptor and has been found to be significantly associated with some cardiometabolic risk factors (Prokopowicz et al., 2017; Wang et al., 2015) and endogenous sex hormones (Chen et al., 2016).

Previous studies have found that the blood lead level (BLL) is associated with cardiovascular outcomes of atherosclerotic origin (Chen et al., 2017; Solenkova et al., 2014) and age-related decline in renal function (Kim et al., 1996) in the general population, suggesting that lead exposure may accelerate vascular complications in diabetic patients. Interestingly, in diabetic individuals, recent trials have found that chelation treatment reduces the risk of CVD-related endpoints by 41% (HR 0.59, 95% CI: 0.44, 0.79) over 5 years (Lamas et al., 2013) and retards the progression of diabetic kidney disease (DKD) with high-normal body lead burdens (Chen et al., 2012). However, epidemiological studies have been very limited, and whether the BLL is associated with diabetic vascular complications still needs to be investigated. We believe that this field of work may enable us to identify a major new modifiable risk factor for primary and secondary prevention of diabetic vascular complications.

Thus, in this study of a large community-based sample, we aimed to investigate whether a higher BLL is associated with CVD and DKD in Chinese diabetic adults.

## 2. Methods

### 2.1. Study design and participants

We designed a cross-sectional study named the METAL study (Environmental Pollutant Exposure and Metabolic Diseases in Shanghai, www.chictr.org.cn, ChiCTR1800017573) to investigate the association between exposure to heavy metals and diabetic complications in Chinese diabetic adults. We enrolled study participants from seven communities in Huangpu and Pudong District, Shanghai, China. The list of diabetic patients who were Chinese citizens ≥18 years old and had lived in their current area for ≥6 months from the registration platform in each community healthcare center was obtained and then we randomly selected 50% of them (n = 5888) to receive the examination using a random number obtained by SPSS Statistics, Version 22 (IBM Corporation, Armonk, NY, USA). In fact, 4813 subjects with diabetes who were 23–99 years of age received examinations from May to August 2018. We excluded participants who were missing BLL data (n = 579). A total of 4234 diabetic participants were included in the baseline analysis. Then, we excluded participants who were missing vascular measurement information values (n = 98), and 4136 participants were included in the analysis of the association between BLL and CVD. In addition, we excluded participants who were missing albumin to creatinine ratio (ACR) data (n = 200) and participants who had chronic nephritis or ≥ 2 WBCs/high-power field in a urine sample (n = 561). A total of 3473 participants were included in the analysis of the association between BLL and DKD (Fig. 1).

The study protocol was approved by the Ethics Committee of Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in the a priori approval by the appropriate institutional review committee. Informed consent was obtained from all participants included in the study.

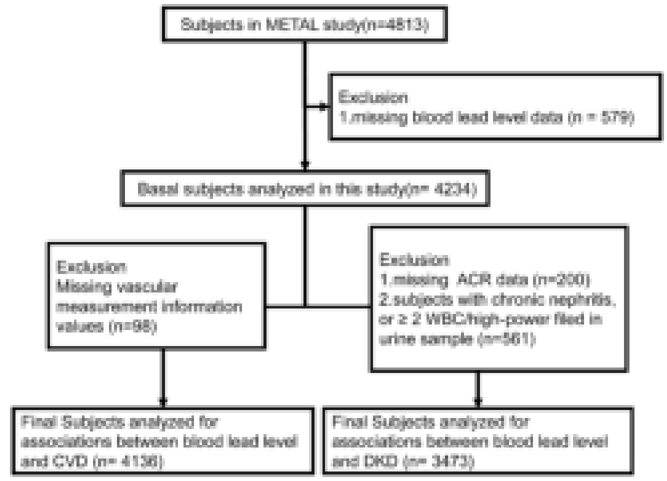


**Fig. 1.** Flowchart of the sampling framework and participants selected from the METAL study.

### 2.2. Measurements

A questionnaire about sociodemographic characteristics, medical history, family history, and lifestyle factors was conducted by interview. The same group of trained and experienced personnel who participated in the SPECT-China study (Wang et al., 2016, 2017) conducted the interviews and clinical examinations, including collecting information about weight, height and blood pressure, according to standard protocols. The detailed protocols had been described in previously published papers (Wan et al., 2019, 2020a). BMI was calculated as weight in kilograms divided by height in meters squared. The estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation for "Asian origin" (Stevens et al., 2011). Current smoking was defined as having smoked at least 100 cigarettes in one's lifetime and currently smoking cigarettes (Xu et al., 2013).

Blood samples were obtained between 6:00 a.m. and 9:00 a.m. after fasting for at least 8 h. Blood was refrigerated immediately after phlebotomy and centrifuged within 2 h, and serum was aliquoted and stored frozen in a central laboratory. The BLL was determined by atomic absorption spectrometry (BH2200, China) (Wang et al., 2015). Glycated hemoglobin (HbA1c) was measured by high-performance liquid chromatography (MQ-2000PT, Medconn, Shanghai, China). Fasting plasma glucose, serum creatinine, triglycerides, total cholesterol, high-density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C) were measured with a Beckman Coulter AU 680 (Brea, USA). Using morning fasting spot urine samples, the concentrations of albumin and creatinine were measured with a Beckman Coulter AU 680 (Brea, USA) using a turbidimetric immunoassay and an enzymatic method, respectively.

### 2.3. Outcome definition

Hypertension was defined as systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg, or self-reported previous diagnosis of hypertension by a physician. Dyslipidemia was defined as total cholesterol ≥6.22 mmol/L (240 mg/dL), triglycerides ≥2.26 mmol/L (200 mg/dL), LDL-C≥ 4.14 mmol/L (160 mg/dL), HDL-C< 1.04 mmol/L (40 mg/dL), or self-reported previous diagnosis of hyperlipidemia by a physician, according to the modified National Cholesterol Education Program-Adult Treatment Panel III.

Carotid atherosclerosis in the common, internal, and bifurcation sites of the bilateral common carotid arteries (CCAs) was assessed by the same trained sonographers who were blinded to any clinical conditions of the participants using a Mindray M7 ultrasound system (MINDRAY, Shenzhen, China) with a 10-MHz probe (Wan et al., 2020b, 2020d). The sonographers were trained by performing carotid ultrasound on same patients before the study began to achieve an interobserver coefficient of variation of less than 10%. As discussed in our previously published paper (Wan et al., 2020c, 2020d), the measurement method was based on the consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force (Stein et al., 2008). A present CCA plaque was identified as focal thickening (≥1.5 mm) of the artery wall. The CCA diameter was measured between the leading edge of the adventitia-media echo of the near wall and the leading edge of the media-adventitia echo of the far wall based on average of end-diastolic diameter measurements of 5–10 mm proximal to the carotid bulb.

CVD was defined as a self-reported diagnosis by a physician, including coronary heart disease, myocardial infarction or stroke. The question in the questionnaire was as follows: “Have you ever been told by a doctor or other healthcare professional that you have coronary heart disease or have suffered from a myocardial infarction or stroke?” The same question was adopted by another large study in China, and the validation rate reached 91% (Lu et al., 2014).

High ACR was defined as an ACR ≥30 mg/g. The DKD definition was suggested by the American Diabetes Association (American Diabetes, 2018) statement: ACR ≥30 mg/g or eGFR <60 ml/min per 1.73 $m^2$.

### 2.4. Statistical analysis

Data analyses were evaluated using IBM SPSS Statistics, Version 22 (IBM Corporation, Armonk, NY, USA). A $P$ value $< 0.05$ indicated significance (two sided). Continuous variables were expressed as the mean ± SD if the data were normally distributed or the median (interquartile range) if the data were not normally distributed, and categorical variables were expressed as percentages (%).

Linear or logistic regression analysis was used to test for a trend in the changes of the variables across the BLL quartiles, providing unadjusted $P$ values. The concentrations of the BLL and urinary ACR were logarithmically transformed to achieve a normal distribution.

BLLs were divided into quartiles, with the first quartile representing the lowest quartile and the fourth quartile the highest quartile. Logistic regression was used to measure the association of the BLL with the prevalence of CVD, DKD and high ACR. Linear regression was used to measure the associations of the BLL with the CCA diameter, Ln ACR and eGFR. The ordinal logistic regression was used to measure the association between the BLL and the prevalence of CCA plaques (none, unilateral or bilateral). The regression coefficients (β) and odds ratios (ORs) (95% confidence interval (CIs)) were calculated. The associations among the BLL, ACR and prevalence of high ACR were also tested in individuals without decreased eGFRs (eGFR ≥90 ml/min per 1.73 $m^2$). The associations were all adjusted for age, sex, duration of diabetes, education status, current smoking, BMI, HbA1c, dyslipidemia and hypertension.

We calculated the minimum detectable ORs for the study. Assuming 36% prevalence of CVD and 27% prevalence of DKD, the study provides 90% power to detect an OR of 1.34 or greater for CVD and 90% power to detect an OR of 1.40 or higher for DKD, when comparing the highest BLL quartile vs the lowest quartile (assuming n = 1034 per quartile for CVD and n = 868 per quartile for DKD).

## 3. Results

### 3.1. Characteristics of the diabetic participants by BLL quartiles

The general demographic and characteristics of the study population are shown in Table 1. This study recruited 4234 diabetic participants. Approximately 46.1% of the participants were men. The median age and BLL of the participants were 67 years (interquartile range, 62–72 years) and 26 μg/L (interquartile range, 18–36 μg/L). The prevalence of CVD, bilateral CCA plaques, and DKD in the participants were 36.5%, 30.1% and 27.5% respectively. Those in the highest quartile were older, were more likely to be men, had a significantly longer duration of diabetes, had higher urine ACR and triglycerides, had lower eGFR and HDL, and had higher prevalence of current smoking, unilateral and bilateral CCA plaques, CVD, high ACR, DKD and hypertension than the participants in the lowest BLL quartile (all with an unadjusted $P$ for trend <0.05)

**Table 1**
Characteristics of study participants according to the blood lead level quartiles.

| Characteristic | Blood lead level, μg/L | | | | Unadjusted $P$ for trend |
|---|---|---|---|---|---|
| | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | |
| | ≤18 | 19–26 | 27–36 | ≥37 | |
| *N* | 1137 | 1106 | 975 | 1016 | – |
| Age, yr | 66.3 ± 8.6 | 66.1 ± 8.6 | 66.3 ± 8.9 | 68.7 ± 8.5 | <0.001 |
| Men, % | 44.6 | 43.7 | 46.5 | 50.2 | 0.005 |
| Duration of diabetes, yr | 9.6 ± 7.7 | 9.4 ± 7.7 | 9.8 ± 7.9 | 11.0 ± 8.5 | <0.001 |
| Beyond high school education, % | 57.1 | 53.1 | 51.9 | 46.1 | <0.001 |
| Current smoking, % | 12.9 | 16.6 | 21.1 | 22.4 | <0.001 |
| BMI, kg/$m^2$ | 24.8 ± 3.6 | 25.0 ± 3.6 | 25.0 ± 3.8 | 25.1 ± 3.4 | 0.052 |
| FPG, mmol/L | 7.8 ± 2.4 | 7.7 ± 2.5 | 7.8 ± 2.6 | 7.8 ± 2.3 | 0.653 |
| HbA1c, % | 7.5 ± 1.5 | 7.4 ± 1.4 | 7.5 ± 1.4 | 7.5 ± 1.2 | 0.862 |
| Vascular measurement | | | | | |
| Unilateral/bilateral CCA plaque, % | 25.8/27.4 | 23.5/28.3 | 26.9/28.7 | 26.3/36.4 | <0.001 |
| Right CCA diameter, mm | 7.71 ± 0.92 | 7.68 ± 0.92 | 7.69 ± 0.91 | 7.85 ± 0.98 | 0.001 |
| Left CCA diameter, mm | 7.51 ± 0.91 | 7.53 ± 0.86 | 7.54 ± 0.90 | 7.68 ± 0.90 | <0.001 |
| CVD, % | 32.2 | 32.1 | 36.5 | 44.1 | <0.001 |
| Renal function | | | | | |
| Urine ACR, mg/g | 12(7–24) | 12(7–26) | 14(7–32) | 15(8–36) | <0.001 |
| High ACR, % | 21.1 | 23.0 | 27.1 | 29.4 | <0.001 |
| eGFR, ml/min per 1.73 $m^2$ | 94.6 ± 14.8 | 93.8 ± 15.9 | 91.3 ± 18.1 | 88.1 ± 18.8 | <0.001 |
| DKD, % | 22.6 | 25.3 | 29.7 | 33.3 | <0.001 |
| Lipids | | | | | |
| Total cholesterol, mmol/L | 5.09 ± 1.16 | 5.12 ± 1.18 | 5.15 ± 1.26 | 5.06 ± 1.22 | 0.815 |
| Triglycerides, mmol/L | 1.47 (1.05–2.12) | 1.57 (1.10–2.23) | 1.58 (1.15–2.30) | 1.56 (1.12–2.25) | 0.001 |
| HDL, mmol/L | 1.23 ± 0.29 | 1.20 ± 0.29 | 1.20 ± 0.30 | 1.19 ± 0.28 | 0.001 |
| LDL, mmol/L | 3.13 ± 0.83 | 3.16 ± 0.84 | 3.18 ± 0.88 | 3.12 ± 0.86 | 0.953 |
| Other prevalent diseases | | | | | |
| Hypertension, % | 75.9 | 75.8 | 78.5 | 83.0 | <0.001 |
| Dyslipidemia, % | 59.2 | 61.6 | 66.1 | 61.5 | 0.078 |

(Table 1).

### 3.2. Association of BLL with vascular measurements and CVD in diabetic participants

Fig. 2 shows that an increased BLL was associated with higher odds of having CCA plaque and CVD after adjusting for possible confounders. The OR (95% CI) of CCA plaques ranging from none to bilateral in the ordinal logistic regression associated with the BLL was 1.53 (1.29, 1.82) for the fourth quartile compared with first quartile ($P$ for trend $< 0.001$). Moreover, the odds of having CVD was significantly increased by 44% for participants with a BLL in the fourth quartile ($\geq$37 μg/L) (1.44 (1.17, 1.76)) ($P$ for trend $< 0.001$). A 1SD increment of Ln BLL was also significantly related to the odds of having CVD (1.25 (1.16, 1.34)). Neither the right nor left CCA diameter was associated with the BLL quartiles or Ln BLL ($P$ for trend $>$0.05 for both). These associations were all adjusted for age, sex, duration of diabetes, education status, current smoking, HbA1c, BMI, dyslipidemia and hypertension.

### 3.3. Association of BLL with DKD in diabetic participants

Fig. 3 indicates that an increased BLL was associated with higher odds of having DKD after adjusting for possible confounders. In all the diabetic participants, compared with those with BLLs in the first quartile, participants having BLLs in the highest quartile ($\geq$37 μg/L) had a 36% and 31% increase in odds of having DKD (1.36, (1.06, 1.74)) and high ACR (1.31, (1.02, 1.69)) ($P$ for trend $< 0.05$ for both), respectively. A 1SD increment of a higher Ln BLL was also significantly related to a higher Ln ACR and a lower eGFR ($P < 0.05$ for both); the regression coefficients (β) (95% CI) were 0.09 (0.05, 0.13) and −0.96 (−1.45, −0.48), respectively. These models were all adjusted for age, sex, duration of diabetes, education status, current smoking, BMI, HbA1c, dyslipidemia and hypertension.

In addition, we analyzed the association between the BLL and urine ACR in diabetic patients with a normal eGFR (eGFR $\geq$90 ml/min per 1.73 $m^2$). Ln ACR and odds of having high ACR were still positively associated with the BLL quartiles, indicating that the BLL may have negative effects on nephrotic changes in the very early stages of diabetes (Supplementary Figure 1).

## 4. Discussion

In this study involving more than 4000 community-dwelling Chinese diabetic adults, we reported positive and significant associations between the BLL and the prevalence of CCA plaques, CVD and DKD. These associations were strong, dose dependent, and consistent even after adjusting for age, sex, duration of diabetes, current smoking, HbA1c, body mass index, dyslipidemia, and hypertension. Our data indicated that lead exposure is probably a modifiable risk factor in both macrovascular disease and DKD in diabetic patients.

China is a large lead-consuming country. Although leaded gasoline in China, a previous primary source of lead exposure, was banned in 2000, the lead acid battery industry in China, which uses over 67% of China's total lead production, has become another considerable source of lead exposure (van der Kuijp et al., 2013). In China, the BLL has gradually decreased in the past several decades, but approximately half of the population aged 0–18 years has a BLL higher than 45 μg/L (Li et al., 2014); furthermore, there is a lead burden in the middle-aged and elderly populations because of the extremely long half-life of lead in the body. In present study, the median BLL was 26 μg/L among the diabetic population, however, it is the fact that there is no biological function of lead in human (Ahamed and Siddiqui, 2007). More importantly, one epidemiological study reported that BLL below 10 μg/L was still associated with increased all-cause and cardiovascular mortality (Menke et al., 2006), although previous studies had demonstrated that BLL above 10 μg/L in adults was associated with increased risk of cardiovascular, cancer, and all-cause mortality (Chen et al., 2017; Lustberg and Silbergeld, 2002; Schober et al., 2006), which suggested that there may be no safe threshold BLL. Thus, the Chinese government still needs to tackle the considerable lead exposure risk it is facing.

Our study found that in diabetes, a higher BLL was associated with a higher prevalence of macrovascular complications, including CVD and bilateral CCA plaques, which are known to be strongly predictive of cardiovascular outcomes (Nambi et al., 2010). The positive association between lead exposure and CVD in the general population was recognized years ago. One previous study reported that increased BLL (median was 37.70 μg/L, which was higher than that in present study) was independently associated with prevalent CVD in women (Chen et al., 2017). More importantly, a trial named TACT (Trial to Assess Chelation Therapy) led to a great advance in this area (Lamas et al., 2013). In the diabetic subgroup, the effect of chelation therapy was even better than

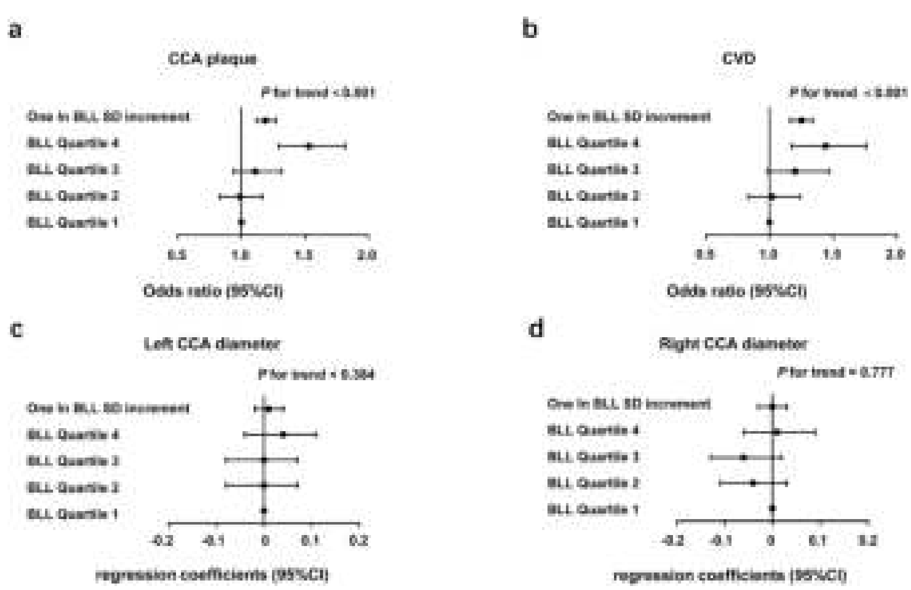


**Fig. 2.** Adjusted associations of the blood lead level with macrovascular measurement changes in diabetic patients. a) BLL and CCA plaque b) BLL and CVD c) BLL and left CCA diameter d) BLL and right CCA diameter.

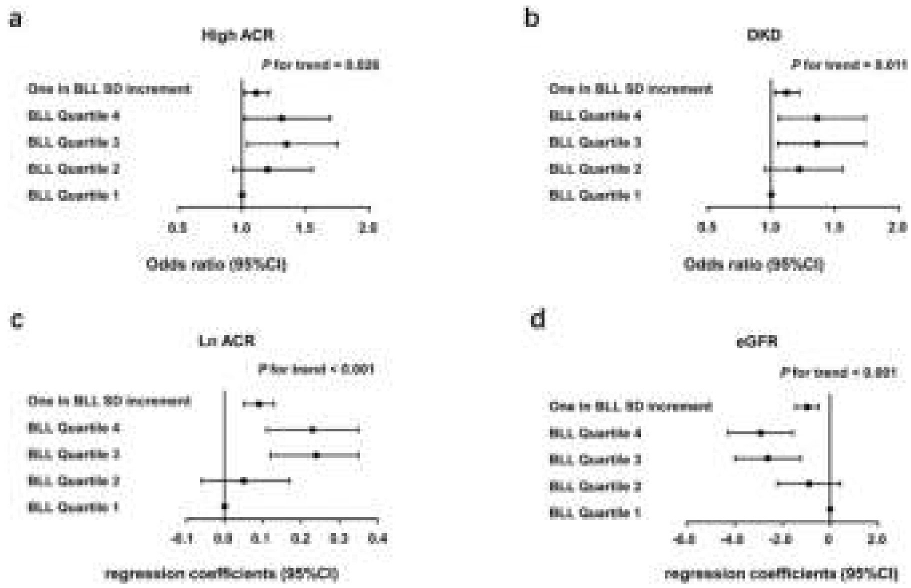


**Fig. 3.** Adjusted associations of the blood lead level with diabetic kidney disease a) BLL and high ACR b) BLL and DKD c) BLL and Ln ACR d) BLL and eGFR.

in the overall subjects. Edetate disodium treatment reduced the risk of the primary endpoints (all-cause mortality, myocardial infarction, stroke, coronary revascularization, or hospitalization for angina) and secondary endpoints (cardiovascular mortality, stroke, or recurrent myocardial infarction) both by approximately 40% in 5 years (Lamas et al., 2013). However, TACT did not provide information about whether the reduction in the BLL is causally associated with the reduced CVD risk. Thus, TACT2 was performed by recruiting solely diabetic patients to provide this causal association, replicate the TACT results, and explore the underlying mechanism (Lamas et al., 2016). These findings are consistent with that from the National Health and Nutrition Examination Surveys, indicating that despite the marked decrease in BLLs over the past several decades, lead exposure remains a significant determinant of cardiovascular mortality in the general population (Menke et al., 2006).

Consistent with CVD, we also found that DKD was associated with a greater BLL. Previous longitudinal studies have suggested that the BLL and bone lead are related to the renal function decline in the general population (Kim et al., 1996) and diabetic patients (Tsaih et al., 2004). A previous cross-sectional study conducted among 736 adolescents (median BLL = 15.1 μg/L) demonstrated a positive association between BLL and urinary albumin (Chaumont et al., 2012). One recent study which enrolled 231 diabetic patients (median BLL = 14.49 μg/L) also suggested that lead exposure might contribute to the development of DKD (Hagedoorn et al., 2020). Another series of enlightening studies conducted in Taiwan found that a high-normal body lead burden (>80 μg and <600 μg), detected by an EDTA mobilization test of 72-h urine samples, accelerated progressive DKD in two years, and more interestingly, they found that chelation therapy could retard the progression of DKD (Chen et al., 2012). However, these studies recruited participants with a relatively small sample size. It is worth mentioning that our results showed this positive association sustained in diabetic patients with a normal eGFR (eGFR ≥90 ml/min per 1.73 $m^2$), indicating that the effects of the BLL on nephrotic changes may start in a very early stage in diabetes. Thus, the prevention role of chelation therapy in DKD should be further investigated in randomized controlled trials.

The mechanism underlying the effect of lead exposure on the development of macro- and microvascular complications remains unclarified. Based on our findings, we suspect that there are common underlying mechanisms in vascular complications that could be affected by lead exposure. Lead is known to promote oxidative stress and inflammation through activation of the MAPK signaling pathway (Simoes et al., 2015), which plays a pivotal role in the development of diabetic microvascular and cardiovascular complications (Giacco and Brownlee, 2010; Wu et al., 2018). This increased superoxide production causes increased formation of advanced glycation end products (AGEs), expression of the AGE receptor, polyol pathway flux, and overactivity of the hexosamine pathway (Giacco and Brownlee, 2010). Lead may also combine with glycation end products to form AGEs and further promote the formation of reactive oxygen species in an autocatalytic reaction (Lamas et al., 2016). The resultant oxidized AGEs accumulate in different vessels, where they further promote oxidative stress (Lamas et al., 2016), leading to atherosclerosis in large vessels, neurodegeneration and retinal microvascular dysfunction in renal fibrosis and activation of the renin-angiotensin system in DKD (Kumar Pasupulati et al., 2016). Thus, lead may deteriorate the vicious cycle of oxidative stress, AGE formation, and accumulation in vessels to increased oxidative stress.

Although the present study has some strengths, including its strong quality control, relatively large sample of community-dwelling participants and novelty, there are also some limitations. First, the nature of this study is cross-sectional; thus, causal relationships cannot be confirmed in the pathophysiological mechanism to link the BLL with diabetic vascular complications. Second, Han Chinese was the ethnic group investigated; thus, the results may not be generalizable to other ethnicities. Third, we measured the BLL, which reflected only recent exposure. The half-life of the BLL (35 days) is much shorter than that of bone lead (years to decades), but bone lead leaks out over time and the BLL and bone lead content form a dynamic homeostasis (Lamas et al., 2016). Future studies of bone lead are still required to confirm our findings.

## 5. Conclusions

We reported that the BLL was positively associated with CCA plaques, CVD and DKD in Chinese middle-aged and elderly individuals with diabetes. Lead exposure is probably a major new modifiable risk factor for diabetic vascular complications, which suggests that reducing lead exposure may be critical for primary or even secondary interventions. The association between the BLL and macro- and microvascular complications observed in this population should be assessed in additional follow-up studies or further double-blind randomized controlled trials with large sample sizes using chelation intervention may be designed and performed.

## Disclosure summary

The authors have nothing to disclose.

Heng Wan, Shihan Chen and Yan Cai contributed equally to this work.

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## Abbreviations

ACR, albumin to creatinine ratio; AGE, advanced glycation end product; BLL, blood lead level; CCA, common carotid artery; CVD, cardiovascular disease; DKD, diabetic kidney disease; eGFR, estimated glomerular infiltration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR: odds ratio; CIs: confidence intervals; SD: standard deviation; regression coefficients (β).

## Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the appropriate institutional review committee. Informed consent was obtained from all participants included in the study.; Consent for publication; Not applicable.

## Availability of data and materials

The data supporting the findings of this study are available upon reasonable request from the corresponding authors.

## Author contributions

Y.L., Y.G. and N.W. designed the study; H.W., S.C., Y.C., Y.C., Y.W., W.Z., and C.C. conducted the research; H.W., S.C., and Y.C. analyzed the data and wrote the manuscript. The final manuscript was read and approved by all the authors.

## Declaration of competing interest

The authors declare that they have no competing interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2020.113663.

The data are summarized as the mean ± SD (if the data were normally distributed) or median (interquartile range) (if the data were not normally distributed) for continuous variables or as a numerical proportion for categorical variables. The unadjusted *P* for trend was calculated by regression tests.

ACR, albumin to creatinine ratio; BLL, blood lead level; BMI, body mass index; FPG, fasting plasma glucose; eGFR, estimated glomerular infiltration rate; HDL, high-density lipoprotein; HbA1c, glycated hemoglobin; LDL, low-density lipoprotein.

Linear (CCA diameter), logistic (CVD) and ordinal logistic (CCA plaque, including none, unilateral or bilateral plaques) regression analyses were used. *P* for trend was calculated to test the association between the trend across the four quartiles of BLL and each outcome by regression analysis.

98 subjects without vascular measurement information were deleted.

Finally, 4136 subjects were enrolled in the analysis. The number of subjects in each BLL quartile (from quartile 1 to quartile 4) was 1100, 1086, 957 and 993.

The model was adjusted for age, sex, duration of diabetes, education status, current smoking, HbA1c, BMI, dyslipidemia, and hypertension. BLL, blood lead level; CCA, common carotid artery; CVD, cardiovascular and cerebrovascular disease; BMI, body mass index; HbA1c, glycated hemoglobin.

Linear regression analysis was used to assess the association between the BLL and Ln ACR and eGFR; logistic regression analysis was used to assess the association between the BLL and the prevalence of DKD and high ACR. *P* for trend was calculated to test the association between the trend across the four quartiles of BLL and each outcome by regression analysis.

Two hundred subjects missing ACR values and 561 with kidney cancer, chronic nephritis or >2 WBCs/high-power filed in a urine sample were excluded.

Finally, 3473 subjects were enrolled in the analysis. The number of subjects in each BLL quartile (from quartile 1 to quartile 4) was 945, 893, 778 and 857. The model was adjusted for age, sex, duration of diabetes, education status, current smoking, BMI, HbA1c, dyslipidemia and hypertension.

BLL, blood lead level; ACR, microalbumin to creatinine ratio; eGFR, estimated glomerular infiltration rate; DKD, diabetic kidney disease; BMI, body mass index; HbA1c, glycated hemoglobin.

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# Long-term time trend of lead exposure in young German adults – Evaluation of more than 35 Years of data of the German Environmental Specimen Bank

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A B S T R A C T

Lead is a ubiquitous pollutant with well-known effects on human health. As there is no lower toxicological threshold for lead in blood and since data gaps on lead exposure still exist in many European countries, HBM data on lead is of high importance. To address this, the European Human Biomonitoring Initiative HBM4EU classified lead as a priority substance. The German Environmental Specimen Bank (German ESB) has monitored lead exposure since more than 35 years. Using data from the early 1980s to 2019 we reveal and discuss long-term trends in blood lead levels (BLLs) and current internal exposure of young adults in Germany. BLLs in young adults decreased substantially in the investigated period. As results from the ESB sampling site Muenster demonstrate, the geometric mean of BLLs of young adults decreased from 1981 (78,7 μg/L) to 2019 (10.4 μg/L) by about 87%. Trends in human exposure closely correlate with air lead levels (ALLs) provided by the European Monitoring and Evaluation Programme (EMEP). Hence, the decrease of BLLs largely reflects the drop in air lead pollution. Known associations of sex, smoking, alcohol consumption, and housing situation with BLLs are confirmed with data of the German ESB. Although internal lead exposure in Germany decreased substantially, the situation might be different in other European countries. Since 2010, BLLs of young adults in Germany levelled out at approximately 10 μg/L. The toxicity of lead even at low levels is known to cause adverse health effects especially in children following exposure of the child or the mother during pregnancy. To identify current exposure sources and to minimize future lead exposure, continuous monitoring of lead intake and exposure levels is needed.

## 1. Introduction

Lead is omnipresent in our environment and largely emitted from anthropogenic sources. The extensive use and emission of lead and lead-containing compounds resulted in considerable exposure of the environment and the human population in the past (Demirbas et al., 2015; Hernberg, 2000; Nadim et al., 2001; Wu and Boyle, 1997). Currently, the production of lead-acid batteries makes up for the majority of global lead consumption (Davidson et al., 2016; Lopez N et al., 2015). Further sources of human lead exposure include drinking water and several foods, e.g. vegetables and cereals (Brizio et al., 2016; European Food Safety Authority, 2010; Mena et al., 1996; Norton et al., 2015; Pirsaheb et al., 2016; Slepecka et al., 2017; Talio et al., 2014; Zietz et al., 2010). The World Health Organizations (WHO) International Agency for Research on Cancer (IARC) classified lead as possibly carcinogenic to humans (IARC, 1987, 2006). In 2006, the Commission for the Investigation of Health Hazards of Chemical Compounds in the work area of the German Research Foundation (DFG) categorized lead and its inorganic compounds as substances that are considered to be carcinogenic for man (DFG, 2006). In 2009, the German Human Biomonitoring Commission (HBM Commission) discontinued the Human Biomonitoring assessment values (HBM-I- and HBM–II–values) because of the lack of a threshold level for lead toxicity (Apel et al., 2017; Kommission Human-Biomonitoring des Umweltbundesamtes, 2002, 2009). Additionally, in 2010 the Panel on Contaminants in the Food Chain (CONTAM Panel) of the European Food Safety Authority (EFSA) concluded

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that use of the provisional tolerable weekly intake (PTWI) set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was no longer appropriate. This conclusion was drawn since no threshold level for toxicological endpoints including developmental neurotoxicity and nephrotoxicity in adults was evident (European Food Safety Authority, 2010; Jeong et al., 2015; Kommission Human-Biomonitoring des Umweltbundesamtes, 2002; 2009; Lanphear et al., 2016; Pawlas et al., 2012). Recent investigations demonstrate that even relatively low blood lead levels (BLL < 50 μg/L) are associated with a reduced cognitive function, e.g. reduced ability to concentrate, and lower IQ in children (European Food Safety Authority, 2010; Jeong et al., 2015; Lanphear et al., 2016; Pawlas et al., 2012). Therefore, serious public health effects are still discussed, especially in children, following exposure of the child or the mother during pregnancy (Clark et al., 2014; Etchevers et al., 2014; Kennedy et al., 2016; Lanphear et al., 2016; Neugebauer et al., 2015). Current examples, e.g. the drinking water contamination of the city of Flint (Michigan, USA) further increase this public concern (Gomez et al., 2018; Pieper et al., 2018). Many countries implemented regulatory actions to reduce human exposure to lead and lead levels in the environment within the last four decades. However, considering Europe, data on lead exposure covering the past five years are only available from seven countries (Rudnai, 2019) and comparable data on exposure levels and trends in exposure are lacking. The European Human Biomonitoring Initiative (HBM4EU, www.hbm4eu.eu) was launched to harmonize Human Biomonitoring efforts in Europe to close data gaps on human exposure in Europe and to provide sound scientific data as a basis for policy decisions, inter alia for lead as a priority substance. Complementary to the representative German Environmental Survey (GerES) the German Environmental Specimen Bank (German ESB) has been monitoring lead exposure in human and environmental samples since the early 1980s. The German ESB is a key element of environmental and human monitoring in Germany and provides valuable policy relevant scientific data on lead exposure in young adults in Germany. In this study, based on the complete dataset of the German ESBs lead monitoring from 1981 to 2019, we show and evaluate the time trend of exposure and evaluate specific factors involved in lead exposure in Germany, to provide an additional building block for evaluating lead exposure in Europe.

## 2. Material and methods

### 2.1. The German Environmental Specimen Bank

The German ESBs main goal is to evaluate time trends in pollutant levels in humans and the environment following real time and retrospective monitoring approaches (Kayser et al., 1982; Kemper and Luepke, 1986; Kemper, 1993; Kolossa-Gehring et al., 2012a; Stoeppler et al., 1984; Umweltbundesamt, 1996). Since its foundation in the early 1980s human and environmental samples are stored in a biobank for the unique opportunity of retrospective time trend analysis (Koch et al., 2017; Lermen et al., 2014; Schröter-Kermani et al., 2016; Wiesmüller et al., 2007; Wittassek et al., 2007). Recruitment and sampling have been carried out according to the respective guidelines and standard operating procedures (SOPs) of the German ESB (Eckard et al., 2011; Lermen et al., 2015; Umweltbundesamt, 1996). Briefly, the concept of the German ESB stipulates an annual cross-sectional sampling of approximately 120 volunteering students on each of the four different sampling sites Muenster, Ulm (both former Western Germany), Halle (Saale), and Greifswald (both former Eastern Germany). In total approximately 480 participants have been recruited annually. Participation is limited to healthy adults aged 20–29 in a balanced sex ratio. Each year young adults, have been recruited by promoting the study at the medical faculties of the universities at the four sampling sites by sending out e-mails to all students of the respective medical faculties, promoting the study before lectures, and by distributing flyers at the respective medical faculty. As only young adults were recruited out of medical students, it can be assumed that the sample is mainly composed of individuals that are not occupationally exposed. Thus, results can be assumed to represent the background exposure in Germany (Umweltbundesamt, 2008). The German ESB focuses on the investigation on long-term trends in exposure and supplements the German Environment Surveys (GerES), Germany's representative large-scale population study on exposure to environmental pollutants being conducted in a five-year cycle (Kolossa-Gehring et al., 2012b). Blood samples were taken under medical supervision. For retrospective analyses, aliquots of the collected samples have been stored at ultra-low temperatures in the German ESB. Samples collected after 2010 are stored in the gas phase of liquid nitrogen (LIN) at temperatures below −130 °C. In 2013, all samples collected before 2010 were transferred from −80 °C freezers into LIN-based storage systems and have since then also been stored at temperatures below −130 °C (Lermen et al., 2014). The concept of the German ESB and the SOPs are available online (https://www.umweltprobenbank.de/en/documents/publications). The current study protocol for the sampling of human samples from young adults was reviewed and approved in 2011 by the ethics committee of the Medical Association Saarland, Germany. Before 2011, the study protocol was reviewed and approved by the ethics committee of the Medical Association Westphalia-Lippe and the Medical Faculty of the University of Muenster, Germany. All study participants gave written informed consent on standardized forms approved by the ethics committees. The right to know and the right not to know were guaranteed. The results were reported to the participants immediately after the analyses were completed.

### 2.2. Questionnaire data

To document the medical history, exposure-relevant behavior (e.g. nutrition, smoking, and drinking habits), and living conditions, a self-administered questionnaire has been used. Limitations in recording exposure-relevant aspects exist for some influencing factors. For example, since many of the German ESB participants are students who mostly live in rented and/or shared flats, information on the material and condition of water pipes is only partly known to participants and therefore not investigated in this study. From 1981 to 2019, questions regarding smoking status were adapted multiple times and consequently evaluation for the whole time period was only possible on a yes or no basis. Since 2007, smoking status was consistently recorded using the following categories: non-smoker (NS), non-smoker but second-hand smoker (NS but SHS), ex-smoker but second-hand smoker (ES but SHS), smoker (S). Questions regarding the consumption of alcoholic beverages were also slightly adapted during the time period inspected. Alcohol consumption was expressed in a binary fashion (yes or no) for analyses.

### 2.3. Blood lead level determinations

After completing all sampling processes of one year at the four sampling sites, the entire batch of samples was analysed to be able to report continuously. In order to be able to distinguish potential geographical differences from analytical effects, samples of one year were measured in randomized order. Since data on blood lead values of the German ESB have been recorded over 35 years, variations exist with regard to the applied methods of chemical analysis. Electrothermal atomic absorption spectrometry at 283.3 nm with Zeeman background compensation using graphite furnace technology (ET-AAS-Z) was used from 1981 until 1999. In 1999, inductively coupled plasma mass spectrometry (ICP-MS) was used for the first time to analyse samples of one sampling site (Muenster). In 2000 and 2001, ICP-MS was used for all four sampling sites followed by high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) in a multi-element analysis mode from 2002 to 2009. In 2010, the analytical method was changed back to ICP-MS, since a limit of quantification (LoQ) of 0.15 μg/L was considered sufficient providing reliable data for blood lead quantification. However, the previously described changes in analytical methods led to changes of the respective limits of quantification (see Table 1 for details on LoQs). This is especially relevant in the years preceding the introduction of ICP-MS (1996–1999), where average blood lead levels approached the LoQ of the ET-AAS-Z method, leading to a high percentage of measurement values below the LoQ (15%–35%). From 1981 to 1994, measurements under LoQ ranged from 0% to 2% and from 1995 to 1999 from 15 to 35%. From 2000 to 2019, all samples were above LoQ. Lead concentration was determined by isotope analysis of $^{207}Pb$ in low (ICP-MS) or medium resolution setting (HR-ICP-MS). Before analysis, whole blood samples were wet-digested by microwave heating with high pressure (Teflon) vessel technology using nitric acid and hydrogen peroxide as oxidation agents. Until 2010, blood lead analysis was performed by the University of Muenster. Since 2011, analysis has been conducted by the Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen (IPASUM). All analytical methods were based on respective guidelines of the German Environmental Specimen Bank (Umweltbundesamt, 1996). Quality assurance was improved over time considering internal and external control schemes. Quality control for lead analysis has been conducted according to external quality assessments schemes (GEQUAS) and the measurement of certified reference material (NIST-CRM) explained in detail elsewhere (Göen et al., 2012). Quality was assured with regularly participation in GEQUAS round robin tests.

### 2.4. *Statistical analyses*

The statistical analyses were carried out using R Version 3.2.3. Blood lead values below LoQ were replaced by LoQ/2 (Hornung and Reed, 1990). P values ≤ 0.05 were considered statistically significant. Trends of geometric mean (GM) BLL values from 1981 to 2019 were assessed separately for male and female participants and tested for the presence of monotonic trends (Mann-Kendall test) using the function "mk.test" from the R package "trend" v1.0.1. Presence of an exponentially declining trend was evaluated by fitting a function of the form GM BLL (year) = a * exp(b*year) + c to the geometric mean BLL values using the non-linear least squares method (function "nls" from R package "stats" v3.4.4). Significance of Pearson's product moment correlation coefficients was assessed using the function "cor.test" from R package "stats" v3.4.4.

To explore further BLL-affecting factors we investigated BLL values of the years 2010–2019. Since BLL values levelled at a plateau following the year 2010, the period from 2010 to 2019 was chosen to minimize confounding differences between locations and sampling years. Due to partly lacking variance homogeneity (Levene test) and deviations from normal distribution (Kolmogorov-Smirnov test), differences between groups were analysed using non-parametric tests. Differences of two groups (e.g. no alcohol consumer vs. alcohol consumer) were analysed with the Mann-Whitney *U* test. For the comparison of smoking categories, a Kruskal-Wallis test, followed by Dunn post hoc tests with Bonferroni correction were used. Housing situation was categorized according to the risk of white lead paint being used as coating. Houses and apartments built before 1949 were assumed to represent a higher risk and were aggregated as "old buildings". Houses and apartments built since 1949 were considered to represent a lower risk and were aggregated as "new buildings" (Falq et al., 2011; Lucas et al., 2012; Meyer et al., 1999).

**Table 1**
Overview of LoQ with respect to the different analytical methods used for blood lead analysis over time.

| Sampling site | until 1998 | 1999 | 2000–2001 | 2002–2009 | since 2010 |
|---|---|---|---|---|---|
| Muenster | 20 μg/L[a] | 0.01 μg/L[b] | 0.01 μg/L[b] | 0.01 μg/L[c] | 0.15 μg/L[b] |
| Halle | 20 μg/L[a] | 20 μg/L[a] | 0.01 μg/L[b] | 0.01 μg/L[c] | 0.15 μg/L[b] |
| Greifswald | 20 μg/L[a] | 20 μg/L[a] | 0.01 μg/L[b] | 0.01 μg/L[c] | 0.15 μg/L[b] |
| Ulm | 20 μg/L[a] | 20 μg/L[a] | 0.01 μg/L[b] | 0.01 μg/L[c] | 0.15 μg/L[b] |

[a] EZ-AAS-Z.
[b] ICP-MS.
[c] HR-ICP-MS.

## 3. Results

### 3.1. *Trend of blood lead concentrations*

German ESB data from Muenster students cover over 38 years (1981–2019) and include 3851 young adults aged 20–29 years (Fig. 1). Geometric mean (GM) BLLs of female and male participants decreased by about 87-88% from 1981 to 2019 (females: 72.2 to 8.4 μg/L, males: 85.3 to 11.0 μg/L) and followed monotonically decreasing trends ($p < 0.001$, Mann-Kendall test). More specifically, the reduction in GM BLLs was closely approximated by a trend function decreasing exponentially over time (Root mean square deviation (RMSD): 4.6 μg/L (females), 5.4 μg/L (males); adjusted $R^2$: 0.94 (females), 0.95 (males)). GM BLLs at the other three sampling sites also followed significant monotonically decreasing ($p < 0.005$, Mann-Kendall test) and similarly shaped trends (Fig. 2). Based on data of all four sampling sites combined, GM BLLs decreased by 53.1% from 24.0 μg/L in 1997 to 11.56 μg/L in 2010. A significant decrease of GM values was not observed following the year 2010 ($p = 0.07$, Mann Kendall test). The whole dataset can be accessed on the German ESBs webpage (German ESB, 2020).

In early years (1986–1990 and 1995–1996), in addition to the sample collections during the winter season, an additional sample collection during summer was carried out each year in Muenster. In the additional dataset, systematic seasonal effects on blood lead levels were not detected (c.f. Supplementary Fig. 1).

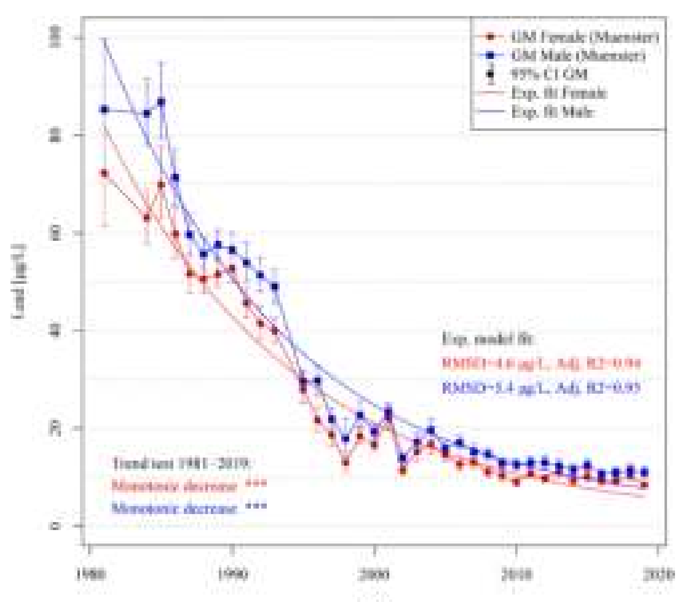


**Fig. 1.** Blood lead concentration of young adults from the sampling site Muenster from 1981 to 2019. Shown are the yearly geometric mean (GM) values of male and female participants with their 95 percent confidence intervals as well as the model fit of an exponentially decreasing trend function.

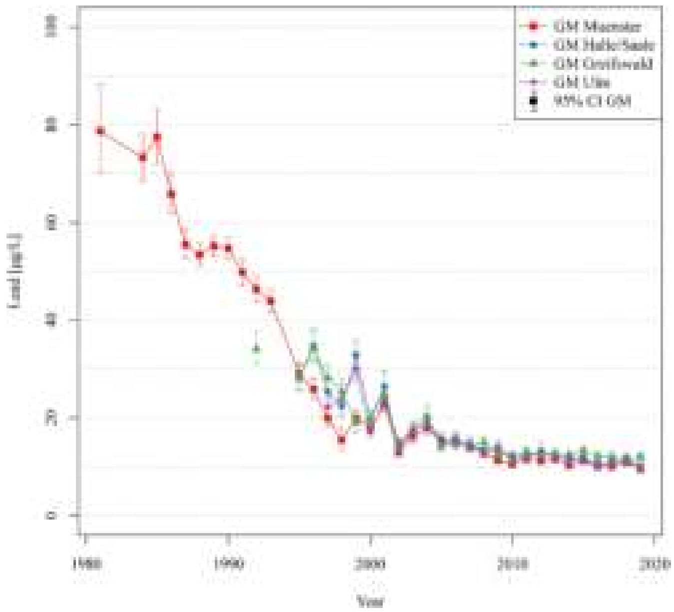


**Fig. 2.** Geometric mean blood lead concentrations from all four sampling sites of the German ESB. Muenster (1981–2019), Greifswald (1992–2019), Halle (1995–2019), and Ulm (1997–2019). 95 percent confidence intervals of GM values are depicted as error bars.

### *3.2. Further factors associated with blood lead levels: sex, housing, smoking status, and alcohol consumption*

To explore further BLL-affecting factors we investigated the years 2010–2019. An overview of BLLs from young adults grouped according to selected factors and considering data of all four sampling sites from 2010 to 2019 is given in Table 2. In general, male participants had significantly higher median BLLs than females (12.4 μg/L versus 10.2 μg/L). Approximately one quarter of participants (males: 24.5%, females: 23.9%) from samplings in 2010–2019 lived in houses build before 1949 and this percentage did not change substantially during the time period investigated. Statistically significant differences between non-smoking participants living in old and new buildings were only found in males (12.9 μg/L (old building) versus 12.0 μg/L (new building); females: 10.1 μg/L versus 9.9 μg/L). Both female and male smokers showed significantly higher BLLs than respective non-smokers (females: 13.3 μg/L versus 9.9 μg/L; males: 14.4 μg/L versus 12.1 μg/L). Although the prevalence of smokers decreased during the investigated period from 2010 to 2019 (females: 15.0%–4.2%, average 10.1%; males: 22.8%–12.8%, average 15.7%), the relative increase in BLLs of smokers versus non-smokers in individual years was comparable to the dataset as a whole.

To evaluate the impact of second-hand-smoke on the BLL we divided the group of non-smoking individuals into the groups of non-smokers (NS), non-smokers but second-hand-smokers (NS but SHS), ex-smokers (ES) and ex-smokers but second-hand-smokers (ES but SHS) and compared their data to the BLLs of smokers (S) (Fig. 3). S had significantly higher BLLs than all other groups in both sexes. An effect of second-hand-smoking was only detectable in females, with NS but SHS having significantly higher BLLs than NS. ES showed elevated BLLs in comparison to NS in both sexes. In the female group, ES and NS but SHS showed similar BLLs at an intermediate level between BLLs of NS and S. In the male group, ES showed BLLs closer to the level of current smokers. The relatively small group size of ES but SHS (males: n = 75; females n = 57) hampered detection of statistically significant differences to other groups. In terms of BLLs however, ES and ES but SHS showed very similar geometric mean values in both sexes. Prevalence of alcohol consumers among participants was high (males: 93.9%; females: 91.9%) and did not change substantially over the time period investigated. Among non-smokers, median BLLs of female and male alcohol consumers were significantly higher compared to female and male participants who did not self-report alcohol consumption (females: 10.1 μg/L vs 8.8 μg/L; males: 12.2 μg/L vs 11.1 μg/L).

## 4. Discussion

### *4.1. Trend of Pb exposure and international comparison*

The German ESB has been monitoring lead exposure of young adults since 1981. Data from 1981 to 2019 show a substantial decrease in lead exposure in young adults in Germany (approx. 87%). A detailed history of European gasoline lead content regulations and especially their implementation in Germany is given in (von Storch et al., 2003). Lead emission decreased in Germany continuously from the mid-80s due to different iterative mitigation steps. Data on regional air lead levels (ALLs) at the four German ESB sampling sites, compiled by the European Monitoring and Evaluation Programme (EMEP) for the same period show trends that corresponded to the observed change in German ESB BLLs remarkably well (see Supplementary Fig. 2). A similar correlation between ALLs and BLLs and a comparable decrease of both after the

**Table 2**
Blood lead concentrations: medians, arithmetic means (±SD), geometric means (95% CI), 95th percentiles, ranges in German young adults aged 20–29 by sex.

| 2010–2019 | | n | MD | AM (±SD) | GM (95% CI) | 95th PE | Min | Max | MW-Test[1] |
|---|---|---|---|---|---|---|---|---|---|
| Female | Total | 2626 | 10.2 | 11.6 (±6.2) | 10.5 (10.3–10.7) | 21.8 | 2.8 | 103.1 | p < 0.001 |
| Male | Total | 2310 | 12.4 | 13.9 (±7.0) | 12.7 (12.4–13.0) | 26.1 | 3.0 | 98.5 | |
| Female participants | | | | | | | | | |
| Non-smoker | | 2345 | 9.9 | 11.3 (±5.8) | 10.3 (10–10.5) | 21.2 | 2.8 | 71.9 | p < 0.001 |
| Smoker | | 265 | 13.3 | 14.5 (±8.3) | 13.1 (12.1–14.1) | 24.7 | 4.2 | 103.1 | |
| Female non-smokers | | | | | | | | | |
| No alcohol consumer | | 205 | 8.8 | 11.1 (±8.0) | 9.6 (8.5–10.7) | 25.6 | 2.9 | 71.9 | p < 0.001 |
| Alcohol consumer | | 2140 | 10.1 | 11.3 (±5.5) | 10.3 (10.1–10.6) | 21.1 | 2.8 | 66.9 | |
| Living in new building[2] | | 1648 | 9.9 | 11.3 (±5.7) | 10.2 (10.0–10.5) | 21.1 | 2.8 | 71.9 | p = 0.363 |
| Living in old building[3] | | 539 | 10.1 | 11.6 (±6.2) | 10.5 (10.0–11) | 21.9 | 3.9 | 66.9 | |
| Male participants | | | | | | | | | |
| Non-smoker | | 1930 | 12.1 | 13.5 (±6.9) | 12.3 (12–12.6) | 24.4 | 3.0 | 98.5 | p < 0.001 |
| Smoker | | 363 | 14.4 | 16.4 (±7.4) | 15 (14.3–15.8) | 30.4 | 5.6 | 55.4 | |
| Male non-smokers | | | | | | | | | |
| No alcohol consumer | | 132 | 11.1 | 12.5 (±8.0) | 10.9 (9.5–12.3) | 23.3 | 3.5 | 61.9 | p = 0.002 |
| Alcohol consumer | | 1798 | 12.2 | 13.5 (±6.8) | 12.4 (12.1–12.7) | 24.5 | 3.0 | 98.5 | |
| Living in new building[2] | | 1387 | 12.0 | 13.2 (±6.7) | 12.1 (11.7–12.4) | 22.8 | 3.0 | 98.5 | p = 0.002 |
| Living in old building[3] | | 444 | 12.9 | 14.4 (±7.6) | 13 (12.3–13.7) | 27.5 | 3.9 | 77.7 | |
| Total | | 4936 | 11.2 | 12.7 (±6.7) | 11.5 (11.3–11.7) | 23.6 | 2.8 | 103.1 | |

Lead concentrations is given in μg/L, [1]Mann-Whitney test, [2]built since 1949, [3]built before 1949.

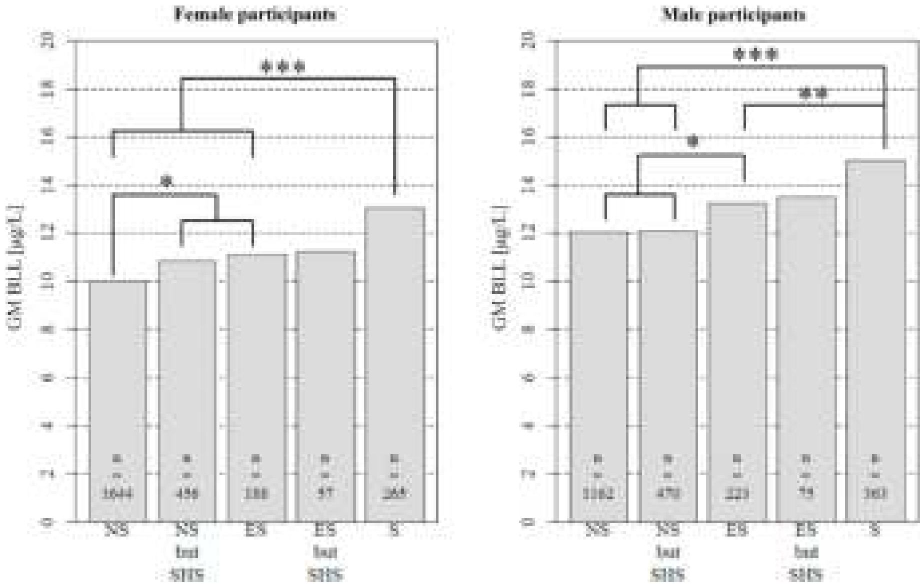


**Fig. 3.** Geometric means of BLLs [µg/L] of male (a) and female (b) non-smokers (NS), non-smokers but second-hand smokers (NS but SHS), ex-smokers (ES), ex-smokers but second-hand smokers (ES but SHS) and smokers (S) at all sampling sites from 2010 to 2019. Statistical significance of differences: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

implementation of political mitigation measures was also found in other European countries (Bono et al., 1995; Petit et al., 2015; Rodamilans et al., 1996), and globally (e.g, USA, Richmond-Bryant et al., 2014, South Korea, Oh et al., 2017). Our findings therefore strongly support that the observed steep decrease of BLLs is to a large degree a reflection of the drop in air lead pollution.

Although the toxicity of lead at low levels is still highlighted due to its adverse health effects on the fetus and children, still data gaps on exposure levels exist on a global scale. In Europe only few Human Biomonitoring studies on lead exposure were conducted in the last decades. However, comparability of results is limited due to different study designs, different target groups, and different points in time (Rudnai, 2019). Comparable data on trends in exposure are highly relevant for the evaluation of exposure in Europe and the implementation of adequate political mitigation measures and the control of its effects. First attempts in harmonizing HBM activities in Europe started already 2005 with the EU project ESBIO (Joas et al., 2012) and was continued subsequently in 2009 with the EU twin project COPHES/DEMOCOPHES (Schindler et al., 2014; Schwedler et al., 2017). Currently the European HBM initiative HBM4EU (www.hbm4eu.eu) is driving harmonization activities to improve collection and assessment of policy relevant data and feed them into open policy processes. Data on a comparable age group (25–35 years) as the German ESB students are provided by the Swedish MONICA study. BLLs in Sweden decreased drastically since the 1990s and show no further significant decrease since 2009 (Wennberg et al., 2017) which is in line with German ESB data. Latest results of the MONICA study reveal median BLLs of Swedish young adults aged 25–35 of 11 µg/L in males and 9.65 µg/L in females in 2014 (Wennberg et al., 2017) which agrees with the BLLs of young adults in Germany at this time.

For the years 2006–2007, the French Nutrition and Health Survey (ENNS) provides a blood lead geometric mean value of 25.7 µg/L (95% CI: 24.9 µg/L – 26.5 µg/L) for the French population aged 18–74 (Falq et al., 2011). For the subgroup of French adults aged 18–39 a GM value of 18.7 µg/L (95% CI: 17.8 µg/L – 19.6 µg/L) is reported. Regarding the years 2009 and 2010, the Spanish BIOAMBIENT study gives a first baseline information with a mean (GM) BLL of 24.0 µg/L (95% CI: 23.0 µg/L – 25.1 µg/L) for Spanish adults aged 18–65 (Cañas et al., 2014). For the age group 18–29, the BIOAMBIENT study reports a GM BLL of 19.1 µg/L (95% CI: 17.9 µg/L – 20.1 µg/L). Although age groups of these two studies are slightly different, compared to the mean values (GM) of 14.7 µg/L in 2006/2007 and of 12.1 µg/L in 2009/2010 for young adults in Germany aged 20–29, French and Spanish adults seem to have slightly higher mean BLLs in the respective years.

Results of the latest Canadian Health Measures Survey (CHMS) are based on data of the years 2016–2017 and report a mean (GM) BLL from adults aged 20–39 of 7.8 µg/L (Health Canada, 2019) while German ESB data indicate a GM BLL value of 11.7 µg/L in 2014/2015.

The US National Health and Nutrition Examination Survey (NHANES) provides data on BLLs of US citizens which are 20 years and older. Nevertheless, regarding the years 2001–2016, BLLs in the US and Germany decreased in a comparable manner (see Table 3). Within 16 years, NHANES data demonstrate that the mean blood lead concentration (GM) of American adults at the age of 20 years and older decreased by 41% from 15.6 µg/L to 9.2 µg/L (Centers for Disease Control and Prevention, 2009, 2014, 2019). During this time period, blood lead values of German ESB participants aged 20-29 decreased by 36.9% from 17.9 µg/L in 2001/2002 to a mean blood lead value (GM) of 11.3 µg/L in 2015/2016.

Data on lead exposure show a reduction in BLLs over time on a global scale and thus confirm the success of multiple mitigation measures implemented on national and international levels like the ban on leaded gasoline. Its impact on lead exposure has been intensively described (Bierkens et al., 2011; Muntner et al., 2005; Smolders et al., 2010; Strömberg et al., 2008; Thomas et al., 1999; von Storch et al., 2003). However, the national differences in concentration levels inevitably raise questions on different methodologies, different exposures and exposure sources and should be subjected to further research.

### 4.2. Further factors associated with blood lead levels

Since 2010 BLLs of young adults in Germany reached a stable plateau. This background exposure might be a complex mix of multiple exposure sources influenced by individual life style and dietary habits as well as social status, as discussed below (Cañas et al., 2014; Grandjean et al., 1981; Richter et al., 2013; Symanski and Hertz-Picciotto, 1995; Vahter et al., 2007; Wennberg et al., 2017; Weyermann and Brenner, 1997).

Amongst lifestyle habits, smoking and alcohol consumption have been discussed to be of highest relevance for human lead exposure

**Table 3**
Blood lead levels for the U.S. population from the NHANES aged 20 and older and from the German ESB aged 20–29 from 2001 to 2016.

| Years | NHANES | | | German ESB | | |
|---|---|---|---|---|---|---|
| | GM (95% CI) | MD (95% CI) | Sample size | GM (95% CI) | MD (95% CI) | Sample size |
| 01-02 | 15.6 (14.9–16.2) | 16.0 (15.0–16.0) | 4772 | 17.9 (17.4–18.5) | 17.9 (17.5–18.2) | 837 |
| 03-04 | 15.2 (14.5–16.0) | 15.0 (14.0–16.0) | 4525 | 18.1 (17.5–18.7) | 17.6 (17.3–17.9) | 896 |
| 05-06 | 14.1 (13.4–14.8) | 14.1 (13.3–14.8) | 4509 | 15.0 (14.6–15.5) | 14.9 (14.7–15.2) | 856 |
| 07-08 | 13.8 (13.1–14.6) | 13.4 (12.6–14.2) | 5364 | 13.9 (13.5–14.3) | 13.9 (13.7–14.2) | 875 |
| 09-10 | 12.3 (11.9–12.8) | 12.0 (11.4–12.5) | 5765 | 12.1 (11.8–12.4) | 12.0 (11.8–12.2) | 876 |
| 11-12 | 10.9 (10.3–11.6) | 10.5 (10.0–11.2) | 5030 | 12.3 (12.0–12.6) | 12.1 (11.9–12.3) | 968 |
| 13-14 | 9.7 (9.2–10.0) | 9.4 (9.0–9.8) | 2695 | 11.7 (11.4–12.1) | 11.2 (11.0–11.4) | 986 |
| 15-16 | 9.2 (8.6–9.8) | 8.8 (8.1–9.6) | 2610 | 11.3 (11.0–11.6) | 11.2 (11.0–11.3) | 995 |

Lead concentrations are given in µg Pb/L whole blood, GM = Geometric Mean, MD = Median, CI = confidence interval.

(Grandjean et al., 1981; Grasmick et al., 1985; Richter et al., 2013; Shaper et al., 1982). In the period of relatively low environmental background exposure since 2010, the German ESB data clearly confirm the association of smoking and alcohol consumption on BLLs. As the investigated group consisted of young adults highly unlikely occupationally exposed to lead, smoking and alcohol consumption can be considered as main factors with high impact on current lead exposure next to food and drinking water, which could not be evaluated in this study. German ESB data also revealed that past smoking is still a determinant for the current internal lead exposure (see Fig. 3). Effects of housing on lead exposure in non-smoking participants could only be found in male participants. In general, male participants had higher BLLs than females what is in line with findings from other studies (Cañas et al., 2014; Falq et al., 2011; Health Canada, 2017, 2019; Muntner et al., 2005; Wennberg et al., 2017). In a recent study with a focus on international comparison limited to female participants (including German ESB BLL data), BMI seems to be somewhat associated with BLL which is, however, in disagreement with findings from other studies (Nakayama et al., 2019). Specific studies need to assess the impact of personal behavior on the BLL and need to clarify the sources for the current low-level background exposure in Germany in more detail, to enable further decrease in lead exposure. Specifically, some foods and drinking water are known exposure sources in this context and have to be included in more detailed investigations (Brizio et al., 2016; European Food Safety Authority, 2010).

### 4.3. Current lead exposure

BLLs in young adults in Germany decreased by more than 85% from 1981 to 2010 (GM: 78.7 to 11.56 µg/L) and since then stabilized on a plateau. Recent studies reported that even a low level of lead exposure is associated with negative impact on human health for adults and children (Canfield et al., 2003; Falck et al., 2019; Grönqvist et al., 2020; Lanphear et al., 2000, 2016, 2018; Zhou et al., 2020). Especially during pregnancy and in young children, blood lead levels below 10 µg/L are found to have detrimental effects (Afeiche et al., 2011; Jakubowski, 2011; Motao Zhu, 2010; Xie et al., 2013). Thus, based on current data no threshold for lead toxicity can be derived. Current BLLs have to be monitored regularly especially in children and women of childbearing age and the ubiquitous exposure has to be further reduced to conciliate public health concerns. Considering potential European regulation measures, the generation of comparable data on lead exposure has to be facilitated and monitoring efforts have to be harmonized to paint a clear picture of lead exposure across borders.

## 5. Conclusions

Overall, human lead exposure decreased drastically in Germany over the last 38 years. Clearly, regulatory actions on lead emissions compassed a significant positive impact on human lead exposure. But current exposure levels still encompass BLLs considered unsafe and no further decrease could be seen in the last decade. Previous regulatory measurements have apparently reached their level of influence and should therefore be improved. A further reduction of lead exposure in the future is still necessary. An extensive monitoring of both, lead intake (monitoring of food and other sources) and exposure (HBM) to uncover the specific sources relevant for current lead exposure will be needed, especially in more sensitive parts of the population like children and women of childbearing age, to characterize their special risks. International harmonization of the national monitoring efforts, like HBM4EU (Ganzleben et al., 2017), will further improve the knowledge base and therefore support action on a global scale.

## Declaration of competing interest

The authors of this manuscript certify that there are no actual or potential competing financial interests and that the authors' freedom to design, conduct, interpret, and publish research is not compromised by any controlling sponsor as a condition of review and publication.


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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2020.113665.

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Short communication

# Occupational exposure to MRI-related magnetic stray fields and sleep quality among MRI – Technicians - A cross-sectional study in the Netherlands

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A R T I C L E I N F O

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A B S T R A C T

We investigated the association between occupational exposure to MRI-related magnetic stray fields with sleep quality in a cross-sectional study among 490 imaging technicians in the Netherlands. Imaging technicians filled in questionnaires about MRI exposure, lifestyle, work practices and sleep quality and quantity (Medical Outcomes Study sleep scale). Of six sleep domains, exposure to MRI-related magnetic stray fields appeared to be associated with increased sleep disturbance (OR 1.93, 95% CI 1.00–3.70) and non-optimal sleep duration (OR 1.95, 95% CI 1.11–3.44). Given earlier findings of possible increased accident risks among exposed imaging technicians, these findings merit follow-up.

## 1. Introduction

Magnetic Resonance Imaging (MRI) has seen a steep increase in the number of MRI units, number of scanning procedures, and in scanner field strength over the past decades (OECD health statistics database (OECD.Stat), 2020; Schaap et al., 2013). This has led to an increase in occupational exposure to MRI-related magnetic stray fields, with increases in the number of exposed staff and increased exposure levels (Schaap et al., 2013, 2016). Occupational exposure to MRI-related magnetic fields has been related to acute symptoms such as vertigo, and neurocognitive effects (Van Nierop et al., 2015; Schaap et al., 2014). Few studies have investigated potential long-term effects of occupational exposure to MRI-related magnetic fields, but increased risks of commuting accidents was observed among MRI system testers and technicians who were exposed to the magnetic stray fields of MRI scanners (Bongers et al., 2016; Huss et al., 2017). In addition, sleeping disorders and tiredness were frequently reported among personnel who worked with or near MRI scanners (Zanotti et al., 2015). The objective of our analysis was to investigate the association between exposure to MRI-related magnetic stray fields and sleep quality in imaging technicians in The Netherlands.

## 2. Methods

In 2013, members of the Dutch Association of Medical Imaging and Radiotherapy (Nederlandse Vereniging Medische Beeldvorming en Radiotherapie, NVMBR) who worked in the field of Medical Imaging were invited to fill in an online questionnaire. The questionnaire inquired about lifestyle, work practices and health, including sleep quality and quantity. Of the 1637 invited imaging technicians, 490 filled in the questionnaire (response rate 30%).

We used proxies to assess occupational exposure to MRI-related magnetic stray fields: we asked participants if they had worked in an MRI-scanner room in the past 12 months or past 4 weeks prior to the survey. We also asked on how many days they had worked in an MRI-scanner room during the past 4 weeks. Furthermore, we tried to disentangle possible exposure to switched gradient stray fields and radiofrequency pulses (RF) from exposure to only static magnetic fields (SF) and time-varying magnetic fields (TvMF), by asking if the participants had been present in an MRI-scanner room during actual image acquisition in the 12 months and 4 weeks prior to the survey.

The 12-item Medical Outcomes Study sleep scale was used to assess the sleep quality in the four weeks prior to the survey (Hays and Stewart, 1992). Sleep items were scored and grouped according to the manual. This resulted in the following sleep domains: 1) sleep disturbance (ability to fall asleep and maintain restful sleep), 2) somnolence

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(daytime drowsiness or sleepiness), 3) sleep adequacy (sufficiency of sleep in terms of wakefulness), 4) snoring, 5) non-optimal sleep duration (non-optimal amount of sleep) and 6) respiratory problems (shortness of breath). The overall sleep problem index II was created by grouping the items from sleep disturbance, sleep adequacy and somnolence domains (Hays and Stewart, 1992).

We used linear regression to study associations between exposure proxies and the overall sleep problem score. Logistic regression was used for the six sleep domains by dichotomizing these domains into 'less affected' and 'more affected' sleep because data were not normally distributed. For sleep domains: sleep disturbance, somnolence, sleep adequacy, snoring and respiratory problems, the 75th percentile was used as the cut-off point to categorize participants as having less or more affected sleep. The 75th percentile was chosen *a priori* to identify stronger affected persons while retaining enough statistical power for the analysis. We grouped participants as having non-optimal sleep duration if they slept, on average, less than 7 h or more than 8 h a day in the past 4 weeks (Hays and Stewart, 1992) and additionally evaluated the group <7 vs 7–8 h as well as >8 vs 7–8 h, so excluding the respective other group from the analysis. We adjusted for *a priori* selected confounders age, sex, current smoking- and alcohol consumption status, current medication use, ever used sleep medication in the past 4 weeks, work-related stress in the past 4 weeks (in tertiles), working on evening- and night shifts in the past 4 weeks (using medians as cut-offs, with categories 0, 1–2, 3–12 evening shifts; 0, 1–2, 3–8 night shifts), and body-mass index categories.

## 3. Results

Characteristics of participants enrolled in this study can be found in Table 1. The study population consisted of 381 women (78%) and 109 men (22%), the average age was 44 years. Of the 490 participants, 216 (44%) reported entering an MRI room in the past 4 weeks and 62 (13%) reported to be present during image acquisition. Adjusted risk estimates did not indicate associations between MRI-related magnetic stray field exposures and the sleep problem summary (sleep problem index II), somnolence, adequacy of sleep, snoring or respiratory problems (Table 2).

We observed increased sleep disturbance among participants who were present in an MRI room during image acquisition in the past 4 weeks (OR 1.93, 95% CI 1.00–3.70). More non-optimal sleep duration was observed among participants who entered an MRI room more often (7–20 days) in the past 4 weeks (OR 1.95, 95% CI 1.11–3.44) than who entered the MRI room sometimes (1–6 days). Most participants with non-optimal sleep duration (n = 108) reported sleeping <7 h (n = 93, 86%). Removing 15 participants reporting to sleep >8 h from the analysis resulted in an adjusted OR of 1.92 (95%CI 1.04–3.51). Reversely, removing participants sleeping <7 h resulted in an OR of 2.33 (95%CI 0.62–8.82). Adjusting for potential confounding had no material effect on the risk estimates.

## 4. Discussion

In our study, occupational MRI exposure was not associated with (overall) sleep quality and most of the evaluated sleep domains. However, imaging technicians who had worked with or near MRI scanners often (7–20 days) during the four weeks prior to the survey reported more often non-optimal sleep duration. Imaging technicians who were present in an MRI room during image acquisition reported more often sleep disturbance.

To the best of our knowledge, this is the first study that evaluated workers' sleep quality and their association with exposure to MRI-related magnetic stray fields. Strength of our study is the inclusion of information on potential confounders. Limitations pertain to the low response rate (30%). It is possible, although unlikely, that study participants were aware of potential detrimental effects of working with or near MRI scanners on their sleep, but the study was not focusing on sleep quality per se. Also, no previous study has attributed decreased sleep quality to working with or near MRI scanners to date.

Exposure measures were correlated to some degree: 29% of imaging technicians who entered an MRI room in the four weeks prior to the survey also reported being present in an MRI room during image acquisition. Imaging technicians who were present in an MRI room during image acquisition could stand in close proximity to the bore, for example when guiding anxious patients. As a consequence, these imaging technicians could be exposed to switched gradient stray fields, but also have a higher SMF peak exposure. Motion-induced TvMF exposure levels depend on body-velocity and positioning relative to the magnet (Crozier and Liu, 2005). As we did not have information on movement patterns and/or speed of our study participants, we were limited in our ability to make a clear distinction between the different MRI-related exposures. We also have no biological explanation for the possible associations between MRI-related magnetic stray field exposures and affected sleep.

We performed a large number of tests, which could have resulted in observing associations by chance, and results should be seen as hypothesis generating. Future studies could be improved by objectively quantifying workers' exposure and sleep, e.g. with dosimetry and sleep actigraphy. Reduced sleep quality affects health, and given previous

**Table 1**
Characteristics of study population.

| | Unexposed to MRI-related stray fields[a] | Past exposure to MRI-related stray fields[a] | Recent exposure to MRI-related stray fields[a] | *P*[b] |
|---|---|---|---|---|
| Age (in years) [a] | 46.58 (9.9) | 42.24 (11.4) | 42.56 (10.6) | <0.001 |
| Sex | | | | 0.519 |
| Female | 186 (79.8) | 30 (73.2) | 165 (76.4) | |
| Male | 47 (20.2) | 11 (26.8) | 51 (23.6) | |
| Smoking status | | | | 0.314 |
| Never | 151 (64.8) | 32 (78.0) | 154 (71.3) | |
| Former | 65 (27.9) | 8 (19.5) | 52 (24.1) | |
| Current | 17 (7.3) | 1 (2.4) | 10 (4.6) | |
| Alcohol use | | | | 0.835 |
| Never | 55 (23.6) | 8 (19.5) | 46 (21.3) | |
| Former | 10 (4.3) | 1 (2.4) | 12 (5.6) | |
| Current | 168 (72.1) | 32 (78.0) | 158 (73.1) | |
| Medication use[c] | | | | 0.322 |
| No | 136 (58.6) | 25 (61.0) | 125 (58.1) | |
| Yes | 39 (16.8) | 9 (22.0) | 50 (23.2) | |
| Past 4 weeks | 57 (24.6) | 7 (17.1) | 40 (18.6) | |
| Sleep medication use | | | | 0.854 |
| No | 216 (92.7) | 39 (95.1) | 201 (93.1) | |
| Yes | 17 (7.3) | 2 (4.9) | 15 (6.9) | |
| Work-related stress | | | | 0.098 |
| Low | 91 (39.1) | 20 (48.8) | 64 (29.6) | |
| Medium | 75 (32.2) | 12 (29.3) | 83 (38.4) | |
| High | 67 (28.8) | 9 (22.0) | 69 (31.9) | |
| Evening shifts | | | | <0.001 |
| 0 days | 119 (51.1) | 24 (58.5) | 62 (61.6) | |
| 1–2 days | 74 (31.8) | 9 (22.0) | 75 (24.1) | |
| 3–12 days | 40 (17.2) | 8 (19.5) | 79 (14.4) | |
| Night shifts | | | | 0.125 |
| 0 days | 161 (69.1) | 28 (68.3) | 133 (61.6) | |
| 1–2 days | 53 (22.7) | 6 (14.6) | 52 (24.1) | |
| 3–8 days | 19 (8.2) | 7 (17.1) | 31 (14.4) | |
| BMI | | | | 0.344 |
| <25 | 142 (60.9) | 30 (73.2) | 144 (66.7) | |
| 25–29.9 | 68 (29.2) | 10 (24.4) | 57 (26.4) | |
| ≥30 | 23 (9.9) | 1 (2.4) | 15 (6.9) | |

[a] All numbers are n (%) with the exception of age, which is shown as mean (SD). Total N = 490.
[b] *P* values of group differences, based on a one-way ANOVA for age and chi-square tests for the other covariates.
[c] There was missing information on medication use (N = 2).

**Table 2**
Associations between entering an MRI room, presence during image acquisition, scanner strength and the seven sleep domains.[a,b,c,d,e]

| | Sleep problem index II | Sleep disturbance | Somnolence | Sleep adequacy | Snoring | Non-optimal sleep duration | Respiratory problems |
|---|---|---|---|---|---|---|---|
| | β adjusted (95% CI) | OR adjusted (95% CI) | OR adjusted (95% CI) | OR adjusted (95% CI) | OR adjusted (95% CI) | OR adjusted (95% CI) | OR adjusted (95% CI) |
| Unexposed | referent | referent | referent | referent | referent | referent | referent |
| Entered MRI room past 12 months, not past 4 weeks | 1.61 (−2.50–5.72) | 1.35 (0.56–3.03) | 1.52 (0.66–3.33) | 0.71 (0.26–1.69) | 0.41 (0.06–1.56) | 2.14 (0.94–4.69) | 0.76 (0.21–2.14) |
| Entered MRI room past 4 weeks | 0.61 (−1.76–2.99) | 1.23 (0.76–1.98) | 1.11 (0.68–1.80) | 1.17 (0.73–1.88) | 1.44 (0.79–2.63) | 1.28 (0.78–2.10) | 0.89 (0.48–1.64) |
| Unexposed | referent | referent | referent | referent | referent | referent | referent |
| Entered MRI room past 12 months, not past 4 weeks | 1.62 (−2.50–5.73) | 1.35 (0.56–3.05) | 1.52 (0.66–3.34) | 0.71 (0.26–1.69) | 0.41 (0.06–1.57) | 2.14 (0.94–4.69) | 0.76 (0.21–2.14) |
| Entered MRI room past 4 weeks, 1–6 days | 1.28 (−1.65–4.20) | 1.52 (0.85–2.69) | 1.25 (0.69–2.25) | 1.15 (0.64–2.04) | 1.69 (0.80–3.49) | 0.68 (0.33–1.34) | 0.91 (0.42–1.90) |
| Entered MRI room past 4 weeks, 7–20 days | −0.01 (−2.87–2.85) | 1.00 (0.55–1.77) | 0.98 (0.54–1.76) | 1.19 (0.68–2.07) | 1.25 (0.59–2.55) | **1.95 (1.11–3.44)** | 0.86 (0.39–1.80) |
| Unexposed | referent | referent | referent | referent | referent | referent | referent |
| Entered MRI room past 12 months (no acquisition) | −0.01 (−4.54–4.52) | 0.94 (0.32–2.41) | 1.21 (0.46–2.91) | 0.79 (0.27–2.02) | 0.60 (0.09–2.37) | 2.37 (0.96–5.59) | 0.67 (0.15–2.13) |
| Entered MRI room past 4 weeks (no acquisition) | −0.58 (−3.55–2.38) | 0.90 (0.48–1.67) | 0.81 (0.42–1.53) | 1.59 (0.90–2.80) | 1.11 (0.49–2.40) | 0.86 (0.43–1.65) | 0.58 (0.23–1.32) |
| Presence during acquisition past 12 months, not past 4 weeks | 1.78 (−1.75–5.31) | 1.34 (0.67–2.63) | 1.52 (0.76–2.97) | 0.68 (0.31–1.42) | 1.30 (0.51–3.07) | 1.72 (0.85–3.40) | 1.49 (0.64–3.27) |
| Presence during acquisition past 4 weeks | 2.32 (−1.18–5.82) | **1.93 (1.00–3.70)** | 1.43 (0.70–2.81) | 1.02 (0.50–2.01) | 1.70 (0.72–3.82) | 1.60 (0.78–3.20) | 0.86 (0.30–2.14) |

[a] Adjusted for age, sex, current smoking- and alcohol consumption status, medication use, sleep medication use, (work-related) stress, evening- and night shifts, and BMI.
[b] Non-optimal sleep duration = on average < 7 h or >8 h of daily sleep in the past 4 weeks.
[c] Sleep problem index II = overall sleep problem summary.
[d] No acquisition = not present during image acquisition.
[e] Increased odds ratios indicate decreased sleep quality.

reports of increased risks of commuting accidents when working with or near MRI scanners, the observation merits follow-up.

## Contributors

HK conceived of the study. EÖ analysed data and drafted a first version of the manuscript. KS was responsible for the data collection. All authors were involved in interpretation of the data and provided input in the consecutive iterations of the manuscript. All authors read and approved of the final version of the manuscript. AH is the guarantor for this work.

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## Ethics board

The ethics committee of the University Medical Centre Utrecht declared that ethical approval was not necessary for this survey (protocol number 13–066/C).

## Declaration of competing interest

All authors declare that they have no conflicts of interest.

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# Proximity to livestock farms and exposure to livestock-related particulate matter are associated with lower probability of medication dispensing for obstructive airway diseases

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A R T I C L E I N F O

*Keywords:*
Asthma
COPD
Particulate matter
Farm animals
Environmental exposure

A B S T R A C T

*Objectives:* The aim of this study is to assess whether medication use for obstructive airway diseases is associated with environmental exposure to livestock farms. Previous studies in the Netherlands at a regional level suggested that asthma and chronic obstructive pulmonary disease (COPD) are less prevalent among persons living near livestock farms.
*Methods:* A nationwide population-based cross-sectional study was conducted among 7,735,491 persons, with data on the dispensing of drugs for obstructive airway diseases in the Netherlands in 2016. Exposure was based on distances between home addresses and farms and on modelled atmospheric particulate matter ($PM_{10}$) concentrations from livestock farms. Data were analysed for different regions by logistic regression analyses and adjusted for several individual-level variables, as well as modelled $PM_{10}$ concentration of non-farm-related air pollution. Results for individual regions were subsequently pooled in meta-analyses.
*Results:* The probability of medication for asthma or COPD being dispensed to adults and children was lower with decreasing distance of their homes to livestock farms, particularly cattle and poultry farms. Increased concentrations of $PM_{10}$ from cattle were associated with less dispensing of medications for asthma or COPD, as well (meta-analysis OR for 10th-90th percentile increase in concentration of $PM_{10}$ from cattle farms, 95%CI: 0.92, 0.86–0.97 for adults). However, increased concentrations of $PM_{10}$ from non-farm sources were positively associated (meta-analysis OR for 10th-90th percentile increase in $PM_{10}$-concentration, 95%CI: 1.29, 1.09–1.52 for adults).
*Conclusions:* The results show that the probability of dispensing medication for asthma or COPD is inversely associated with proximity to livestock farms and modelled exposure to livestock-related $PM_{10}$ in multiple regions within the Netherlands. This finding implies a notable prevented risk: under the assumption of absence of livestock farms in the Netherlands, an estimated 2%–5% more persons (an increase in tens of thousands) in rural areas would receive asthma or COPD medication.

## 1. Introduction

Previous research in the Netherlands suggests that persons living in the vicinity of livestock farms are less likely to have asthma or chronic obstructive pulmonary disease (COPD) (Borlée et al., 2015; de Rooij et al., 2019; Smit et al., 2014). The reduced asthma prevalence may be explained by more diverse microbial exposures in livestock-farming areas, leading to a reduced risk for development of allergic

*Abbreviations:* COPD, chronic obstructive pulmonary disease; GCN, large-scale concentration-maps the Netherlands [grootschalige concentratie-kaarten Nederland]; PAF, population-attributable fraction; PM, particulate matter; SES, socio-economic status.

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sensitization (von Mutius, 2016). This explanation is largely based on studies in children growing up on livestock farms (Ege et al., 2011; Riedler et al., 2001), who tend to have a lower risk of atopic asthma and allergies. A lower atopy prevalence was also recently found among Dutch adults living in the vicinity of livestock farms (Borlée et al., 2018). In contrast, no biologically plausible explanation could be provided for the reduced prevalence of COPD in the vicinity of livestock farms (Smit et al., 2014). Furthermore, while prevalence was lower close to farms, the frequency of exacerbations among patients with COPD was higher and pulmonary function was lower, particularly with higher livestock-related air pollution levels (Borlée et al., 2015, 2017; van Dijk et al., 2016a; van Kersen et al., 2020).

In countries other than the Netherlands, evidence is mixed regarding a protective effect of living in the vicinity of livestock farms (Casey et al., 2015; Douglas et al., 2018; Kauffmann et al., 2002; Schultz et al., 2019). Studies among farmers themselves are also inconclusive, as some have shown a protective effect with increased farming exposure, whereas others have indicated a higher risk of asthma with increasing farming exposures, particularly for non-atopic asthma (Wunschel and Poole, 2016). Studies among farmers generally have shown an increased prevalence of COPD compared to those in non-farmers, which is attributed to long-term dust exposure (Fontana et al., 2017; Guillien et al., 2019).

In previous research in the Netherlands on associations between asthma and COPD in relation to proximity to livestock farms, several measures of exposure were used, including nearest distances to several types of animal farms and particulate matter (PM) emissions from these farms (Smit et al., 2014). Recently, such exposure measures were extended with modelled concentrations of PM and endotoxins, which is a constituent of organic PM (de Rooij et al., 2019). Such modelled concentrations take into account the proximity to multiple farms and may better approximate the exposures behind the previously observed associations that are currently unknown.

The previous studies focussed on a study population of 92,548 persons (22,406 children; 70,142 adults) living in a livestock-dense area in the southeast of Netherlands and a subset of that population (Borlée et al., 2015; de Rooij et al., 2019; Smit et al., 2014). This region may not be representative of other regions in the Netherlands, because it differs in the density of livestock farms and has relatively higher particulate matter concentrations.[1] This follows from the relatively larger contribution of the agricultural sector in this area besides for instance traffic sources and a relatively high contribution from abroad (with industry and traffic being the most important contributors).

Hence, previous research may be complemented by studies including the entire Netherlands, with a larger study population and modelled PM concentrations as an additional measure of exposure besides distance to the nearest livestock farm. The use of nationwide available data on medication dispensing for asthma and COPD allows full coverage of the Netherlands. Therefore, the aim of this study is to investigate the association between medication dispensing for asthma and COPD and environmental exposure related to livestock farms in the Netherlands.

## 2. Material and methods

### 2.1. Study population

The basis of our study population was the Dutch population (16, 670, 000 individuals), with available data on anonymized address locations (key register for addresses and buildings: BAG), individual-level variables, and medication of all persons that were reimbursed by their statutory basic medical insurance.[2] Medication data covered the calendar year 2016. Persons were included if they were registered as living in the Netherlands on 01-01-2015 and had been living at the same home address for at least two years prior to that date. This and several other selection criteria are listed in Table S1, together with the excluded number of persons. Persons with data missing in either the address locations dataset or individual-level variables were excluded. Also, persons were excluded who lived in districts[3] that included houses within 2 km of the border with Belgium or Germany, as emissions of and distances to foreign livestock farms could not be accounted for in the analysis. Persons living in residential care homes were excluded, because of uncertainty whether their medication use is always registered through insurance. Two criteria were defined to exclude persons likely to be occupationally exposed to livestock: persons that lived at the same address as a farm registered in the farm location data and those that were registered as working in the livestock sector.[4] Lastly, persons living in urban agglomerations[5] were excluded.

### 2.2. Health outcome

The health outcome measure was the dispensing of drugs prescribed for obstructive airway diseases in 2016, indicated as a binary variable. Medication for obstructive airway diseases includes both inhalants and drugs for systemic use, which are indicated by an ATC-code (Anatomical Therapeutic Chemical classification: R03) in data collected by the administrative body responsible for Dutch health insurance (CVZ, Zorginstituut Nederland) for risk equalization among insurance companies. These data do not contain information about duration of use or dosage. The data include persons that are eligible to receive medication according to the standard health insurance policy, excluding medication dispensed during hospital admission and in nursing homes but including medication dispensed by outpatient pharmacies and in residential homes for the elderly. Children aged 0–5 years, those aged 6–17 and adults were distinguished, because diagnosis and treatment of asthma are generally different for children under 6 years of age compared to older children and adults, whereas older children will not be occupationally exposed and are less likely to smoke than adults.

### 2.3. Exposure indicators

Two proxies of environmental exposure related to livestock farms were used: one based on distances of homes to livestock farms in the Netherlands and one based on the modelled particulate matter concentration originating from livestock farms. Both proxies are conceptually related and provide different perspectives on possible exposure. Particulate matter concentration is not only an indicator of exposure to particulate matter but also to other farm-related emissions. Moreover, modelled concentrations, besides accounting for meteorological conditions, implicitly take into account the proximity of addresses to multiple farms of various sizes, whereas the distance-measure used only takes into account the proximity of the nearest farm. Both proxies should have expressed exposure in 2015, which would then indicate exposure of at least one year prior to medication use in 2016.

[1] The yearly produced concentration maps (such as $PM_{2.5}$ and $PM_{10}$) can be viewed and/or downloaded via links on https://www.rivm.nl/gcn-gdn-kaarten/concentratiekaarten.

[2] "Risicovereveningsbestanden van het College voor Zorgverzekeringen".

[3] Dutch: "wijk"; no administrative unit but statistical unit used by Statistics Netherlands.

[4] Economic activity data collected by Statistics Netherlands: SECSMBIBUS; company classification code (SBI-code): A 014.

[5] The mapping of Statistics Netherlands is used, which defines urban agglomerations as connected areas with urban buildings where most human activities take place, where most jobs are present and where most public facilities are located.

### 2.4. Distance to livestock farms

Exposure variables based on distances to farms were defined as the distance to the nearest livestock farm in meters, represented by fixed distance intervals (initially 0–500; 500–1000; 1000–1500; 1500–2000; and >2000 m). The distances between the residences and livestock farms, were calculated with ArcGIS (ESRI (Environmental Systems Research Institute), 2011), on the basis of locations of farms (landbouwtelling, Netherlands Enterprise Agency: RVO) and address locations (BAG) from 2015. Only farms with a minimum number of animals were taken into account, as in previous studies (Borlée et al., 2015; Smit et al., 2014) (Table S2), and a distinction was made between livestock farms of any type, as well as by type: cattle, pig, poultry, goat, sheep, and farms with any other animals.

### 2.5. Modelled particulate matter concentration

The exposure to livestock-related particulate matter up to 10 μm ($PM_{10}$) was calculated with the OPS (Operational Priority Substances) model (Sauter et al., 2018), which is an atmospheric transport and dispersion model for airborne pollutants. One of the applications of OPS, is the production of annual-averaged maps of concentration and deposition for the Netherlands at a 1 km by 1 km resolution for air quality monitoring purposes (e.g. (RIVM, 2016)) referred to as GCN and GDN maps (Largescale Concentration/Deposition maps of The Netherlands). The model uses Gaussian plumes to describe the relation between an individual source and an individual receptor. The contributions of the individual sources are summed to obtain the total concentration at a certain location or grid cell. It uses trajectories for long-range transport. The long-term version of the model is employed, which is statistical in the sense that calculations are performed for a number of typical meteorological situations (classes) occurring in, for example, a year. The sum of the values per class, weighted according to their relative frequency of occurrence, is the long-term value.

For this study, a resolution of 250 m by 250 m and meteorological conditions of 2015 were used. The $PM_{10}$ emission strengths of point sources of the various farm locations throughout the Netherlands were requested from the Pollutant Release and Transfer Register[6] for the year 2015. These emissions are calculated by multiplying the number of animals per location with animal-specific and housing type-specific emission factors (Vonk et al., 2016). Emissions from abroad were not included for this model exercise. We distinguished between $PM_{10}$ emissions from housing for goats, poultry, cattle, pigs, horses and ponies, donkeys, mink and rabbits; data for sheep were not available. The sum of the concentrations resulting from these emissions is referred to as "livestock-related $PM_{10}$ exposure". This sum does not include secondary inorganic aerosol, which can partly be attributed to ammonia emissions from livestock farms. However, it does not have a role in microbial or other exposures that are of interest in this study. For analyses of specific animal categories, only goats, poultry, cattle, pigs and the combined concentrations from other animals were distinguished.

To improve the dispersion modelling on the local scale, animal category-specific particle size distributions were implemented. These distributions differ from those implemented by default that are used to obtain the large-scale picture of the air quality in the Netherlands. Specification of such distributions is important, as small and light particles are transported over longer distances than larger and heavier particles. The particle-size distributions were determined on the basis of measurement data of Lai et al. (2014) and Winkel et al. (2015) (see Supplementary Methods).

Exposure to $PM_{10}$ from sources other than Dutch livestock farms as well as to secondary inorganic aerosols was determined by subtracting $PM_{10}$ concentrations originating from livestock farms from the total $PM_{10}$ concentration from all sources. These data were retrieved from the standard available annual GCN map for 2015, with meteorological conditions of 2015, emissions of 2014 and a grid, 1 km by 1 km, which includes emissions from all sectors within the Netherlands and abroad, including aggregated emissions from agriculture per country (RIVM, 2016). A comparison of these livestock-related $PM_{10}$ concentrations from GCN maps and the calculations performed for this study showed only small differences, due to differences in resolution of both model grid and emission sources and year of emission data. Further, the subtraction assures that non-livestock $PM_{10}$ concentrations exclude livestock-related emissions that do not originate from housing (e.g., supply of concentrates to farms). The nitrogen dioxide ($NO_2$) concentration, as another important source of air pollution was directly obtained from the standard available GCN maps.

### 2.6. Confounding variables

We included individual covariates relevant for studies on respiratory health because they are potential determinants and are available for analysis within a secure environment provided by Statistics Netherlands. Only age, sex, marital status, migration background and household income fulfilled these requirements. Data on sex, age, marital status and migration background originate from registry data (Basisregistratie Personen), and data on household income were compiled by Statistics Netherlands based on information from tax authorities and other sources. In addition, an indicator for neighbourhood socioeconomic status (SES) in 2016, which is derived every 4 years by the Netherlands Institute for Social Research (Knol, 1998), was used. This indicator is constructed on the four-digit postal code level, with each postal code area comprising on average about 4000 inhabitants. It is based on a principal-component analysis of the income level, unemployment rate and education level of the inhabitants of the postal-code area, which is rescaled to 5 categories, with 1 indicating highest and 5 indicating lowest SES.

### 2.7. Data privacy regime

Data were analysed within the secure environment provided by Statistics Netherlands, where researchers had access to information on the individual level but not to directly identifiable information such as address locations. No data used outside this secure environment contained information with which individuals could be identified. Exposure variables were calculated outside the secure environment and linked to a general address code (BAG), which was then re-coded by Statistics Netherlands and linked to the health outcome and associated demographic and socioeconomic variables under study.

### 2.8. Analyses

Logistic regression analyses were conducted separately for 14 regions with different agricultural characteristics as defined by Statistics Netherlands (Dutch: groepen van landbouwgebieden, Fig. 1). Such separate analyses were performed because of computational limitations that hampered the joint analysis of 7.7 million individuals. Separate analyses were performed for each distance-exposure variable and $PM_{10}$-exposure variable, and results were combined across the 14 regions in meta-analyses. Analyses were conducted separately for children and adults with three different levels of adjustment: personal-level adjustment models included, in addition to the exposure variable, sex, age, marital status (not for children), migration background and household income; fully adjusted models included, in addition to these variables,

[6] www.emissieregistratie.nl. (Pollutant Release and Transfer Register). The Pollutant Release and Transfer Register is responsible for collecting, processing, managing, registering and reporting emission data, so that the Netherlands can meet (inter)national obligations in the field of emission reporting. Emission registration is a cooperative program between various parties; the management and control of emission registration is the responsibility of RIVM.

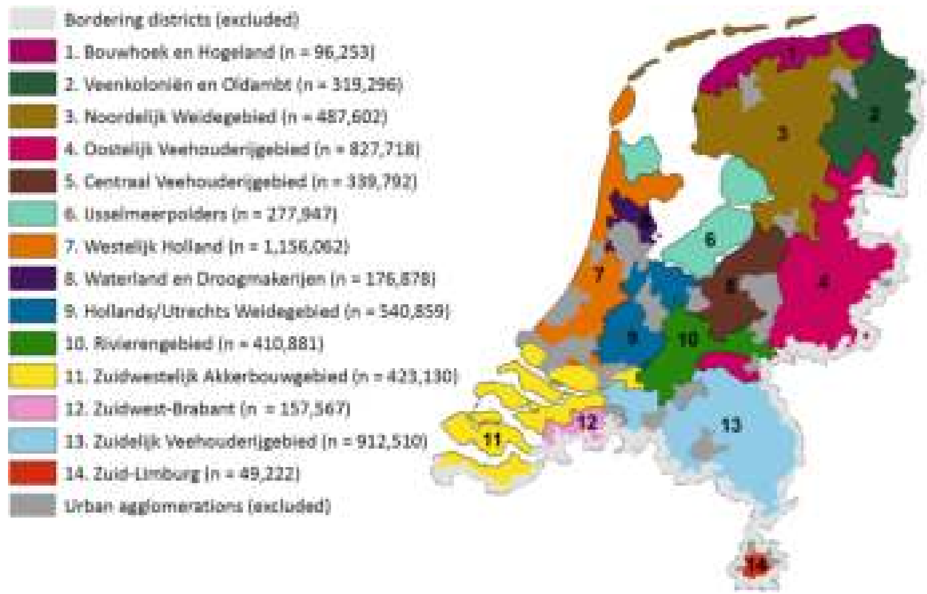


**Fig. 1.** Specific regions in the Netherlands are shown. The numbers refer to those regions as noted in the manuscript, their Dutch names and number of adults living in the region within brackets. Dark grey indicates the urban agglomerations; light grey indicates the areas close to the border from which inhabitants were excluded.

SES of the postal code area and exposure to non-livestock $PM_{10}$ and $NO_2$; mutually adjusted models included all animal categories simultaneously, as well as all other covariables. Distance-exposure and livestock-related $PM_{10}$-exposure variables were not adjusted for each other as this may lead to over-adjustment. Logistic regression analyses were performed with the glm function of the stats package in R (R Core Team). For $PM_{10}$-exposure analyses, random effects meta-analyses were performed with the metafor package in R (Viechtbauer, 2010). For distance-based exposure analyses, multivariate random effects meta-analyses were performed by including covariance matrices of the distance-categories in the mvmeta package in R (Gasparrini, 2018) to account for the covariance of distance categories. Heterogeneity between regions was assessed with the $I^2$-statistic.

Models based on distance-based exposure variables, included distance intervals of 500 m (0–500; 500–1000; 1000–1500; 1500–2000 and > 2000 m). For the exposure variables “distance to nearest livestock farm” and “distance to nearest cattle farm”, the interval “1500–2000 m” was not included because of the low number of persons living further than 2000 m from cattle farms in some regions; hence, the largest distance category for these variables was “>1500 m”. For the mutually adjusted model based on distance variables, the distance intervals were refined by first making a model including distance intervals of 500 m. Because with this model most effects were observed within 1000 m, a new model was made including distance intervals of 250 m (0–250; 250–500; 500–750; 750–1000 and > 1000 m). When high variance within a distance interval or little difference between adjacent distance intervals was observed, distance intervals were merged, keeping a minimum of three distance intervals per animal category.

### 2.9. Sensitivity analyses

Several types of sensitivity analyses concerning the analytical model, health outcome, exposure and selection of the study population were performed. Sensitivity analyses were compared to fully adjusted analyses. Sensitivity to the analytical model was studied by running multilevel analyses in which the district (Dutch: “wijk”[3]) was included as random effect as a proxy to adjust for potential differences in medication prescription practices between general practitioners and for differences between districts that could not be explained by the other covariables (van de Kassteele et al., 2017). The GLIMMIX procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used for these multilevel analyses.

The health outcome in the main analyses concerns the prevalence of medication dispensing in 2016, thus assuming a consistent relation between exposure and health outcome over time. To distinguish new prescriptions from such a prevalence measure, incidence measures of medication dispensing were defined with a run-in time of either 2 or 5 years, thus excluding all persons who received medication in 2014 and 2015 or in 2011–2015. A further refinement in health outcome to help distinguishing asthma and COPD is an analysis of a subset of adults younger than 40 years who are unlikely to have COPD.

Sensitivity to the application of different exposure measures was performed by use of a different source of farm location data provided by the Pollutant Release and Transfer Register[6]. Sensitivity to different selection criteria was assessed by performing additional analyses in which persons that moved in the past two years, those that lived close to the border, those that were assumed to be living or working on a farm, or those that lived in urban agglomerations were added to the study population. For the last selection criterion, persons living in urban agglomerations were not included in analyses of the 14 regions, but logistic regression analyses were performed for the entire population in urban agglomerations, the results of which were included as a 15th region in meta-analyses.

### 2.10. Population attributable fractions

Where the odds ratios calculated from logistic regression analyses provided an indication of risk, the population attributable fractions (PAF) provided an indication of the *impact* of a risk factor on the total population; the PAFs took into account both the relative risk of an exposure and the number of persons exposed. Since the number of persons and effect sizes varied by region, the PAFs better reflected the overall impact than the odds ratios (ORs) from meta-analyses. PAFs were calculated using the following equation:

$$PAF = (C_p - C_0) / C_p \quad (1)$$

Here $C_p$ is the predicted number of cases of medication reception in the region under the original data (population), and $C_0$ is the number of predicted cases based on the model coefficients under the counterfactual situation that no livestock farms were present, i.e., no exposure to livestock-related particulate matter and all distances to the nearest

**Table 1**
Association between medication dispenses and distance to nearest livestock farms, expressed as odds-ratios (95% confidence interval) from meta-analyses over the 14 regions, for different levels of adjustment.

| Adults (>17, n = 6,175,717) | Exposure§ | Personal-level adjusted‡ | Fully adjusted |
|---|---|---|---|
| Distance to nearest livestock farm (m) | | | |
| 0–500 | 29.2% (10–47%) | 0.91 (0.87–0.96)*** | 0.95 (0.91–0.99)** |
| 500–1000 | 37.6% (19–43%) | 0.96 (0.93–0.99)* | 0.98 (0.95–1.01) |
| 1000–1500 | 20.2% (10–29%) | 0.99 (0.97–1.00) | 0.99 (0.98–1.01) |
| >1500 | 13.0% (1–44%) | 1 | 1 |
| Distance to nearest cattle farm (m) | | | |
| 0–500 | 20.2% (4–37%) | 0.90 (0.85–0.95)*** | 0.94 (0.90–0.97)*** |
| 500–1000 | 32.9% (10–42%) | 0.95 (0.91–0.99)* | 0.97 (0.95–1.00) |
| 1000–1500 | 22.5% (12–30%) | 0.98 (0.95–1.01) | 0.99 (0.97–1.01) |
| >1500 | 24.4% (3–74%) | 1 | 1 |
| Distance to nearest pig farm (m) | | | |
| 0–500 | 3.4% (0–11%) | 0.93 (0.89–0.97)*** | 0.95 (0.92–0.98)** |
| 500–1000 | 10.4% (0–30%) | 0.96 (0.92–1.01) | 0.99 (0.96–1.02) |
| 1000–1500 | 13.3% (1–27%) | 0.97 (0.92–1.02) | 0.99 (0.98–1.01) |
| 1500–2000 | 12.0% (1–20%) | 0.98 (0.96–1.00) | 0.99 (0.97–1.01) |
| >2000 | 61.1% (16–98%) | 1 | 1 |
| Distance to nearest poultry farm (m)± | | | |
| 0–500 | 1.5% (0–6%) | 0.91 (0.87–0.95)*** | 0.92 (0.89–0.95)*** |
| 500–1000 | 5.7% (0–21%) | 0.94 (0.90–0.99)* | 0.97 (0.94–1.00)* |
| 1000–1500 | 8.7% (0–26%) | 0.98 (0.93–1.02) | 1.00 (0.97–1.03) |
| 1500–2000 | 9.4% (0–21%) | 0.99 (0.95–1.03) | 1.00 (0.98–1.03) |
| >2000 | 74.6% (27–100%) | 1 | 1 |
| Distance to nearest goat farm (m) | | | |
| 0–500 | 0.4% (0–1%) | 0.88 (0.79–0.98)* | 0.91 (0.85–0.99)* |
| 500–1000 | 1.6% (0–4%) | 0.93 (0.88–0.99)* | 0.95 (0.90–1.00)* |
| 1000–1500 | 3.4% (0–8%) | 0.95 (0.90–1.00) | 0.97 (0.92–1.02) |
| 1500–2000 | 4.9% (1–15%) | 0.94 (0.87–1.01) | 0.97 (0.93–1.01) |
| >2000 | 89.8% (73–99%) | 1 | 1 |
| Distance to nearest sheep farm (m) | | | |
| 0–500 | 8.0% (1–22%) | 0.95 (0.89–1.01) | 0.98 (0.92–1.04) |
| 500–1000 | 15.8% (7–34%) | 0.97 (0.93–1.02) | 0.98 (0.93–1.03) |
| 1000–1500 | 18.5% (10–28%) | 0.97 (0.94–1.01) | 0.98 (0.94–1.01) |
| 1500–2000 | 17.3% (12–28%) | 0.99 (0.97–1.01) | 0.99 (0.97–1.01) |
| >2000 | 40.3% (9–64%) | 1 | 1 |
| Distance to nearest farm with other animals (m) | | | |
| 0–500 | 8.1% (2–13%) | 0.95 (0.92–0.97)*** | 0.96 (0.94–0.99)*** |
| 500–1000 | 21.5% (7–29%) | 0.97 (0.94–0.99)* | 0.98 (0.96–1.00)* |
| 1000–1500 | 24.2% (12–29%) | 0.99 (0.96–1.02) | 0.99 (0.97–1.01) |
| 1500–2000 | 20.2% (14–34%) | 1.00 (0.97–1.02) | 0.99 (0.97–1.01) |
| >2000 | 25.9% (4–66%) | 1 | 1 |

*p < 0.05, **p < 0.01, ***p < 0.001.
§Exposure: mean percentage of persons living in distance-interval, with lowest and highest exposure over the regions in brackets.
‡Personal-level adjusted: adjusted for age, sex, marital status, household income and migration background.
¶Fully adjusted: adjusted for the same variables as in the personal-level adjusted models as well as for SES-category, $NO_2$ exposure and exposure to non-livestock-related particulate matter.
±Left out region 8 because of insufficient exposure.

livestock farm in the highest distance category.

Confidence intervals were obtained by simulating 1000 Monte Carlo estimates per region, with a different set of model coefficients for each simulation based on the variance-covariance matrix of the logistic regression model, with use of the mvrnorm function in R from the MASS package (Venables and Ripley, 2002). From these 1000 estimates, 1000 different numbers of predicted cases per region were calculated. The total number of predicted cases was determined by summing the predicted cases over the regions and over age groups. From these 1000 different numbers of predicted cases, PAFs were calculated by equation (1). The 95% confidence interval (CI) is assumed to be the range between the 2.5 and 97.5 percentiles of the 1000 PAFs.

We calculated PAFs for two different sets of models; one set included the distances to nearest farms in six animal categories, and the other set included exposure to animal-type specific particulate matter. Each of the two PAFs was based on summing the number of predicted cases from 42 models for three age groups and 14 regions.

## 3. Results

### 3.1. Characteristics of the study population

In total 7,735,491 persons (6,175,717 adults; 1,228,242 children between 6 and 17 years old; 331,532 children under 6) were included; 8,934,509 persons that did not fit the selection criteria were excluded (Table S1). In 2016, 608,173 adults (9.8%), 72,044 children between 6 and 17 (5.9%) and 25,727 children under age 6 (7.8%) received R03 medication (Table S3). In total, 29.2% of the included adults lived within 500 m from any livestock farm, with percentages ranging from 10% to 47% over the regions (Table 1). The median concentration of livestock-related $PM_{10}$ for adults was 0.16 $\mu g/m^3$ (10th-90th percentile: 0.04–0.54 $\mu g/m^3$; Fig. 2), similar to that for children. This particulate matter mostly originated from poultry farms (median: 0.13 $\mu g/m^3$; 10th-90th percentile: 0.03–0.41 $\mu g/m^3$). Concentrations of particulate matter from other types of farms were much lower, with a median for pig farms of 0.019 $\mu g/m^3$ (10th-90th percentile: 0.005–0.10 $\mu g/m^3$), for cattle 0.01 $\mu g/m^3$ (10th-90th percentile: 0.005–0.02) and concentrations for goats lower than 0.002 in 99% of cases. Personal characteristics of the study population can be found in Table S3.

### 3.2. Association with distance variables

Proximity to livestock farms was associated with lower R03 medication dispensing in 2016 for adults in personal-level adjusted and fully adjusted analyses, with a pooled odds ratio (OR) of 0.95 (95% confidence interval [CI]: 0.91–0.99) for living within 500 m of a livestock farm, compared to living further than 1500 m away (Table 1). Such an association was also found for children between ages 6 and 17 years, but no significant association was found for younger children (Table S4).

Also, proximity to the nearest cattle, poultry, pig, or goat farm or farm with other animals (except sheep) was significantly associated with lower R03 medication dispensing in meta-analyses among adults (Table 1). Yet, after mutually adjusting for different animal categories, associations remained significant only for cattle and poultry (Table 2). For both cattle and poultry farms, lower R03 medication dispensing with decreasing distance was apparent in multiple regions across the Netherlands (Fig. 3; Fig. S2). Such negative associations were also found in several regions for other animal categories, yet none of these associations remained statistically significant in the meta-analyses (Fig. S3-6). Significantly positive associations were found for distances to the nearest sheep farm and nearest pig farm for some regions, but in other regions significantly negative associations were found (Fig. S3,S5).

The value of $I^2$ for meta-analyses of mutually adjusted regression outcomes was 67%–94% across animal categories, indicating considerable heterogeneity, which may be driven by the small confidence intervals for some regions. Importantly, regions with fewer than 20% of persons living within 500 m of a cattle farm (2,6,7,8,11,12) tend to have the lowest ORs for small distances (Fig. 3).

In children, significantly negative associations with proximity to cattle farms were found in meta-analyses of mutually adjusted models, as well (Table S5). Meta-analyses for children also showed decreasing medication dispensing with decreasing distance in several other animal categories, but this was not consistently significant across age groups and analysis types (Table S4-5). Proximity of young children (0–5) to sheep appeared positively associated with medication dispensing, yet significant only when mutually adjusted for proximity to other animals (Table S4-5).

### 3.3. Association with particulate matter exposure

A weak non-significant negative association was found between receipt of R03 medication in 2016 and exposure to $PM_{10}$ from all livestock farms combined (Table 3). Exposure to $PM_{10}$ from cattle farms was significantly negatively associated with receipt of R03 medication in multiple regions across the Netherlands and in a meta-analysis (OR from meta-analysis of mutually adjusted models: 0.92; 95% CI: 0.86–0.97; $I^2$ = 97%; Table 3; Fig. 4). The $I^2$ value indicates considerable heterogeneity across regions, as seen in Fig. 4. In regions with relatively low average cattle-$PM_{10}$ concentrations (2,6,7,11, 12, 14), estimates were generally lower than in regions with higher concentrations (Figs. 2 and 4). For poultry, in some regions significantly negative associations were found as well, but meta-analyses results were not significant (Fig. S6). For pigs and goats, both significantly negative and positive associations were found in individual regions (Fig. S8,9), with positive but not significant associations from meta-analyses for both children and adults (Table 3, Table S6). $PM_{10}$ from sources other than livestock farms was positively associated with receipt of R03 medication in several regions and in a meta-analysis, but only for adults (Table 3, Table S6; Fig. S10). Calculated heterogeneity across regions was considerable, ranging from 52% to 97% across animal categories.

### 3.4. Sensitivity analyses

#### 3.4.1. Sensitivity to model formulation

Inclusion of district as a random effect in multilevel models gave similar results as the single-level model, although coefficients varied between the models (Table S7,8).

#### 3.4.2. Sensitivity to health outcome

Results of inclusion of a run-in time of either two or five years were similar to results of no inclusion of a run-in time. However, somewhat larger effect sizes were found for the distance to the nearest pig farm, whereas the association with pig-related particulate matter turned significantly negative (Table S9,11). The number of cases included for a run-in time of two years was 116,449 (21 per 1000) and for five years 83,952 (16 per 1000). Only inclusion of adults less than 40 years of age or older persons had a limited effect on the estimates, yet for adults less than 40, the positive association between medication dispensing and particulate matter from non-livestock sources was not significant (Table S10,11).

#### 3.4.3. Sensitivity to exposure variables and selection criteria

Results were hardly sensitive to the use of different farm location data or different selection criteria: not excluding persons that moved in 2014 or 2015 or those living close to the border, excluding persons living in a district within five km from the border, or including those that were expected to live on a farm (Table S12,13). Including urban agglomerations as a 15th region also had little effect on meta-analysis

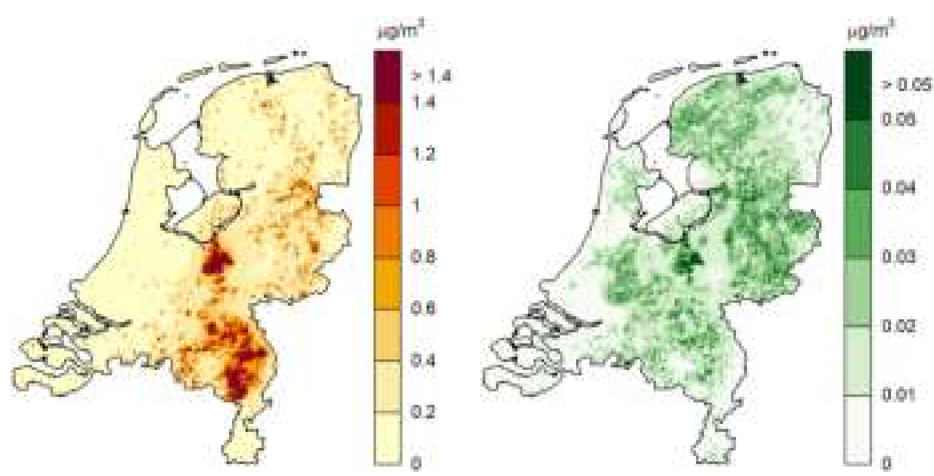


**Fig. 2.** Modelled particulate matter concentration from all livestock farms (left) and cattle farms (right). The pattern of total livestock-related particulate matter concentrations is driven by emissions from poultry farms (Fig. S1A). Fig. S1 also shows modelled particulate matter concentrations from pig, goat, and other livestock farms.

**Table 2**
Association between medication dispenses and distance to nearest livestock farms, expressed as odds-ratios (95% confidence interval) from a meta-analysis over the 14 regions of models in which exposures related to different animal categories were mutually adjusted for each other.

| Adults (>17 years of age, n = 6,175,717) | Mutually adjusted§ |
|---|---|
| Distance to nearest cattle farm (m) | |
| 0–250 | 0.93 (0.91–0.95)*** |
| 250–500 | 0.96 (0.94–0.97)*** |
| 500–750 | 0.97 (0.96–0.99)** |
| 750–1000 | 0.99 (0.97–1.01) |
| >1000 | 1 |
| Distance to nearest pig farm (m) | |
| 0–500 | 0.99 (0.96–1.02) |
| 500–1000 | 1.03 (0.99–1.06) |
| >1000 | 1 |
| Distance to nearest poultry farm (m)± | |
| 0–250 | 0.89 (0.82–0.97)** |
| 250–500 | 0.96 (0.92–0.99)* |
| 500–750 | 0.96 (0.92–1.00)* |
| 750–1000 | 0.98 (0.96–1.01) |
| >1000 | 1 |
| Distance to nearest goat farm (m) | |
| 0–500 | 0.95 (0.89–1.00) |
| 500–1000 | 0.98 (0.95–1.01) |
| >1000 | 1 |
| Distance to nearest sheep farm (m) | |
| 0–500 | 1.01 (0.98–1.05) |
| 500–1000 | 1.00 (0.98–1.03) |
| >1000 | 1 |
| Distance to nearest farm with other animals (m) | |
| 0–250 | 0.98 (0.96–1.01) |
| 250–500 | 0.99 (0.97–1.00) |
| 500–1000 | 0.99 (0.98–1.01) |
| >1000 | 1 |

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
§Mutually adjusted: adjusted for age, sex, marital status, household income, migration background, SES-category, $NO_2$ exposure, exposure to non-livestock-related particulate matter and for exposure to particulate matter from other animals; all values in this table are the results of the same model.
±Left out region 8 because of insufficient exposure.

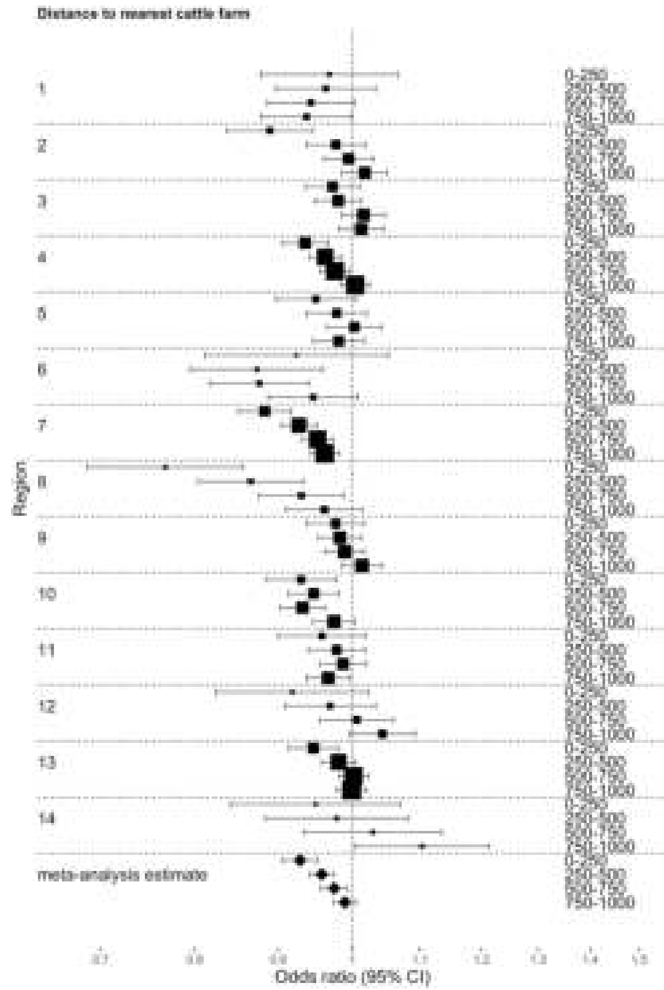


**Fig. 3.** Forest plot for odds ratios of medication dispensing against distance to nearest cattle farm for mutually adjusted models for adults (adjusted for age, sex, marital status, household income, migration background, socio-economic status, exposure to nitrogen dioxide, distance to nearest poultry farm, distance to nearest pig farm, distance to nearest goat farm, distance to nearest sheep farm, distance to nearest farm with other animals, and exposure to non-livestock-related particulate matter). Regions refer to the regions in Fig. 1, the reference category for all regions is > 1000 m. Meta-analysis summary results can also be found in Table 2.

estimates, but the estimates with pig-related particulate matter turned from non-significantly positive to non-significantly negative when agglomerations were included. Furthermore, associations within urban agglomerations were significantly positive for distance to the nearest sheep farm and for goat-related particulate matter in mutually adjusted analyses (not shown).

### 3.5. Population attributable fractions

The predicted fraction of persons (adults and children) that receives R03 medication attributable to the presence of livestock farms was −0.016 (95% confidence Interval, CI: -0.013 to −0.019) for a model with distance-based measures and −0.052 (95% CI: -0.045 to −0.059) for a model based on $PM_{10}$-exposure measures. Hence, on the basis of these models, the number of persons in the study-population receiving R03 medication could increase from 1.6% to 5.2% when no livestock farms were present. Cattle-related exposure contributed most to both estimates, because of both the strength of the associations and the number of persons living close to cattle farms (Fig. 5). The population attributable fraction was positive, assuming no exposure from proximity to sheep farms or from pig-related particulate matter.

## 4. Discussion

This study shows that environmental exposure to livestock farms is negatively associated with medication dispensing for chronic obstructive airway diseases; persons living close to livestock farms and those with higher modelled exposure to $PM_{10}$ from livestock farms receive less medication than persons living further away and those with lower PM levels. A positive association between medication dispensing and exposure to non-livestock $PM_{10}$ (including secondary inorganic aerosols) was found. The protective association seems most evident for environmental exposure related to cattle farms, for which associations with both distance and $PM_{10}$ remained significant after mutually adjusting for exposure related to other animal categories; such protective association was found to a lesser extent for poultry farms, for which associations were most clear for the distance variables. Results differed per region, but for most regions negative associations were found for distance-based and particulate-matter-based exposure variables and for exposure related to cattle and poultry farms. Such consistency across regions shows that associations are not limited to a previously studied area in the southeast of the Netherlands. Results were only slightly sensitive for the model formulation or use of different selection criteria.

While effect sizes are relatively small, about two-thirds of the Dutch rural population lives within 1 km of a livestock farm (55% including urban agglomerates). Hence, with the assumption that the observed associations are causal, 2%–5% more persons living in rural areas might receive medication for obstructive airway diseases if no livestock farms

**Table 3**
Association between medication dispenses and livestock-related $PM_{10}$-exposure, expressed as odds ratios (95% confidence interval) from meta-analyses over the 14 regions, for different levels of adjustment.

| Adults (>17, n = 6,175,717) | Personal-level adjusted‡ | Fully adjusted | Mutually adjusted§ |
|---|---|---|---|
| Livestock-related $PM_{10}$# | 0.98 (0.95–1.02) | 0.97 (0.94–1.00) | n.a. |
| Non-livestock $PM_{10}$#± | 1.27 (1.08–1.49)** | 1.27 (1.07–1.51)** | 1.29 (1.09–1.52)** |
| Cattle-related $PM_{10}$# | 0.92 (0.88–0.96)*** | 0.92 (0.87–0.97)** | 0.92 (0.86–0.97)** |
| Pig-related $PM_{10}$# | 1.40 (0.79–2.47) | 1.00 (0.96–1.05) | 1.06 (0.99–1.13) |
| Poultry-related $PM_{10}$# | 0.99 (0.96–1.01) | 0.98 (0.95–1.00) | 0.99 (0.96–1.02) |
| Goat-related $PM_{10}$# | 1.00 (0.99–1.01) | 1.00 (0.99–1.00) | 1.00 (1.00–1.01) |
| Other animal-related $PM_{10}$# | 0.99 (0.98–1.00) | 0.99 (0.98–1.00) | 1.00 (0.99–1.00) |

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
# per 10–90 percentile increase in exposure.
± in fully-adjusted model, adjusted for livestock-related $PM_{10}$; in Mutually adjusted model adjusted for $PM_{10}$ from animal categories. The estimates correspond to an OR of 1.06 (1.02–1.10) per 1 $\mu g/m^3$ increase.
‡Personal-level adjusted: adjusted for age, sex, marital status, household income and migration background.
¶Fully adjusted: adjusted for the same variables as in the personal-level adjusted models as well as for SES-category, $NO_2$ exposure and exposure to non-livestock-related particulate matter.
§Mutually adjusted: adjusted for the same variables as in the fully adjusted models as well as for exposure to particulate matter from other animals; all values in this column are the results of the same model.

were present in the Netherlands, which equates to several tens of thousands of persons. The lower bound of this estimate is based on models including the distances to the nearest livestock farms of several types while the upper bound of the estimate is based on models including livestock-related particulate matter. The difference between these estimates may be explained by the inability of the distance to the nearest farm to take into account combined effects of proximity to multiple farms and characteristics such as farm size, which are implicitly accounted for in the modelled livestock-related particulate matter concentrations. The PAFs are in the same order of magnitude as what could be inferred from the odds ratios for asthma and COPD in relation to persons living within 500 m of a livestock farm from the study by Smit et al. (2014). The PAFs that can be calculated from these odds ratios are about −0.053 for COPD and −0.016 for asthma (based on exposure of the entire Dutch population), with a factor 10 uncertainty around these estimates, depending on the assumptions (Post et al., 2020).

Some PAFs appeared to deviate from zero, even though the corresponding null-exposure variables did not show significant associations in meta-analyses. This difference can be explained by a difference in weights of regions between meta-analyses, in which weights are based on standard errors of the estimates, and PAF calculation, in which weights are based on the number of inhabitants of the region. The positive PAFs for pig-related particulate matter and distance to nearest sheep farm and the negative PAFs for distance to nearest pig farm, distance to nearest farm with other animals, and other animal-related particulate matter should thus be interpreted with caution. The inconsistent results between PAFs and meta-analysis results for these animals make such results less strong than results for cattle and poultry farms, for which results are more consistent.

In the present study, no distinction could be made between medication dispensing for asthma and that for COPD. Yet, COPD is generally not diagnosed among persons less than 40 years old, and in adults less than 40 the association between medication dispensing and environmental exposure related to livestock farms was similar to the association among all adults. Moreover, since the associations similar to those in adults were found in children, they likely apply to asthma. Persons 40 years or older receiving R03 medication can have either asthma or COPD, yet analysis in this group showed similar effect sizes compared to analysis in adults younger than 40.

Associations with both proxies used in this study support previous findings in the Netherlands regarding an inverse association between asthma and COPD and proximity to livestock farms that were based on self-reported and general practitioner diagnoses of asthma and COPD

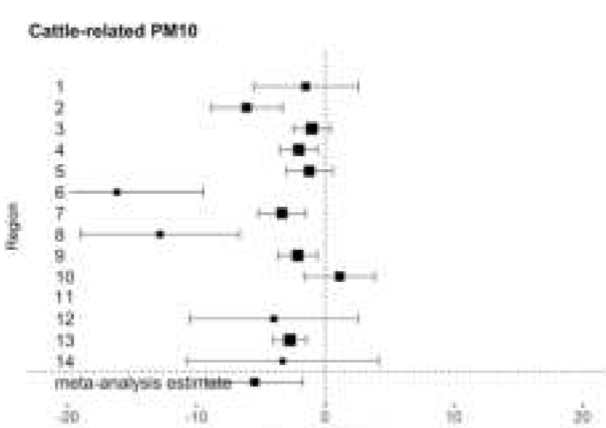


**Fig. 4.** Forest plot for mutually-adjusted models for the association between medication dispenses and cattle-related $PM_{10}$ exposure among adults (adjusted for age, sex, marital status, household income, migration background, socio-economic status, exposure to nitrogen dioxide, exposure to poultry-related, pig-related, goat-related and other animal-related particulate matter, and exposure to non-livestock-related particulate matter). Regions refer to the regions in Fig. 1. Meta-analysis results can also be found in Table 3.

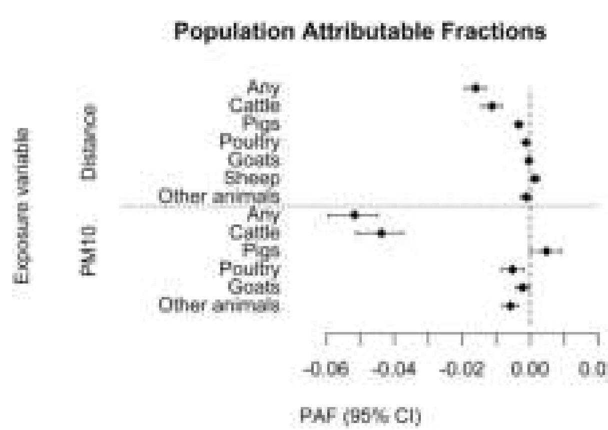


**Fig. 5.** Population attributable fractions (PAFs) for models with distance to nearest livestock farm as exposure and those with livestock-related $PM_{10}$ as exposure. The exposure variables refer to the counterfactuals in the two sets of 42 mutually adjusted models (14 regions, 3 age groups) from which the PAFs were estimated (mutually adjusted for exposure related to the animal categories, as well as for age, sex, marital status, household income, migration background, socio-economic status, exposure to nitrogen dioxide and exposure to non-livestock-related particulate matter).

(Borlée et al., 2015; de Rooij et al., 2019; Smit et al., 2014). The findings therefore support the hypothesis that the inverse association between asthma and residence in the vicinity of livestock farms is caused by more diverse microbial exposure leading to reduced allergic sensitization (Ege et al., 2011; Ehrenstein et al., 2000; von Mutius, 2016). This hygiene hypothesis is not a plausible explanation for an inverse association with COPD, which is currently supported only by previous research in the Netherlands; most studies among farmers show an increased risk of COPD (Fontana et al., 2017; Guillien et al., 2019). The inverse association between medication dispensing for asthma and COPD and livestock-related exposure is not likely explained by a difference between urban and rural areas, because analyses were performed within regions and persons living in urban agglomerations were excluded.

An alternative explanation for fewer occurrences of medication dispensing close to livestock farms are individual differences in healthcare seeking behaviour. In previous research, such healthcare seeking behaviour appeared lower among persons living close to livestock farms but was not affected by distance to general practitioners (van Dijk et al., 2016b). Regional differences in healthcare seeking behaviour are not likely to have driven inverse associations, because these were observed for multiple regions and remained when adjusted for differences at district level in multilevel analyses. Another alternative explanation for the associations is that persons that have asthma or COPD or that are sensitive to such diseases or parents of asthmatic patients may be more inclined to move away from farms. However, this explanation appears in contradiction to sensitivity analyses in which a run-in time was implemented. These analyses suggest that not only prevalent but also new cases of asthma and COPD are inversely associated with livestock-related exposure, which is unlikely if moving is the result of being diagnosed with asthma or COPD.

In contrast to the association with livestock-related particulate matter, the association of medication dispensing for asthma and COPD with non-livestock-related particulate matter was positive. This finding appears to align well with existing evidence of increased or worsening asthma and COPD among persons exposed to air pollution (Salvi and Barnes, 2009; Viegi et al., 2001; World Health Organization, 2013), yet epidemiological evidence for association of incidence and prevalence of asthma and COPD in adults with air pollution exposure is less strong (Atkinson et al., 2015; EPA, 2019; Gowers et al., 2012; Hendryx et al., 2019; Liu et al., 2017; Schikowski et al., 2014). A positive association is biologically plausible, as several mechanisms have been identified by which particulate matter may induce asthma or COPD (Gowers et al., 2012; Schikowski et al., 2014).

Only associations with cattle farms remained significant for both distance and $PM_{10}$-related variables after mutually adjusting for other animal categories. Cattle farms are the most widely distributed type of farms in the Netherlands, with more than 20% of the study population living within 500 m from such farms. Hence, more persons live close to only a cattle farm and no other farms than live close to other farms but not to a cattle farm. This distribution may have hindered finding associations with other animal categories in mutually adjusted analyses, but makes it unlikely that associations with cattle-specific exposure are affected by correlated proximity to other farms. A significant inverse association between both COPD and asthma and the presence of a cattle farm within 500 m from a home address was also found in a previous study in the Netherlands (Smit et al., 2014), yet in that study the association did not remain significant when adjusted for proximity to other categories of animal farms. Significantly negative associations with proximity to pigs and goats, but not poultry, were also found by Smit et al. (2014) and Borlée et al. (2015), yet they were not consistently significant across studies and asthma and COPD health outcomes. Internationally, in studies on asthma among persons growing up on farms, traditional farms with cattle have been suggested to be an important factor in the protective effect (Illi et al., 2012).

The associations for cattle-related exposures are not likely explained by the amount of $PM_{10}$ emitted from cattle farms, with the 10th-90th percentile interval for cattle-related $PM_{10}$ concentration for adults more than 20 times lower than that of poultry farms and more than 270 times lower than that of the non-livestock $PM_{10}$ concentration. These associations suggest that livestock-related $PM_{10}$ is a proxy that best aligns with an air transmission route. Exposure to air containing molds, bacteria and endotoxins may indeed be a likely route causing a variety of positive and negative respiratory conditions (May et al., 2012; von Mutius and Vercelli, 2010). Besides air exposure, important exposure factors for a protective effect for asthma are consumption of unprocessed milk and contact with straw and animals (Brooks et al., 2013; Wlasiuk and Vercelli, 2012). A possible correlation of such exposure through farm visits with proximity to livestock farms cannot be ruled out, yet most of the persons living on farms should have been excluded from the analyses.

Although the models in this study were adjusted for several covariables, some potentially important confounders were not included. Smoking, for example, is the primary risk factor for COPD (Kohansal et al., 2009; Viegi et al., 2001). We adjusted for several socio-economic factors known to correlate with such behaviour (van de Kassteele et al., 2017), yet we cannot be certain that some bias may be present in the results due to lack of adjustment for lifestyle factors. Small associations between lifestyle factors and general air pollution have previously been shown to influence risk estimates for mortality (Strak et al., 2017).

Other weaknesses of this study are the limited precision in both the health outcome and the exposure measures. The health outcome of medication dispensing for asthma and COPD can be regarded as a proxy for obstructive airway disease, but it may not be as accurate as information on prescriptions or use; it does not provide an indication about the severity of the disease because of lacking information on doses; and it does not provide sufficient information to distinguish between asthma and COPD. Lack of information on the severity of asthma and COPD hindered the investigation of exacerbations among persons with COPD, which were found to be increased among those living close to livestock farms (Borlée et al., 2015; van Dijk et al., 2016a).

Exposure misclassification may have occurred for various reasons. Such misclassification may be particularly large if exposures are not airborne, as airborne exposures aligns best with our exposure proxies. If airborne exposures have a role in the observed associations, misclassification of $PM_{10}$ exposure may still have occurred. Such misclassification may arise, for example, because of exposure at the home address only, since most persons are not at their home address all day. In addition, exposure was determined only on a grid, 250 m by 250 m. Further, assumptions in dispersion modelling may have led to some misclassification. For example, plume rise due to either heat content or momentum was not included, as no general information on source characteristics required for this plume rise is available. Including plume rise has a diluting effect near the source, hence its exclusion may lead to an overestimation of source-specific concentrations, especially close to farms. Among other characteristics such as emission height, standard animal housing characteristics as used for the GCN maps were applied because no detailed database on this exists. The different forms of potential exposure misclassification make it difficult to give an overall estimate about their possible impact. Effects of exposure misclassification on reduction of statistical power is probably not an important issue, given the large number of persons under study.

In conclusion, the results of this study show that medication dispensing for asthma or COPD decreases with decreasing distance to livestock farms or increasing particulate matter exposure from cattle farms, in particular, in multiple regions within the Netherlands. As medication dispensing is likely indicative of prevalence of asthma or COPD, the results suggest an inverse association between asthma or COPD and livestock exposures. On the assumption that this association is causal, the number of persons with asthma or COPD in rural areas might be up to 5% higher with no livestock-related exposure. This number is considerable in view of the more than 700,000 cases we included in this study and hence is a motivation for additional research regarding potential underlying mechanisms. Such research could focus on ruling out alternative explanations such as healthcare seeking behaviour or finding more evidence of the role of microbial exposures in the associations and how such exposure relates to farming practices.

## Declaration of competing interest

None.

## Acknowledgements

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2020.113651.

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# Revalue associations of short-term exposure to air pollution with respiratory hospital admissions in Lanzhou, China after the control and treatment of current pollution

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ABSTRACT

Significant progress has been made in air pollution control Lanzhou, China recently, however, there was only one study so far on the assessment on health gains from air quality improvement after adopting strict air pollution control measures. The present study aimed to estimate the short-term effects of six criteria air pollutants including $PM_{2.5}$, $PM_{10}$, $NO_2$, $SO_2$, CO and $O_3$ on respiratory admissions in Lanzhou, China, then compare the results of our study with those earlier studies conducted in Lanzhou before the implementation of air pollution control measures. Data on daily hospital admissions from the three largest hospitals in Lanzhou and daily air pollution concentration and meteorological variable were collected during a 4-year period (2014–2017). A generalized additive model; adjusted for long-term trend, seasonality, and other potential confounders was done to quantitatively assess the influences of air pollutants on daily respiratory admissions and analyze the influences of different seasons, sexes, and age groups. The most apparent effects for $PM_{2.5}$, $PM_{10}$, $SO_2$, CO and $O_3$ on respiratory hospitalizations were observed at lag6, and lag7, respectively, and a 10μg/$m_3$ increase in $PM_{2.5}$, $PM_{10}$, $SO_2$, CO and $O_3$ concentration were associated with 0.885% (95%CI: 0.414%~1.358%), 0.328% (95%CI: 0.145% ~0.511%), 3.005% (95%CI: 1.689%~4.339%), 3.199% (95%CI: 0.912%~5.537%) for CO, 0.733% (95%Cl: 0.263%~1.205%) increase in respiratory admission, respectively. No remarkable association was found between $NO_2$ and respiratory disease hospitalisation. Females and younger groups were more susceptible to air pollutant than males and elderly groups. Together, we demonstrated that the positive associations were more pronounced in the cold season than in the warm season. The findings in present study suggest that even in Lanzhou, where air quality has been improved dramatically, positive associations still exist between air pollution and daily number of total respiratory admission.

## 1. Introduction

With rapid urbanisation and industrialisation over the past four decades, China has experienced deteriorating air quality due to vehicle exhaust emissions and energy consumption. Moreover, air pollution has become one of the most serious environmental problems in urban areas. In recent years, several epidemiological studies have found positive associations between air pollution and the occurrence and exacerbation of respiratory diseases, including bronchiectasis (Raji et al., 2020), asthma (Fusco et al., 2001; Santus et al., 2013; Luo et al., 2018; Phosri et al., 2019; Chang et al., 2020), upper respiratory diseases (Santus et al., 2013; Chang et al., 2020), pneumonia (Wong et al., 1999; Fusco et al., 2001; Santus et al., 2013; Luo et al., 2018; Chang et al., 2020; Raji et al., 2020) and chronic obstructive pulmonary disease (COPD) (Wong et al., 1999; Fusco et al., 2001; Santus et al., 2013; Phosri et al., 2019; Chang et al., 2020; Raji et al., 2020). For example, in a study conducted in Ahvaz, Iran, from 2008 to 2018, a significant relationship was observed between exposure to air pollutants and a higher number of hospital admissions because of asthma, COPD and bronchiectasis (Raji et al., 2020). A study in Bangkok, Thailand, revealed that short-term exposure to particulate matter ≤10 μm ($PM_{10}$), nitrogen dioxide ($NO_2$) and carbon monoxide (CO) caused pneumonia, COPD and asthma that required hospitalisation. Moreover, sulphur dioxide ($SO_2$) and ozone ($O_3$) levels were found to be associated with hospital admission for COPD and

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asthma (Phosri et al., 2019). A recent study in Shenyang reported that increased daily concentrations of $PM_{10}$, $NO_2$ and CO were associated with the number of hospital admissions for acute respiratory diseases and exacerbation of obstructive lung diseases (Chang et al., 2020).

However, associations of ambient air pollution with morbidity outcomes have been extensively reported in western countries and the eastern and coastal economically developed cities in China, such as Shanghai, Guangzhou, Wuhan, Chengdu, Beijing, Taiyuan and Hefei (Chen et al., 2010; Zhang et al., 2014; Wang, 2017; Qiu et al., 2018; Ma et al., 2018; Luo et al., 2018; Xie et al., 2019). It is possible that the characteristics of outdoor air pollution (e.g., air pollution level and mixture, local terrain and emission sources) and the sociodemographic status of local residents (e.g., disease pattern, age structure and socio-economic characteristics) between these areas and Lanzhou may be different. For these reasons, the effect size of air pollution in other areas may not apply in Lanzhou. Moreover, the types of respiratory diseases that are likely to be affected by variations in the concentrations of air pollutants and seasonal periods in Lanzhou have not been identified.

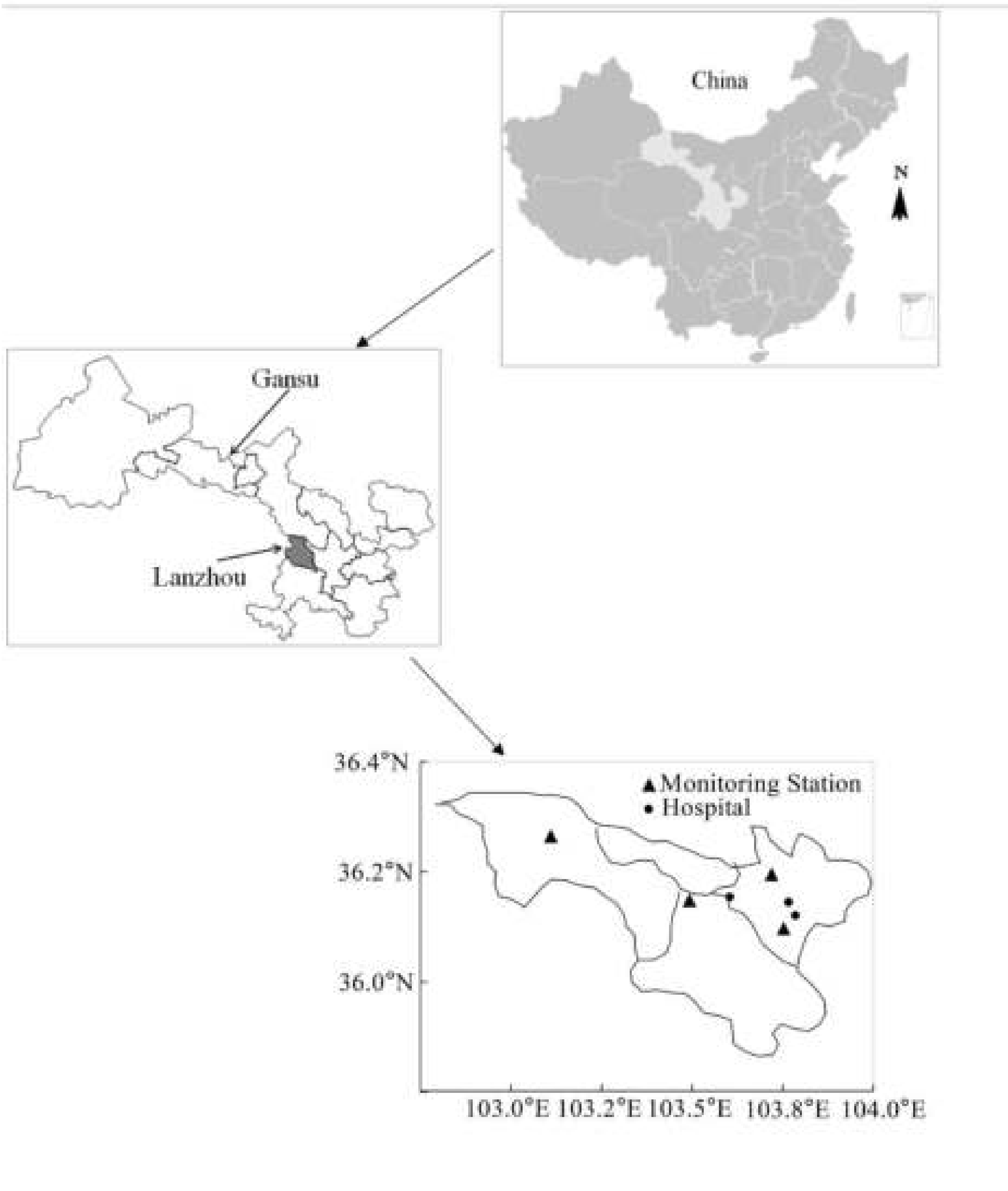


**Fig. 1.** The locations of air pollutants monitoring stations and hospitals.

Thus, epidemiological evidence on the association between air pollution and its health effects in Lanzhou needs to be added to the scientific literature. Several studies have been published on air pollution in Lanzhou, and two of them used data collected from 2001 to 2005 and 2007–2009 that linked air pollution to adverse population health in cities (Chu et al., 2008; Yu et al., 2011; Tao et al., 2013, 2014). However, those studies did not assess the lag–-response relationship between daily respiratory hospital admissions and air pollutants as well as the adverse health effects of exposure to PM $\leq$ 2.5 μm ($PM_{2.5}$), CO and $O_3$8h or during seasonal variations.

Lanzhou, located at 36°02N and 103°48E, is the capital of Gansu Province in China and has experienced severe atmospheric pollution. This phenomenon is caused by large-scale emissions from the petrochemical industry and valley basin, which is conducive to formation of stagnant meteorological conditions. Some interventions, such as mountain-slope afforestation and local terrain modification, have disrupted the long-standing boundary-layer temperature inversion, but there was only minimal improvement in air quality (Chu et al., 2008; Yu et al., 2011). With the implementation of the Air Pollution Prevention Battle in 2012 and the Plan for the Control of Air Pollution in 2015, the air quality in Lanzhou significantly improved and the phenomenon of Lanzhou Blue (graphical abstract) frequently appeared. This city won the Award for Today's Transformative Step in the 2015 World Climate Conference at Paris.

To date, only a few studies have examined the association between air pollution and respiratory hospital admissions in Lanzhou since 2013. The health effects of air pollutants (i.e. $PM_{2.5}$, $O_3$8h and CO) on respiratory morbidity also have not yet been investigated. Thus, the study aim was to conduct a time series study to investigate the association between six ambient air pollutants ($PM_{2.5}$, $PM_{10}$, $SO_2$, $NO_2$, O3-8h and CO) and respiratory hospital admissions in Lanzhou, China. We further examined the change of air pollutants in Lanzhou (2001–2017) and evaluated the change in morbidity risks due to air pollutants during three periods of 2001–2005, 2007–2009 and 2014–2017. Finally, the effects of modifying air pollutants were investigated via a subtype analysis according to sex, age, season and disease type.

## 2. Materials and methods

### 2.1. Study area and data collection

This time-series study was conducted in the urban districts in Lanzhou, the capital of northwestern Gansu province in China (Fig. 1). Lanzhou covers a land area of 13,100 $km^2$, with an urban area of 321.75 $km^2$ and total population of 304 million. Lanzhou is a temperate semi-arid continental climate with four distinct seasons: a dry spring, a hot sunny summer, a cool autumn and a cold winter. As a heavy chemical industry base, Lanzhou has suffered from serious air pollution historically. However, with implementation of a number of stringent measures and actions against air pollution, air quality has improved considerably. With the passage of time, it is possible that the concentrations of air pollutants in Lanzhou have decreased significantly as a result of those actions over the past 5 years, and Lanzhou won the Prize for Today Change Progress award at the United Nations climate-change conference in Paris in 2015. This remarkable transformation enables us to investigate whether the adverse effects of air contaminants ($PM_{2.5}$, $PM_{10}$, $SO_2$, $NO_2$, CO, and $O_3$) on hospital admissions still exist under the condition of low concentrations of air pollutants in Lanzhou.

### 2.2. Data collection

Electronic medical records (admission date, diagnosis, diagnosis code, discharge date, departments, home address, sex and age) of hospital admissions because of respiratory diseases were obtained from three large general top-level hospitals between January 1, 2014 and December 31, 2017 from three hospitals in urban Lanzhou (Fig. 1). These hospitals are famous for their good medical treatment of respiratory diseases. More than 75% of local residents are willing to seek treatment in these hospitals. The respiratory inpatients' data were defined according to the 10th version of the International Classification of Diseases, with codes J00–J99. We further considered five specific or classified diseases: upper respiratory tract infection (URTI; ICD-10: J00-J06 and J30-J39), lower respiratory tract infection (LRTI; ICD-10: J20-J22 and J40-J47), chronic obstructive pulmonary disease (COPD, ICD-10: J40–J44), pneumonia (ICD-10: J12-J18) and asthma (ICD-10: J45-J46). In addition, hospital admissions for different sexes and different age groups (<65 years and ≥65 years) were also separately analysed.

Ambient air quality data for the study period were obtained from the website of China's National Urban Air Quality Real Time Publishing Platform (http://106.37.208.233:20035/). These data included $PM_{2.5}$, $PM_{10}$, $SO_2$, $NO_2$, CO and $O_3$. The daily concentration (24-h for $PM_{2.5}$, $PM_{10}$, $SO_2$, $NO_2$ and CO; 8- h for $O_3$) were averaged from four fixed-site monitors operated under the Lanzhou Ministry of Ecology and Environment, which are distributed in urban districts (Fig. 1). These stations are far away from major roads, industrial sources, buildings, and sources of pollution, which ensures that monitoring results reflect the general air pollution levels in the Lanzhou urban area. All measurements of atmospheric pollutants conformed with national standards (GB3095–2012). Meteorological information, including daily average temperature and humidity, were provided by the Lanzhou meteorological bureau.

### 2.3. Statistical analyses

Given that the daily hospital respiratory admissions data are generally considered rare events and have a Poisson distribution, the over-dispersed generalized additive model (GAM) based on a Poisson distribution with log-link function was used to estimate the associations between $PM_{2.5}$, $PM_{10}$, $SO_2$, $NO_2$, CO, and $O_3$8h and cause-specific respiratory hospital admissions. Because of the potential non-linear effects, penalised smoothing spline functions were used to eliminate seasonality and long-term trends in daily morbidity as well as temperature and relative humidity. We included day of the week and public holidays in the model as dummy variables. The degree of freedom (df) of each model variable was chosen on the basis of Akaike's information criterion, and referred to previous studies (Capraz et al., 2017; Wang, 2017; Phosri et al., 2019). A penalised smoothing spline of 7 df per year was used for the temporal trends. Considering the distributed and delayed effects of temperature, the moving average temperature of the current day ($Temp_0$) and the moving average of lag1 through 3 days of temperatures ($Temp_{1-3}$) were used in the model (Zhao et al., 2017, 2019a, 2019b; Liao et al., 2020). We used 3 df for the current day ($Temp_0$) and moving average of the previous 3 days ($Temp_{1-3}$). Because no evidence of lagged confounding by relative humidity has been shown in air pollution epidemiology, a df of 3 for the current day's relative humidity ($RH_0$) was incorporated into the models (Liao et al., 2020). Briefly, the core model formula can be defined as follows:

$$Log[E(Y_t)] = \alpha + \beta Z_t + S(time, df = 7\times \ no.of\ year) + S(Temp_0, df = 3) + S(Temp_{1-3},\ df = 3) + S(RH_0, df = 3) + dow + holiday$$

where $Y_t$ was the number of hospital admissions for respiratory diseases on day $t$, $a$ was the intercept, $\beta$ is the regression coefficient, $Z_t$ indicates the pollutant concentrations on day $t$, s () represents a spline smoothing function for nonlinear variables, df is the degree of freedom, time is the days of calendar time on day $t$ and *dow* and *holiday* are dummy variables that represent the day of week and a public holiday, respectively.

To examine the delayed effects of air pollutants, we further introduced various lag structures, including a single-day lag (from lag0 to lag7) and cumulative lags (from lag01 to lag07). Single lag and cumulative lag were defined according to the definitions in the relevant literature (Zhang et al., 2014; Capraz et al., 2017; Wang, 2017; Luo et al., 2018; Phosri et al., 2019).

We performed additional analyses as well. First, we performed separate analyses to examine the potential modifying effect by sex (male and female), age (<65 years, and ≥65years) and season (warm: April to September and cool: October to March). We further evaluated significant differences between effect estimates from stratified analyses by calculating the 95% confidence interval (CI) as

$$\left(\widehat{Q}_1 - \widehat{Q}_2\right) \pm 1.96\sqrt{\left(\widehat{SE}_1^2 + \widehat{SE}_2^2\right)}$$

where $\widehat{Q}_1$ and $\widehat{Q}_2$ are the estimates for two categories, and $\widehat{SE}_2^2$ are their standard errors (Phosri et al., 2019). Second, because a linearity assumption between air pollution levels and hospital respirator admissions may not be justified, we plotted the non-linear exposure–response (E–R) curves of air pollutant effects on respirator admissions by using a penalised spline function with 3 df. Third, sensitivity analyses were also performed to check the robustness of the results by: (a) fitting two-pollutant models and (b) modifying the df for temperature (6–10 df) in the penalised spline function of time.

All of the data analyses were performed in R Programming Language (version 3.4.4; R Core Team, 2017) using the "mgcv" packages. The results are presented as the excess risk (*ER*) and 95% CIs for daily respiratory hospital admissions, per 10 μg/$m^3$ increase in $PM_{2.5}$, $PM_{10}$, $SO_2$, $NO_2$ and $O_3$8h and per 1 mg/$m^3$ increase in CO.

## 3. Results

Table 1 shows the summary statistics of daily respiratory hospital admissions, air pollution concentrations, and meteorological factors in Lanzhou from 2014 to 2017. In total, 39,505 hospital admissions because of respiratory diseases were recorded, with an average of 27.3 inpatients per day. The daily average numbers of respiratory admissions because of URTI, COPD, pneumonia and asthma were 6.7 ± 4.9, 9.2 ± 6.2, 5.8 ± 4.1, 5.5 ± 3.5 and 2.2 ± 1.5, respectively. The admissions were commonly attributed to respiratory infections, such as URTI and LRTI. The subgroups of respiratory diseases in this study were different from those in previous studies conducted in Lanzhou (Tao et al., 2013, 2014). In this study, LRTI is the most common cause of daily respiratory hospital admissions. The proportion of male participants who were admitted because of respiratory conditions was higher than that of the female participants. The sex ratio was 1.44:1 (23,298:16,206). Approximately 58.5% of individuals admitted because of respiratory conditions were ≥65 years old. The daily mean concentrations of $PM_{2.5}$, $PM_{10}$, $SO_2$, $NO_2$, $O_3$8h and CO were 53.3, 122.9, 22.4, 45.9, 84.0 μg/$m^3$ and 1.3 mg/$m^3$, respectively. During the study period, the daily mean temperature and relative humidity in Lanzhou were 11.3 °C and 50.8%, respectively.

Table 2 shows the Spearman correlation coefficient between air pollutants and meteorological variables. A moderate positive correlation was observed between $PM_{2.5}$, $PM_{10}$, $SO_2$, $NO_2$ and CO but not with $O_3$8h, with Spearman's correlation coefficients ranging from 0.41 to 0.81. However, a negative association was noted between these pollutants and daily mean temperature. Moreover, $O_3$8h was negatively correlated with $PM_{2.5}$ , $PM_{10}$, $SO_2$ and CO (Spearman correlation coefficients ranging from −0.48 to −0.13) and slightly correlated with relative humidity and moderately correlated with temperature ($r = 0.58$, $p < 0.05$).

Fig. 2 shows the variations in annual average concentrations of $PM_{10}$, $SO_2$ and $NO_2$ from 2001 to 2017 in Lanzhou and the decreasing trends of ambient $SO_2$ levels over time. Specifically, from 2001 to 2017, annual concentrations decreased for $SO_2$ by 75.9% to 20.3 μg/$m^3$ (corresponding to 63.7 μg/$m^3$ reduction) and for $PM_{10}$ by 44.8% (106.6 μg/$m^3$ reduction). However, $NO_2$ concentrations remained at 57.1 μg/$m^3$ in 2017 with a modest rise in recent years, which indicated that the air pollution control actions in Lanzhou were effective to some extent.

For period stratified analyses, Table 3 presents continuous and significant variations in the average concentration levels of $PM_{10}$, $SO_2$ and $NO_2$ during the periods of 2001–2005, 2007–2009 and 2014–2017 in Lanzhou, with similar temporal trends shown in Fig. 2. Compared with the period 2001–2005, the annual average concentrations of $PM_{10}$ and $SO_2$ declined by 37.5% and 71.7%, whereas the concentration of $NO_2$ has risen by 0.20% during the period 2014–2017.

Table 4 depicts the estimated effects of single-pollutant models using different lag structures. $PM_{2.5}$, $PM_{10}$, $SO_2$, $O_3$8h and CO, but not $NO_2$, had significant effects on respiratory hospitalisation. The most significant associations between respiratory hospital admissions and $PM_{2.5}$, $PM_{10}$, $SO_2$ and CO was on lag6 day, and the corresponding increases were 0.885% (95% CI: 0.414%–1.358%), 0.328% (95% CI: 0.145%–0.511%), 3.005% (95% CI: 1.689%–4.339%) and 3.199% (95% CI: 0.912%–5.537%), respectively. The strongest positive

**Table 1**
Descriptive statistics on respiratory hospital admissions, air pollutant levels and meteorological variables in Lanzhou during 2014–2017.

| | $\bar{X} \pm S$ | Minimum | Percentile | | | Maximum |
|---|---|---|---|---|---|---|
| | | | $P_{25}$ | $P_{50}$ | $P_{75}$ | |
| Respiratory admissions | | | | | | |
| Total | 27.3±16.2 | 1.0 | 14.0 | 24.0 | 37.0 | 95.0 |
| URTI | 6.7±4.9 | 1.0 | 3.0 | 5.0 | 9.0 | 31.0 |
| LRTI | 9.2±6.2 | 1.0 | 4.0 | 8.0 | 13.0 | 35.0 |
| COPD | 5.8±4.1 | 1.0 | 3.0 | 5.0 | 8.0 | 24.0 |
| Pneumonia | 5.5±3.5 | 1.0 | 3.0 | 5.0 | 7.0 | 23.0 |
| Asthma | 2.2±1.5 | 1.0 | 1.0 | 2.0 | 3.0 | 11.0 |
| Total admission by sex | | | | | | |
| Male | 16.0±10.1 | 1.0 | 8.0 | 14.0 | 22.0 | 60.0 |
| Female | 11.3±7.4 | 1.0 | 5.0 | 10.0 | 15.0 | 49.0 |
| Total admission by age | | | | | | |
| <65 | 15.7±9.3 | 1.0 | 9.0 | 14.0 | 21.0 | 57.0 |
| ≥65 | 11.6±8.5 | 1.0 | 5.0 | 10.0 | 17.0 | 55.0 |
| Air pollutants | | | | | | |
| $PM_{2.5}$(μg/$m^3$) | 53.3±27.2 | 12.7 | 35.4 | 45.7 | 63.6 | 269.4 |
| $PM_{10}$μg/$m^3$) | 122.9±78.3 | 18.9 | 81.0 | 107.9 | 145.2 | 1484.5 |
| $SO_2$(μg/$m^3$) | 22.4±14.5 | 3.5 | 10.9 | 18.3 | 30.4 | 81.9 |
| $NO_2$(μg/$m^3$) | 45.9±15.7 | 7.8 | 36.40 | 45.36 | 52.90 | 146.6 |
| CO(mg/$m^3$) | 1.3±0.7 | 0.3 | 0.8 | 1.1 | 1.6 | 4.7 |
| $O_3$8h (μg/$m^3$) | 84.0±36.5 | 8.0 | 56.0 | 78.0 | 106.0 | 221.0 |
| Meteorological factors | | | | | | |
| Mean temperature(°C) | 11.3±9.6 | -12.3 | 2.5 | 12.9 | 19.8 | 29.9 |
| Relative humidity (%) | 50.8±15.1 | 18.0 | 39.0 | 50.0 | 61.7 | 96.1 |

**Table 2**
Spearman's correlation coefficients between daily air pollutants and weather conditions in Lanzhou, 2014–2017.

| Variables | $PM_{10}$ | $SO_2$ | $NO_2$ | CO | $O_3$8h | Temperature | Humidity |
|---|---|---|---|---|---|---|---|
| $PM_{2.5}$ | 0.79* | 0.62* | 0.39* | 0.68* | −0.33* | −0.42* | −0.16* |
| $PM_{10}$ | | 0.54* | 0.51* | 0.50* | −0.13* | −0.38* | −0.26* |
| $SO_2$ | | | 0.41* | 0.81* | −0.48* | −0.63* | −0.21* |
| $NO_2$ | | | | 0.44* | 0.05 | −0.20* | −0.12* |
| CO | | | | | −0.47* | −0.55* | 0.01 |
| $O_3$8h | | | | | | 0.58* | −0.33* |
| Temperature | | | | | | | −0.09* |

*$P < 0.01$.

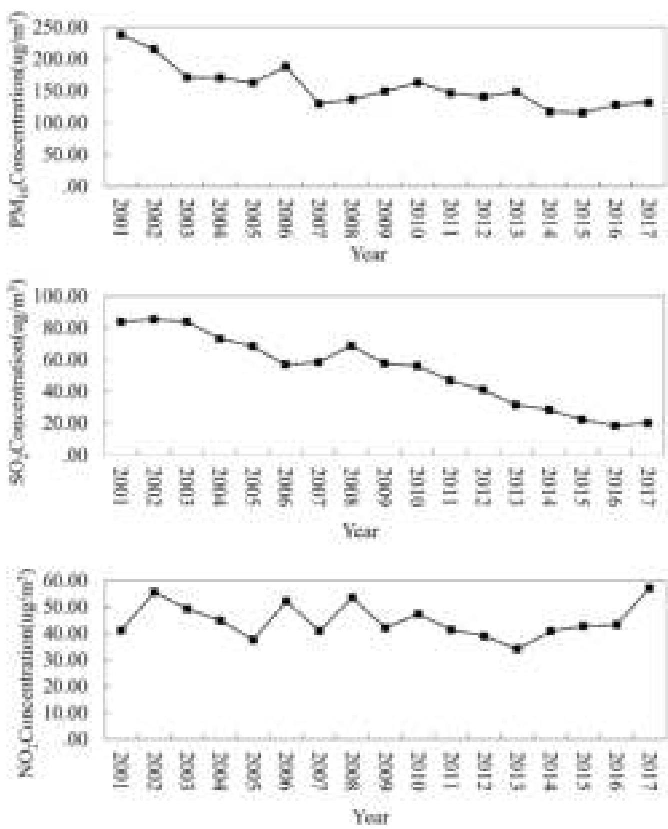


**Fig. 2.** Temporal trends in annual concentrations of $PM_{10}$, $SO_2$ and $NO_2$ in Lanzhou, China, 2001–2017.

**Table 3**
Descriptive statistics of the average concentration levels of $PM_{10}$, $SO_2$ and $NO_2$ in Lanzhou, China, stratified for three periods.

| Pollutant | Period | Mean ± SD | Min | Max | IQR |
|---|---|---|---|---|---|
| $PM_{10}$ | 2001–2005 | 196.6 ± 169.5 | 16.0 | 2561.0 | 139.0 |
| | 2007–2009 | 137.0 ± 81.0 | 12.0 | 1765.0 | 80.0 |
| | 2014–2017 | 122.9 ± 78.3 | 18.9 | 1484.5 | 64.2 |
| $SO_2$ | 2001–2005 | 79.1 ± 61.5 | 2.0 | 371.0 | 69.0 |
| | 2007–2009 | 63.0 ± 43.7 | 6.0 | 267.0 | 48.0 |
| | 2014–2017 | 22.4 ± 14.5 | 3.5 | 81.9 | 19.5 |
| $NO_2$ | 2001–2005 | 45.8 ± 29.2 | 4.0 | 260.0 | 31.0 |
| | 2007–2009 | 46.0 ± 25.5 | 4.0 | 162.0 | 30.0 |
| | 2014–2017 | 45.9 ± 15.7 | 7.8 | 146.6 | 16.5 |

association between $O_3$8h and respiratory admissions was on lag7 day (0.733%; 95% CI: 0.263%–1.205%). In contrast, there were no significant associations between respiratory hospital admission at any lag structure and $NO_2$.

Table 5 presents the results from a single-day lag (lag0–lag7) and single lags and moving average lag days (lag01–lag07) for respiratory hospital admissions with every 10 μg/m$^3$ and 1 mg/m$^3$ increase in air pollutants by sex. In male participants, statistically significant associations were observed between some lag structures for the concentrations of air pollutants. Respiratory hospitalisation was associated with same day pollution caused by $PM_{2.5}$, $PM_{10}$, $SO_2$ and CO at lag6 and lag7. For lag7, a 10 μg/m$^3$ increase in $PM_{2.5}$, $PM_{10}$ and $SO_2$ and 1 mg/m$^3$ increase in CO corresponded to 0.867% (95% CI: 0.235%–1.503%), 0.279% (95% CI: 0.035%–0.523%), 2.478% (95% CI: 0.773%–4.213%) and 4.703% (95% CI: 1.764%–7.727%) increases in the total number of respiratory admissions, respectively. We found statistically significant relationships between ER in $O_3$8 h at lag7 with a 0.864% (95%CI: 0.253%~1.478%) increase in respiratory admissions. However, $NO_2$ was not associated with respiratory admissions. In single-day lags, the most significant associations between $PM_{2.5}$, $PM_{10}$, $SO_2$, CO and $O_3$8h levels and hospitalisations in the female participants were observed on lag6 with increases of 0.968% (95% CI: 0.236%–1.704%), 0.351% (95% CI: 0.072%–0.631%), 3.751% (95% CI: 1.697%–5.846%), 0.886% (95% CI: 0.162%–1.615%) and 3.947% (95% CI: 0.400%–7.619%), respectively. $NO_2$ was not associated with respiratory hospital admissions in the female participants. In addition, the differences between sexes were insignificant for $PM_{2.5}$, $PM_{10}$, $NO_2$, $SO_2$, $O_3$8h and CO.

Table 6 shows the ERs and 95% CIs for respiratory admissions at 10 μg/m$^3$and 1 mg/m$^3$ increments for the concentrations of air pollutants for various lag structures among different age groups. In the group aged <65 years, $PM_{2.5}$, $PM_{10}$, $SO_2$ and CO had similar lag patterns for the association between hospitalisations and respiratory diseases. The maximum estimates for the effects of these four pollutants were observed on lag6 day, and the corresponding ERs were 0.955% (95% CI: 0.332%–1.582%), 0.385% (95% CI: 0.146%–0.625%), 3.390% (95% CI: 1.655%–5.155%) and 3.553% (95% CI: 0.575%–6.618%), respectively. In addition, $O_3$8h was associated with hospitalisations, and the maximum effect estimates were observed at lag7 day (0.754% [95% CI: 0.141%–1.371%]). A negative association was observed between $NO_2$ and hospitalisations. In addition, the ≥65-year-old age group was more vulnerable to higher $PM_{2.5}$ levels, and statistically significant results were observed at lag5, 6 and 7 days for $PM_{2.5}$. The effect estimates from lag5–7 days for $PM_{2.5}$ were 0.756% (95% CI: 0.049%–1.468%), 0.771% (95% CI: 0.050%–1.497%) and 0.815% (95% CI: 0.076%–1.559%), respectively. The effects of $PM_{10}$ at lag3 day and $SO_2$ at lag6 day were significantly associated with an increase in the number of respiratory hospitalisations (0.275% [95% CI: 0.026%–0.525%] and 2.292% [95% CI: 0.285%–4.339%], respectively) in elderly individuals. The effects of all pollutants on respiratory hospitalisation were not significantly different ($p > 0.05$, all) across all age groups (<65 vs. ≥65 years).

Fig. 3 shows a season-specific analysis of the effects of different air pollutants on respiratory admissions at lag7. Overall, we found that increments of $PM_{2.5}$, $PM_{10}$, $SO_2$ and CO concentrations had harmful effects on the total number of respiratory admissions, with larger effect estimates in the cold season. The effect of $O_3$8h was greater in the warm season (Fig. 3). For example, an increase in the ER of respiratory hospital admissions by 1.068% (95%CI: 0.443%–1.697%), 0.318% (95%CI: 0.320%–0.606%), 2.293% (95%CI: 1.688%–4.402%) and 2.498% (95% CI: 0.667%–3.901%) in the cold season was associated with 10 μg/m$^3$ increases in $PM_{2.5}$, $PM_{10}$ and $SO_2$ and a 1 mg/m$^3$ increase in CO,

**Table 4**
Percent change (95% CI) of respiratory hospital admissions associated with a 10 μg/m$^3$ increase in air pollutant concentrations with different lag days.

| Lag days | $PM_{2.5}$ | | $PM_{10}$ | | $NO_2$ | | $SO_2$ | | CO | | $O_3$8h | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ER | 95% CI | ER | 95% CI | ER | 95% CI | ER | 95% CI | ER | 95% CI | ER | 95% CI |
| lag0 | −0.713 | (-1.190,-0.233) | −0.139 | (-0.304,0.027) | −0.019 | (-0.975,0.947) | −3.027 | (-4.326,-1.710) | −5.366 | (-7.479,-3.204) | −0.006 | (-0.527,0.519) |
| lag1 | −0.213 | (-0.695,0.270) | −0.118 | (-0.286,0.050) | −2.225 | (-3.158,-1.284) | −2.375 | (-3.660,-1.073) | −4.989 | (-7.113,-2.816) | −0.334 | (-0.830,0.165) |
| lag2 | −0.122 | (-0.622,0.381) | −0.146 | (-0.324,0.033) | −0.456 | (-1.382,0.478) | −1.394 | (-2.661,-0.111) | −3.037 | (-5.178,-0.847) | −0.250 | (-0.727,0.229) |
| lag3 | 0.104 | (-0.375,0.586) | 0.019 | (-0.146,0.185) | 0.649 | (-0.295,1.603) | −2.332 | (-3.625,-1.021) | −1.114 | (-3.369,1.194) | −0.463 | (-0.945,0.021) |
| lag4 | −0.478 | (-0.944,-0.009) | −0.241 | (-0.399,-0.082) | −2.092 | (-3.021,-1.155) | −3.503 | (-4.796,-2.192) | −3.222 | (-5.470,-0.921) | −0.520 | (-0.985,-0.053) |
| lag5 | 0.228 | (-0.235,0.693) | 0.067 | (-0.092,0.226) | −1.534 | (-2.434,-0.627) | −0.814 | (-2.117,0.507) | −0.700 | (-2.933,1.584) | 0.178 | (-0.286,0.644) |
| lag6 | **0.885** | **(0.414,1.358)** | **0.328** | **(0.145,0.511)** | −0.801 | (-1.713,0.119) | **3.005** | **(1.689,4.339)** | **3.199** | **(0.912,5.537)** | 0.443 | (-0.021,0.909) |
| lag7 | **0.572** | **(0.087,1.061)** | **0.195** | **(0.009,0.382)** | −1.325 | (-2.239,-0.402) | **1.827** | **(0.527,3.145)** | **2.579** | **(0.370,4.837)** | **0.733** | **(0.263,1.205)** |
| lag01 | −0.706 | (-1.278,-0.131) | −0.185 | (-0.383,0.014) | −1.766 | (-2.910,-0.607) | −3.796 | (-5.332,-2.235) | −7.353 | (-9.810,-4.830) | −0.364 | (-1.018,0.294) |
| lag02 | −0.749 | (-1.410,-0.084) | −0.249 | (-0.477,-0.020) | −1.890 | (-3.184,-0.578) | −3.923 | (-5.636,-2.179) | −8.155 | (-10.900,-5.326) | −0.580 | (-1.311,0.157) |
| lag03 | −0.644 | (-1.392,0.109) | −0.213 | (-0.464,0.040) | −1.080 | (-2.524,0.384) | −4.969 | (-6.843,-3.056) | −8.017 | (-11.100,-4.827) | −0.939 | (-1.738,-0.134) |
| lag04 | −0.642 | (-1.484,0.208) | −0.319 | (-0.593,-0.045) | −2.267 | (-3.861,-0.646) | −6.015 | (-8.085,-3.900) | −9.077 | (-12.553,-5.463) | −1.097 | (-1.943,-0.243) |
| lag05 | −0.416 | (-1.360,0.538) | −0.268 | (-0.566,0.031) | −3.171 | (-4.921,-1.389) | −6.616 | (-8.893,-4.283) | −10.384 | (-14.331,-6.256) | −0.856 | (-1.751,0.048) |
| lag06 | 0.173 | (-0.874,1.232) | −0.076 | (-0.401,0.251) | −4.329 | (-6.236,-2.384) | −5.443 | (-7.966,-2.851) | −10.733 | (-15.204,-6.026) | −0.606 | (-1.557,0.354) |
| lag07 | 0.569 | (-0.587,1.738) | 0.047 | (-0.307,0.403) | −5.968 | (-8.012,-3.878) | −4.281 | (-7.046,-1.433) | −10.831 | (-15.780,-5.592) | −0.189 | (-1.200,0.833) |

[a] Noting: except CO, CO Units: 1 mg/m$^3$ increase in CO.

respectively. The effect estimates of $NO_2$ on the total number of respiratory admissions did not significantly differ between the two seasons. Between-season differences in respiratory hospitalisations were insignificant for all six pollutants.

Fig. 4 shows the ER relationships for respiratory hospital admissions associated with exposure to air pollutants (lag7 for $PM_{2.5}$, $PM_{10}$, $NO_2$, $SO_2$, $O_3$8h and CO). The ER relationship curves for $PM_{2.5}$ had a steep slope at concentrations of 35–40 μg/m$^3$. Then, the curve decreased at concentrations >40 μg/m$^3$. The curve had thresholds for associations between hospital admissions and respiratory disease at 60–100 μg/m$^3$ for $PM_{10}$, 5–20 μg/m$^3$ for $SO_2$, 30–60 μg/m$^3$ for $O_3$8h and 0.5–0.8 mg/m$^3$ for CO. Notably, although the $O_3$8h and CO concentrations are significantly lower than the current air quality standard for residential areas in China ($O_3$8h: 160 μg/m$^3$ for $O_3$8h and 24-h average: 4 mg/m$^3$ for CO), $O_3$8h and CO still had an effect on respiratory hospital admission (see Fig. 5).

Table 7 shows the estimated ERs and 95% CI of each air pollutant with the single- and double-pollutant models adjusted for co-pollutants at lag7. After adjustment, the results showed that associations between $PM_{2.5}$, $PM_{10}$, $NO_2$, $SO_2$, $O_3$8h and CO hospital admissions were robust after adjusting for co-pollutants.

Table S1 presents the estimated ERs and 95% CI of each pollutant in the GAM models in the sensitivity analyses using the alternative of time. The adverse effects of air pollution on respiratory hospital admissions did not change significantly by modifying the df value of temporal trends from 6 to 10 per year. The effect estimates remained relatively stable (Supplementary Table S1).

In terms of hospital admission for the sub-diagnoses of respiratory diseases, hospital admission for URTI was associated with exposure to $NO_2$ (3.430% increase, lag2), $NO_2$ (4.677% increase, lag02) and $NO_2$ (5.290% increase, lag03). A significant association was found for $SO_2$24 h, lag6. Hence, an increase of 10 μg/m$^3$ was associated with an increment of 3.081% (95% CI: 0.278%–5.963%) for hospital admissions caused by URTI (Supplementary Table S2). Moreover, elevated $PM_{2.5}$, $PM_{10}$, $SO_2$ and CO levels were significantly associated with an increase in the number of hospitalisations for LRTI at lag7 day (Supplementary Table S3). A 10 μg/m$^3$ increment in $PM_{2.5}$ at lag7 day corresponded to a 1.225% (95% CI: 0.149–2.313) increase in the number of daily hospital admissions for COPD (Supplementary Table S4). However, the effects of six air pollutants on pneumonia hospitalisation differed from those of the above-mentioned respiratory diseases. The association between $PM_{2.5}$ concentration and pneumonia hospitalisation was statistically significant at lag6 (1.841% [95% CI: 0.823–2.870]), lag7 (1.325% [95% CI: 0.281–2.380]) and lag07 (95% CI: 2.603% [0.013–5.261]). For $PM_{10}$, a 10 μg/m$^3$ increment in concentrations at lag3, lag6 and lag7 corresponded to 0.422% (95% CI: 0.108%–0.736%), 0.541% (95% CI: 0.156%–0.928%) and 0.432% (95% CI: 0.034%–0.832%) increases in the number of hospitalisations, respectively. In terms of $SO_2$ and $O_3$8h, significant effects were observed at days 6 and 7 in the single-day models (Supplementary Table S5). However, all air pollutants did not significantly affect respiratory morbidities (Supplementary Table S6).

## 4. Discussion

The 4-year data on Lanzhou, China, showed positive associations of elevated $PM_{2.5}$, $PM_{10}$, $SO_2$, $O_3$8h and CO levels with respiratory hospital admissions. However, there was no significant association between $NO_2$ levels and respiratory hospital admissions. Among all air pollutants, CO was most strongly associated with hospital admissions, followed by $SO_2$, $PM_{2.5}$, $O_3$8h and $PM_{10}$. Moreover, the adverse effect estimates of some air pollutants, such as $PM_{2.5}$, $PM_{10}$, $SO_2$, CO and $O_3$8h, could be detected even below the air quality standard of China. Therefore, the current air quality standard might not be sufficient for protecting the health of individuals in Lanzhou.

Statistically significant associations were observed in some lag structures between air pollutant concentrations. In this study, the most

**Table 5**
Percent change (95% CI) of respiratory hospital admissions associated with a 10 μg/$m^3$ increase[a] in air pollutant concentrations with different lag days by sex.

| | Lag days | $PM_{2.5}$ [b] | | $PM_{10}^{b}$ | | $NO_2^{b}$ | | $SO_2^{b}$ | | $CO^{ab}$ | | $O_3 8h^{b}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ER | 95% CI | ER | 95% CI | ER | 95% CI | ER | 95% CI | ER | 95% CI | ER | 95% CI |
| Male | lag0 | −1.198 | (-1.822,-0.570) | −0.192 | (-0.411,0.027) | −0.557 | (-1.797,0.698) | −3.191 | (-4.888,-1.463) | −6.674 | (-9.392,-3.874) | −0.044 | (-0.721,0.639) |
| | lag1 | −0.916 | (-1.548,-0.279) | −0.239 | (-0.464,-0.013) | −3.107 | (-4.310,-1.889) | −2.773 | (-4.444,-1.074) | −7.282 | (-9.985,-4.497) | −0.376 | (-1.021,0.272) |
| | lag2 | −0.113 | (-0.765,0.542) | −0.140 | (-0.373,0.094) | −0.132 | (-1.341,1.091) | −0.855 | (-2.512,0.832) | −1.712 | (-4.542,1.201) | −0.076 | (-0.696,0.547) |
| | lag3 | 0.219 | (-0.403,0.844) | 0.093 | (-0.122,0.307) | 0.874 | (-0.357,2.120) | −1.983 | (-3.675,-0.261) | −0.471 | (-3.435,2.584) | −0.303 | (-0.928,0.326) |
| | lag4 | 0.055 | (-0.546,0.659) | −0.042 | (-0.241,0.158) | −2.382 | (-3.586,-1.162) | −3.175 | (-4.867,-1.453) | −2.902 | (-5.847,0.135) | −0.671 | (-1.275,-0.063) |
| | lag5 | 0.547 | (-0.052,1.151) | **0.235** | **(0.033,0.437)** | −1.685 | (-2.854,-0.502) | 0.242 | (-1.473,1.987) | 1.448 | (-1.525,4.511) | 0.104 | (-0.498,0.709) |
| | lag6* | **0.799** | **(0.185,1.416)** | **0.273** | **(0.037,0.521)** | −1.222 | (-2.406,-0.024) | **2.725** | **(1.014,4.464)** | **3.121** | **(0.138,6.192)** | 0.236 | (-0.367,0.842) |
| | lag7 | **0.867** | **(0.235,1.503)** | **0.279** | **(0.035,0.523)** | −0.743 | (-1.940,0.469) | **2.478** | **(0.773,4.213)** | **4.703** | **(1.764,7.727)** | **0.864** | **(0.253,1.478)** |
| | lag01 | −1.538 | (-2.284,-0.786) | −0.306 | (-0.570,-0.042) | −2.823 | (-4.300,-1.324) | −4.192 | (-6.193,-2.149) | −9.792 | (-12.909,-6.563) | −0.466 | (-1.317,0.392) |
| | lag02 | −1.482 | (-2.340,-0.615) | −0.352 | (-0.653,-0.050) | −2.606 | (-4.281,-0.901) | −3.902 | (-6.139,-1.612) | −9.437 | (-12.956,-5.777) | −0.550 | (-1.501,0.411) |
| | lag03 | −1.296 | (-2.265,-0.317) | −0.266 | (-0.596,0.064) | −1.653 | (-3.521,0.252) | −4.766 | (-7.215,-2.252) | −9.022 | (-12.985,-4.879) | −0.805 | (-1.844,0.244) |
| | lag04 | −0.831 | (-1.921,0.272) | −2.194 | (-0.575,0.138) | −2.899 | (-4.957,-0.796) | −5.337 | (-8.049,-2.545) | −9.556 | (-14.047,-4.831) | −1.070 | (-2.170,0.042) |
| | lag05 | −0.565 | (-1.792,0.677) | −0.121 | (-0.509,0.269) | −3.791 | (-6.050,-1.479) | −5.506 | (-8.502,-2.412) | −9.927 | (-15.073,-4.470) | −0.987 | (-2.147,0.187) |
| | lag06 | −0.294 | (-1.655,1.085) | 0.003 | (-0.424,0.431) | −5.312 | (-7.761,-2.799) | −4.952 | (-8.249,-1.537) | −10.595 | (-16.394,-4.393) | −0.945 | (-2.176,0.301) |
| | lag07 | 0.185 | (-1.320,1.714) | 0.151 | (-0.313,0.617) | −6.554 | (-9.189,-3.841) | −3.506 | (-7.124,0.254) | −8.938 | (-15.456,-1.917) | −0.559 | (-1.869,0.767) |
| Female | lag0 | −0.020 | (-0.755,0.720) | −0.074 | (-0.326,0.179) | 0.733 | (-0.759,2.247) | −2.189 | (-4.208,-0.128) | −2.896 | (-6.237,0.563) | 0.101 | (-0.713,0.921) |
| | lag1 | 0.957 | (0.204,1.716) | 0.301 | (0.011,0.592) | −0.957 | (-2.425,0.533) | −1.493 | (-3.497,0.552) | −1.490 | (-4.883,2.024) | −0.295 | (-1.070,0.485) |
| | lag2 | −0.170 | (-0.949,0.615) | −0.137 | (-0.413,0.141) | −0.864 | (-2.298,0.591) | −2.111 | (-4.060,-0.122) | −4.956 | (-8.197,-1.600) | −0.415 | (-1.158,0.334) |
| | lag3 | −0.050 | (-0.799,0.705) | −0.073 | (-0.332,0.187) | 0.437 | (-1.035,1.930) | −2.163 | (-4.180,-0.104) | −1.196 | (-4.680,2.416) | −0.527 | (-1.282,0.234) |
| | lag4 | −1.236 | (-1.970,-0.496) | −0.541 | (-0.799,-0.282) | −1.367 | (-2.824,0.111) | −3.221 | (-5.236,-1.163) | −2.780 | (-6.266,0.836) | −0.339 | (-1.067,0.393) |
| | lag5 | −0.255 | (-0.980,0.474) | −0.204 | (-0.460,0.053) | −1.055 | (-2.460,0.371) | −1.720 | (-3.729,0.331) | −2.817 | (-6.193,0.681) | 0.317 | (-0.408,1.046) |
| | lag6* | **0.968** | **(0.236,1.704)** | **0.351** | **(0.072,0.631)** | 0.050 | (-1.377,1.498) | **3.751** | **(1.697,5.846)** | **3.947** | **(0.400,7.619)** | **0.886** | **(0.162,1.615)** |
| | lag7 | 0.283 | (-0.472,1.045) | 0.115 | (-0.173,0.404) | −1.845 | (-3.259,-0.410) | 1.050 | (-0.948,3.088) | 0.102 | (-3.223,3.541) | 0.564 | (-0.169,1.303) |
| | lag01 | 0.594 | (-0.296,1.492) | 0.113 | (-0.200,0.427) | −0.258 | (-2.055,1.572) | −2.588 | (-4.982,-0.132) | −3.253 | (-7.202,0.863) | −0.199 | (-1.216,0.828) |
| | lag02 | 0.395 | (-0.642,1.442) | 0.016 | (-0.347,0.380) | −0.839 | (-2.857,1.221) | −3.367 | (-6.018,-0.641) | −5.856 | (-10.189,-1.315) | −0.537 | (-1.672,0.611) |
| | lag03 | 0.392 | (-0.785,1.584) | −0.019 | (-0.417,0.382) | −0.180 | (-2.430,2.123) | −4.377 | (-7.285,-1.378) | −5.750 | (-10.617,-0.617) | −0.925 | (-2.166,0.332) |
| | lag04 | −0.244 | (-1.568,1.097) | −0.354 | (-0.788,0.081) | −1.081 | (-3.579,1.483) | −5.834 | (-9.030,-2.525) | −7.098 | (-12.568,-1.285) | −0.959 | (-2.276,0.376) |
| | lag05 | −0.179 | (-1.666,1.331) | −0.407 | (-0.878,0.067) | −1.891 | (-4.642,0.939) | −6.839 | (-10.353,-3.187) | −9.193 | (-15.364,-2.572) | −0.480 | (-1.877,0.936) |
| | lag06 | 0.786 | (-0.870,2.469) | −0.141 | (-0.654,0.376) | −2.305 | (-5.327,0.813) | −4.750 | (-8.691,-0.639) | −8.483 | (-15.574,-0.796) | 0.116 | (-1.371,1.626) |
| | lag07 | 1.139 | (-0.687,2.998) | −0.030 | (-0.588,0.531) | −4.285 | (-7.514,-0.943) | −4.073 | (-8.380,0.436) | −10.705 | (-18.398,-2.288) | 0.560 | (-1.021,2.167) |

[a] Noting: except CO, CO Units: 1 mg/m [3] increase in CO [b] Single-day lag of lag6 pollutants concentrations were used. *Insignificant for between-group difference.

**Table 6**
Percent change (95% CI) of respiratory hospital admissions associated with a 10 μg/m$^3$ increase[a] in air pollutant concentrations with different lag days by age.

| | Lag days | $PM_{2.5}$ [b] | | $PM_{10}$[b] | | $NO_2$[b] | | $SO_2$[b] | | CO[ab] | | $O_3$8h[b] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ER | 95% CI | ER | 95% CI | ER | 95% CI | ER | 95% CI | ER | 95% CI | ER | 95% CI |
| <65 | lag0 | −0.651 | (-1.281,-0.016) | 0.002 | (-0.211,0.216) | 0.766 | (-0.486,2.033) | −3.408 | (-5.097,-1.689) | −5.884 | (-8.605,-3.081) | −0.700 | (-1.381,-0.014) |
| | lag1 | −0.730 | (-1.363,-0.092) | −0.267 | (-0.493,-0.040) | −2.118 | (-3.329,-0.892) | −2.926 | (-4.593,-1.229) | −6.677 | (-9.377,-3.898) | −0.186 | (-0.836,0.469) |
| | lag2 | −0.410 | (-1.061,0.245) | −0.391 | (-0.629,-0.152) | −0.170 | (-1.372,1.047) | −2.671 | (-4.306,-1.007) | −4.248 | (-6.980,-1.435) | −0.290 | (-0.913,0.337) |
| | lag3 | 0.048 | (-0.579,0.678) | −0.156 | (-0.377,0.065) | 0.512 | (-0.706,1.746) | −2.257 | (-3.946,-0.539) | −1.910 | (-4.798,1.066) | −0.691 | (-1.320,-0.058) |
| | lag4 | −0.594 | (-1.210,0.025) | −0.272 | (-0.482,-0.061) | −1.728 | (-2.933,-0.508) | −4.158 | (-5.841,-2.445) | −4.059 | (-6.950,-1.079) | −0.572 | (-1.181,0.041) |
| | lag5 | −0.097 | (-0.710,0.520) | 0.027 | (-0.183,0.238) | −2.289 | (-3.448,-1.117) | −1.698 | (-3.390,0.024) | −2.917 | (-5.751,0.002) | 0.051 | (-0.556,0.661) |
| | lag6 * | **0.955** | **(0.332,1.582)** | **0.385** | **(0.146,0.625)** | −1.660 | (-2.837,-0.469) | **3.390** | **(1.655,5.155)** | **3.553** | **(0.575,6.618)** | 0.408 | (-0.199,1.019) |
| | lag7 | 0.450 | (-0.193,1.098) | 0.211 | (-0.033,0.457) | −2.119 | (-3.298,-0.926) | **2.230** | **(0.516,3.973)** | 2.542 | (-0.332,5.499) | **0.754** | **(0.141,1.371)** |
| | lag01 | −1.030 | (-1.783,-0.271) | −0.185 | (-0.446,0.077) | −1.120 | (-2.613,0.397) | −4.454 | (-6.440,-2.426) | −8.904 | (-12.033,-5.665) | −0.772 | (-1.624,0.088) |
| | lag02 | −1.213 | (-2.080,-0.340) | −0.385 | (-0.687,-0.082) | −1.149 | (-2.838,0.570) | −5.286 | (-7.486,-3.034) | −10.202 | (-13.678,-6.585) | −0.952 | (-1.900,0.007) |
| | lag03 | −1.110 | (-2.088,-0.123) | −0.449 | (-0.782,-0.115) | −0.693 | (-2.568,1.219) | −6.346 | (-8.753,-3.875) | −10.610 | (-14.490,-6.555) | −1.578 | (-2.611,-0.535) |
| | lag04 | −1.061 | (-2.165,0.055) | −0.519 | (-0.881,-0.160) | −1.593 | (-3.670,0.528) | −7.599 | (-10.249,-4.870) | −12.084 | (-16.436,-7.505) | −1.800 | (-2.896,-0.692) |
| | lag05 | −1.090 | (-2.326,0.161) | −0.480 | (-0.874,-0.083) | −2.758 | (-5.030,-0.430) | −8.460 | (-11.362,-5.463) | −14.660 | (-19.516,-9.510) | −1.578 | (-2.738,-0.404) |
| | lag06 | −0.580 | (-1.952,0.812) | −0.266 | (-0.697,0.167) | −4.485 | (-6.947,-1.957) | −7.321 | (-10.535,-3.991) | −15.097 | (-20.591,-9.222) | −1.297 | (-2.532,-0.048) |
| | lag07 | −0.230 | (-1.750,1.313) | −0.100 | (-0.568,0.371) | −6.487 | (-9.118,-3.780) | −6.106 | (-9.636,-2.438) | −15.307 | (-21.392,-8.751) | −0.769 | (-2.080,0.561) |
| ≥65 | lag0 | −0.776 | (-1.502,-0.045) | −0.336 | (-0.598,-0.074) | −0.902 | (-2.382,0.601) | −1.741 | (-3.776,0.336) | −3.681 | (-7.032,-0.209) | 0.867 | (0.056,1.684) |
| | lag1 | 0.543 | (-0.193,1.284) | 0.082 | (-0.167,0.330) | −2.002 | (-3.460,-0.522) | −0.876 | (-2.897,1.186) | −1.434 | (-4.873,2.129) | −0.466 | (-1.237,0.312) |
| | lag2 | 0.228 | (-0.551,1.012) | 0.188 | (-0.080,0.457) | −1.001 | (-2.446,0.454) | 0.233 | (-1.749,2.255) | −1.772 | (-5.156,1.732) | 0.001 | (-0.741,0.748) |
| | lag3 | 0.278 | (-0.470,1.032) | **0.275** | **(0.026,0.525)** | 0.770 | (-0.717,2.278) | −1.952 | (-3.960,0.099) | 0.184 | (-3.381,3.881) | 0.074 | (-0.678,0.831) |
| | lag4 | −0.150 | (-0.863,0.568) | −0.146 | (-0.386,0.094) | −2.257 | (-3.715,-0.777) | −2.224 | (-4.246,-0.159) | −1.717 | (-5.266,1.965) | −0.456 | (-1.176,0.269) |
| | lag5 | **0.756** | **(0.049,1.468)** | 0.157 | (-0.086,0.400) | −0.183 | (-1.605,1.260) | 0.782 | (-1.255,2.860) | 2.832 | (-0.753,6.547) | 0.391 | (-0.324,1.111) |
| | lag6 * | **0.771** | **(0.050,1.497)** | 0.257 | (-0.028,0.542) | 0.338 | (-1.098,1.796) | **2.292** | **(0.285,4.339)** | 2.237 | (-1.286,5.886) | 0.482 | (-0.323,1.202) |
| | lag7 | **0.815** | **(0.076,1.559)** | 0.268 | (-0.018,0.556) | −0.175 | (-1.614,1.285) | 1.174 | (-0.808,3.197) | 2.054 | (-1.344,5.569) | 0.585 | (-0.141,1.317) |
| | lag01 | −0.220 | (-1.093,0.660) | −0.180 | (-0.484,0.125) | −2.248 | (-4.029,-0.434) | −1.788 | (-4.222,0.707) | −3.676 | (-7.648,0.468) | 0.194 | (-0.826,1.224) |
| | lag02 | −0.105 | (-1.120,0.920) | −0.060 | (-0.407,0.289) | −2.683 | (-4.691,-0.632) | −1.168 | (-3.893,1.634) | −4.239 | (-8.678,0.415) | 0.094 | (-1.054,1.256) |
| | lag03 | 0.060 | (-1.095,1.229) | −0.188 | (-0.263,0.501) | −1.464 | (-3.713,0.838) | −1.992 | (-4.978,1.088) | −3.249 | (-8.269,2.046) | 0.270 | (-0.997,1.553) |
| | lag04 | 0.098 | (-1.201,1.414) | −0.014 | (-0.429,0.403) | −2.901 | (-5.379,-0.357) | −2.563 | (-5.872,0.862) | −3.497 | (-9.193,2.557) | 0.174 | (-1.168,1.534) |
| | lag05 | 0.695 | (-0.766,2.177) | 0.060 | (-0.391,0.514) | −3.285 | (-6.019,-0.472) | −2.587 | (-6.255,1.225) | −2.628 | (-9.243,4.471) | 0.432 | (-0.979,1.863) |
| | lag06 | 1.403 | (-0.215,3.047) | 0.229 | (-0.266,0.727) | −3.620 | (-6.619,-0.525) | −1.364 | (-5.421,2.867) | −2.826 | (-10.324,5.299) | 0.597 | (-0.895,2.112) |
| | lag07 | 1.927 | (0.144,3.742) | 0.332 | (-0.207,0.874) | −4.744 | (-7.978,-1.395) | −0.386 | (-4.807,4.241) | −3.200 | (-11.432,5.797) | 0.726 | (-0.859,2.337) |

[a] Noting: except CO, CO Units: 1 mg/m $^3$ increase in CO [b] Single-day lag of lag6 pollutants concentrations were used. *Insignificant for between-group difference.

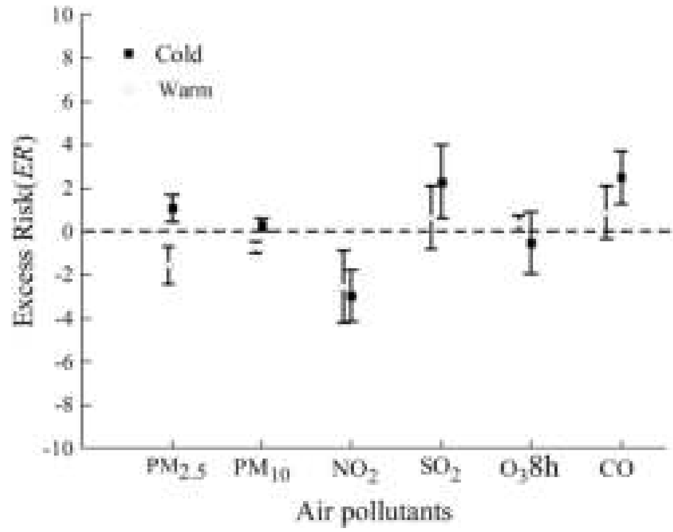


**Fig. 3.** Percent change (95% CI) of respiratory diseases hospital admissions associated with every 10 μg/m$^3$ increase in $PM_{2.5}$, $PM_{10}$, $NO_2$, $SO_2$, $O_3$8h, or 1 mg/m$^3$ CO (lag7) concentration by season (cool, warm).

significant associations between air pollution and hospital admissions were mainly found at lag6 and lag7 in the single-day lag effect model. This finding was not in accordance with that of previous studies. That is, earlier studies have reported that the strongest effects were observed on the current day (lag0) or in multiple lag days (lag01, lag02, lag03 and lag05) (Wong et al., 1999; Fusco et al., 2001; Petroeschevsky et al., 2001; Chen et al., 2010; Bao, 2013; Zhang et al., 2014; Phung et al., 2016; Capraz et al., 2017; Vahedian et al., 2017; Wang, 2017; Luo et al., 2018; Phosri et al., 2019). Some studies have shown that air pollutants had the greatest effect from lag2 to lag5 (Phung et al., 2016; Capraz et al., 2017; Vahedian et al., 2017; Wang, 2017; Luo et al., 2018; Phosri et al., 2019). Our results revealed that the effects of these five pollutants may be longer and cumulative, and the causes might be complex. One possible reason is that $PM_{2.5}$ and irritating gaseous air pollutants in the atmosphere can cause inflammation in the respiratory tract after several days. Another plausible explanation for the lag effects is long exposure to high concentrations of air pollutants, which may lead to decreased sensitivity to pollution among local residents.

The results of the current and previous studies conducted in Lanzhou (Tao et al., 2013, 2014) showed that, in general, the effect size of three air pollutants varied during three periods (Fig. 4). There were minimal changes in ER for $PM_{10}$ during the three periods (2001–2005, 2007–2009 and 2014–2017). This phenomenon might have resulted from a local resident having been exposed to high $PM_{10}$ levels for a long time, which can reduce their susceptibility to pollution. Moreover, a particle source analysis performed in Lanzhou revealed that the main source of $PM_{10}$ is ground dust (Tao et al., 2013, 2014), which commonly comprises inorganic minerals with low toxicity.

The effect of $SO_2$, compared with that of $PM_{10}$, on respiratory hospitalisation significantly increased from 0.492% (95% CI: 0.028%–0.971%) at lag1 in 2001–2005 to 1.104% (95% CI: 0.333%–1.917%) at lag3 in 2007–2009. Then, it further elevated 1.827% (95% CI: 0.527%–3.145%) at lag7 in 2014–2009. Although the average $SO_2$ concentration in Lanzhou decreased remarkably in 2001, it significantly increased recently. This phenomenon might be because $SO_2$ is highly soluble in the upper respiratory tract and can irritate the respiratory mucosa even at small concentrations. In addition, although the emission of $SO_2$ has been effectively controlled, coal is still widely used in urban and surrounding areas where there is no central heating. We found that a 10 μg/m$^3$ increase in $NO_2$ was associated with respiratory hospital admission increments of 1.064% (95% CI: 0.226%–1.903%) at lag1 in 2001–2005 and 1.400% (95% CI: 0.133%–2.700%) at lag0 in 2007–2009. Nevertheless, no significant association was observed from 2014 to 2017. This phenomenon is mainly attributed to two reasons. Firstly, the government advocates the idea of green travel. Thus, a higher number of individuals choose public transport carriers as their primary means of transportation when going to work or school every day. Secondly, some individuals want to convert from using oil to gas and utilise natural gas when driving their cars in urban areas. Hence, the harmful effect of $NO_2$ was not statistically significant.

We found that the effects of most air pollutants ($PM_{2.5}$, $PM_{10}$, $SO_2$ and CO) were stronger in the cold season than in the warm season. Two other gaseous pollutants ($O_3$8h and $NO_2$) had higher estimates in the cold season than in the warm season. However, $O_3$8h and $NO_2$ were not

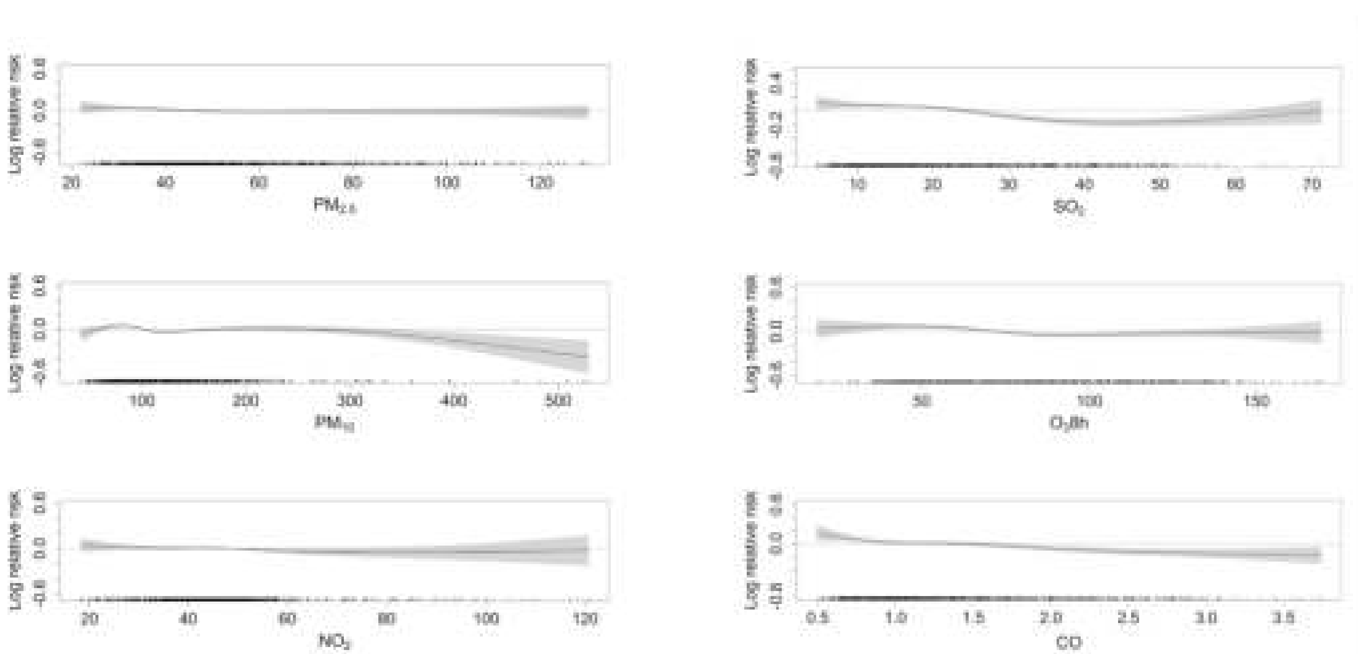


**Fig. 4.** The exposure-response relationship curves between daily respiratory hospital admissions and air pollutants.

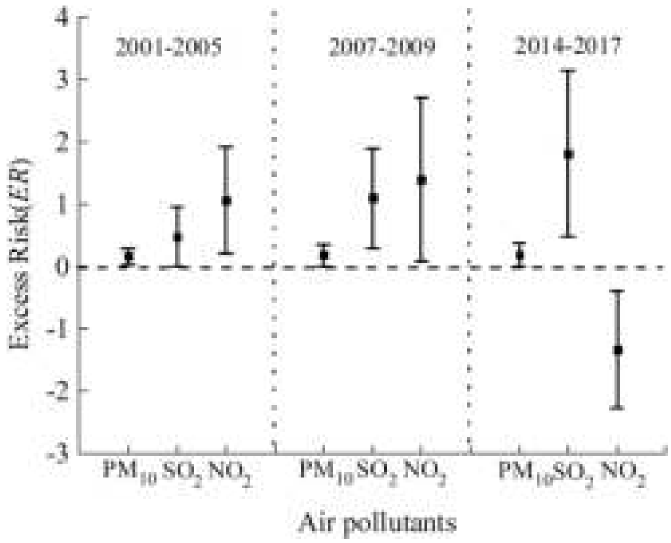


**Fig. 5.** Association between a 10 μg/$m^3$ increase in air pollutants and the increase in respiratory diseases hospital admissions during the three periods (2001–2005, 2007–2009, and 2014–2017).

significantly associated with the outcomes. This finding is consistent with those of two other studies (Chen et al., 2010; Rodopoulou et al., 2014). Conversely, some studies have reported contrasting results (Fusco et al., 2001; Zhang et al., 2014; Qiu et al., 2018). Therefore, the effect of air pollution on respiratory hospital admissions in terms of seasonal variations has not been validated. There are a few possible causes of the varying effects of air pollutants on respiratory hospital admissions in the cold and warm seasons. Firstly, the concentrations of air pollutants in Lanzhou might be seasonally dependent. Previous studies have revealed that low or high temperatures could increase respiratory morbidity (Song et al., 2017; Zhao et al., 2018). Secondly, the composition of air pollutants in Lanzhou might change with the seasons. Thirdly, the meteorological variables and pollutants were correlated with each other. Thus, the associations between air pollution and respiratory morbidity might be caused by different meteorological conditions in different periods.

Regarding sex-specific estimates, the estimated effect sizes of all pollutants were slightly larger in women than in men. This finding is in accordance with those of previous studies showing that women are more sensitive to air pollutants (Zhang et al., 2014; Phung et al., 2016; Capraz et al., 2017) and might be attributed to the differences in physical structures between men and women. That is, women have smaller airways and more vulnerable airway reactivity than men. Hence, they are more sensitive to physical conditions (Mitsakou et al., 2007). In addition, differences in health according to sex are also affected by genetic and biological differences, lifestyle habits (smoking, drinking, exercise and diet) and individual characteristics (routine activity and occupation).

In the age-specific analysis, the estimated effects of $PM_{2.5}$, $PM_{10}$, $SO_2$, $O_3$8h and CO were stronger in the group <65 years old. Our findings differed from those of previous studies showing that elderly individuals were more sensitive to the effect of air pollution (Wong et al., 1999; Capraz et al., 2017; Vahedian et al., 2017). A few studies have found that adults (<65 years old) were more vulnerable than elderly individuals (≥65 years old), which was consistent with the finding in our study (Phung et al., 2016; Luo et al., 2018; Phosri et al., 2019). The main cause for this finding was time of exposure to ambient air pollutants. Younger people spend more time outside engaging in physical activities than elderly individuals, so they are exposed to a higher dose of air pollutants. In addition, older people in China generally have greater willingness to see a doctor because of the popularity of medical insurance. Personal lifestyle habits (smoking, drinking and exercise) might also contribute to different effects.

The ER relationship curves have important public health implications. That is, the estimated percentage changes may be helpful in monitoring the adverse health effects caused by air pollution. Thus, further health risk assessment should be performed. In the current study, the curves for $PM_{2.5}$, $PM_{10}$, $SO_2$, $O_3$8h and CO were nonlinear, thereby indicating the thresholds for their associations with respiratory hospital admissions. Notably, even though the curves were below the air quality standard levels in China, significant effects of $O_3$8h and CO were still noted. Therefore, the current air quality standards should be revised to better protect public health.

Moreover, this study found that every 10 μg/$m^3$ increase in $PM_{2.5}$ at lag7 day was associated with a 0.885% (95% CI: 0.414–1.358) increase in the number of hospital admissions for respiratory diseases. The effect estimates of $PM_{2.5}$ on the number of respiratory hospital admissions may be lower in the current study than those in other studies (Fig. S1 in the supplemental materials). For example, in a study conducted in Istanbul, Turkey, the results showed that a 10 μg/$m^3$ increase in $PM_{2.5}$ corresponded to a 7.22% (95% CI: 5.37%–9.09%) increase in the number of respiratory admissions (Capraz et al., 2017). Another study found that $PM_{2.5}$ had an effect on respiratory admissions, as shown by a 2.0% (95% CI: 0.7%–4%) increase in ER at lag7 days (Vahedian et al., 2017). Further, in a study in Hefei City, China, $PM_{2.5}$ caused a 6.8% increase in the number of respiratory hospital admission (Xie et al., 2019). Another study showed a 0.87% increase in the number of respiratory hospital admissions for every 10 μg/$m^3$ increase in $PM_{2.5}$ (Wang, 2017). In addition, Rodopoulou et al. conducted a study in Doña Ana County, New Mexico and showed that a 10 μg/$m^3$ increase in $PM_{2.5}$ was associated with a 1.30% increase in the number of respiratory admissions. As reported in our study, $PM_{2.5}$ levels had a minimal effect on respiratory

**Table 7**
Association with a 10 μg/$m^3$ increase in air pollutants for respiratory hospital admissions using single, two pollutant models during 2014–2017.

| pollutant | Single pollutant model[a] | double pollutant model | | | | | |
|---|---|---|---|---|---|---|---|
| | | $PM_{2.5}$ | $PM_{10}$ | $NO_2$ | $SO_2$ | $O_3$8h | CO |
| $PM_{2.5}$ | 0.572 (0.087,1.061) | – | 0.585 (0.140,1.189) | 0.604 (0.336,1.355) | 0.526 (0.039,0.994) | 0.571 (0.077,1.068) | 0.550 (0.080,1.052) |
| $PM_{10}$ | 0.195 (0.009,0.382) | 0.157 (0.030,0.396) | – | 0.207 (0.039,0.415) | 0.199 (0.010,0.399) | 0.177 (0.016,0.370) | 0.156 (0.070,0.322) |
| $NO_2$ | −1.325 (-2.239,-0.402) | −1.543 (-2.689,-0.788) | −1.464 (-2.383,-0.536) | – | −1.181 (-2.178,-0.173) | −1.481 (-2.407,-0.546) | −1.298 (-2.336,-0.248) |
| $SO_2$ | 1.827 (0.527,3.145) | 1.697 (0.300,2.918) | 1.393 (0.009,2.795) | 2.028 (1.570,4.506) | – | 1.633 (0.011,2.672) | 1.507 (0.368,3.418) |
| $O_3$8h | 0.733 (0.263,1.205) | 0.663 (0.138,1.170) | 0.678 (0.111,1.048) | 0.786 (0.310,1.264) | 0.706 (0.075,1.222) | – | 0.654 (0.139,1.071) |
| CO | 2.579 (0.370,4.837) | 2.963 (0.839,4.946) | 2.703 (0.601,4.261) | 3.068 (2.453,6.750) | 2.311 (0.813,3.536) | 2.339 (0.275,4.502) | – |

Note. a Single-day lag of lag7 pollutants concentrations were used.

hospital admissions, and this might be attributed to the integrated control measures for PM implemented by the administration.

The associations between $PM_{10}$ and respiratory admissions in this study were consistent with those of previous studies (Fig. S2 in the supplemental materials). For example, a previous study in Hong Kong, China, showed that $PM_{10}$ had significant effects on the number of respiratory admissions, with an estimated increase of 1.6% (Wong et al., 1999). In 2010, Bao conducted a study in Urumchi, China, on the effect of exposure to $PM_{10}$ on respiratory function and showed that an increase of 10 μg/$m^3$ corresponded to a 6.7% (95% CI: 4.6%–9.5%) increase in hospital admissions (Bao 2013). In the study of Luo et al. in Hefei, a 3.1% (95% CI: 0.2%–6.0%) increase in respiratory hospital admissions was observed for every 10 μg/$m^3$ increase in daily $PM_{10}$ concentrations (Xie et al., 2019). Other studies performed in Christchurch, New Zealand (Mcgowan et al., 2002), Doña Ana County, New Mexico (Rodopoulou et al., 2014), Ho Chi Minh City, Vietnam (Phung et al., 2016), Arak, Iran (Vahedian et al., 2017), Istanbul, Turkey (Capraz et al., 2017) and Bangkok, Thailand (Phosri et al., 2019) showed that a 10 μg/$m^3$ increase in $PM_{10}$ was associated with 2.28%, 0.8%, 0.7%, 1.0%, 3.28% and 1.57% increments in the number of respiratory hospitalisations. Compared with the abovementioned studies, our study obtained a lower estimate of 0.328% (95% CI: 0.145%–0.511%). The variations in the adverse effect size might be attributed to the differences in research participants and $PM_{10}$ levels and its chemical constituents.

This study showed that $NO_2$ was negatively correlated with hospital admissions due to respiratory diseases. The causes of this interesting phenomenon might be complex and have not been fully elucidated. However, previous studies have supported our findings. For example, two other studies conducted by Chen et al. (2010) in Shanghai, China, and Vahedian et al. (2017) in Arak, Iran, did not find any significant relationship between $NO_2$ levels and respiratory hospitalisations. However, a significant association was observed between $NO_2$ and respiratory conditions in other previous studies. Wong et al. evaluated the correlation between $NO_2$ and respiratory hospitalisations in Hong Kong, China, and observed a positive association between them at different lag days (Wong et al., 1999). Bao conducted a study in Urumchi, China, and found an association between $NO_2$ and respiratory admissions. Moreover, a 10 μg/$m^3$ increase in $NO_2$ concentration at lag4 was correlated with a 5.10% increase in the number of respiratory inpatients (Bao, 2013). The study by Zhang et al. in Guangzhou, China, revealed an increase in the number of respiratory inpatients with elevated $NO_2$ concentrations (Zhang et al., 2014). Moreover, some studies conducted in Wuhan (Wang, 2017), Taiyuan (Luo et al., 2018), Rome, Italy (Fusco et al., 2001), Brisbane, Australia (Petroeschevsky et al., 2001), Ho Chi Minh City, Vietnam (Phung et al., 2016), Istanbul, Turkey (Capraz et al., 2017) and Bangkok, Thailand (Phosri et al., 2019) showed significant associations between $NO_2$ concentration and respiratory morbidity (Fig. S3 in the supplemental materials). Hence, the inconsistencies in the effect of $NO_2$ concentrations on respiratory admissions may be attributed to the differences in the type of $NO_2$ due to industrial emissions and motor vehicle exhaust between Lanzhou and other areas. For example, the local government in Lanzhou City encourages drivers to use clean energy cars, which may reduce the harmful impact of $NO_2$ on human health. However, more research should be conducted to identify any associations.

$SO_2$ is one of the primary contaminants in industrial areas, which are affected by both soot and motor vehicle exhaust. The $SO_2$ concentration in Lanzhou has been decreasing for several years. However, the adverse effect of $SO_2$ on respiratory diseases was substantially below the air quality standard levels in China. The results of this study were comparable with those of previous studies in China (Fig. S4 in the supplemental materials). Our findings were similar to those for other cities, such as Hong Kong (Wong et al., 1999), Urumchi (Bao, 2013) and Taiyuan (Luo et al., 2018), but not for Shanghai. Our study obtained a higher effect estimate of 3.005% (95% CI: 1.689%–4.339%). A comparison of the effect estimate between our study and those conducted in Rome (Fusco et al., 2001), Brisbane (Petroeschevsky et al., 2001), Ho Chi Minh (Phung et al., 2016), Arak (Vahedian et al., 2017) and Bangkok (Phosri et al., 2019) showed that the estimate of ER in Lanzhou was significantly larger than those in the first four cities. Only Bangkok had a lower estimate (Fig. S4 in the supplemental materials). Consequently, more effective and efficient governance measures must be taken to enhance the regulation of $SO_2$ pollution and prevent its detrimental effects in Lanzhou.

In the current study, the effects of CO on health were more evident and stronger than those of $PM_{2.5}$, $PM_{10}$, $NO_2$, $SO_2$ and $O_3$8h. This finding has been reported previously in other studies conducted worldwide (Fusco et al., 2001; Vahedian et al., 2017; Phosri et al., 2019) (Fig. S5 in the supplemental materials). Fusco et al. conducted a study in Rome, Italy and found that CO had adverse effects on the total number of respiratory admissions. In a study in 2017 in Arak, Iran, the total respiratory admissions increased by 9% for every 1 mg/$m^3$ increase in CO levels (Vahedian et al., 2017). In another study conducted in Bangkok, Thailand, a 1 mg/$m^3$ increase in CO was associated with a 7.69% (95% CI: 5.20%–10.23%) increase in the total number of respiratory cases (Phosri et al., 2019). However, a study in Wuhan, China, found a nonsignificant association between CO concentrations and the total number of respiratory hospital admissions (Wang, 2017). Toxicological studies have shown that when CO enters the body, it may not directly induce respiratory tract disorders, but it will significantly weaken red blood cells, impair oxygen carrying capacity and inhibit oxygen release. Therefore, respiratory symptoms may be exacerbated. CO pollution is still an environmental problem in Lanzhou, which is consequently associated with respiratory admissions, as revealed in this study. However, no studies conducted in Lanzhou have assessed the harmful effect of this pollutant. Therefore, the establishment of policies and protective measures should be based on scientific evidence regarding the effect of CO on local populations to reduce the incidence of respiratory diseases.

The association between $O_3$ and respiratory hospital admissions had been reported in different studies conducted worldwide. However, the results were inconsistent (Fig. S6 in the supplemental materials). One study in Hongkong (Wong et al., 1999) assessed the association between CO and respiratory hospital admission and showed a 2.20% increase in respiratory hospital admissions at lag03 for every 10 μg/$m^3$ increase in CO (Wong et al., 1999). In a study in 2001 in Brisbane, Australia, $O_3$ was found to be positively associated with respiratory hospital admissions (0.357% increase, lag2) (Petroeschevsky et al., 2001). Another study (Vahedian et al., 2017) found that $O_3$ was significantly associated with daily respiratory hospitalisations ($ER = 1.9\%$). Further, a study in Bangkok, Thailand, reported that a 10 μg/$m^3$ increase in $O_3$ concentration was associated with a 1.13% increase in the total number of respiratory hospitalisations (Phosri et al., 2019). Conversely, other studies conducted by Fuscoet al. (2001) in Rome, Italy, Wang (2017) in Wuhan and Luo et al. (2018) in Taiyuan, China, found that increments in $O_3$ level were negatively associated with the total number of respiratory hospital admissions. However, the estimated effect of $O_3$ on all respiratory hospitalisations in our study was smaller than those in other cities, such as Hong Kong, Arak and Bangkok. Differences in the results might be attributed to the varying $O_3$ levels worldwide, geographical locations, people's lifestyle and personal characteristics (such as age, sex, work and socioeconomic status).

Nevertheless, our study had several limitations. Firstly, air pollution data were collected from four fixed monitoring stations in Lanzhou. Hence, there might be some inevitable assessment error. Secondly, the respiratory admission data were from three large hospitals in the main urban area of Lanzhou. Therefore, the results have limited applicability to other situations. Thirdly, individual characteristics such as genetic factors, occupational exposure and smoking history, would affect the morbidity of respiratory diseases. Finally, our study did not perform analysis of individual information because of data unavailability, which might have affected the magnitude of the calculated estimates.

## 5. Conclusions

Our study results suggest that short-term exposure to ambient air pollutants except to $NO_2$ was associated with all respiratory hospitalisations in Lanzhou, and the detrimental effects of air pollutants on human health were detectable even below the air quality standard of China. This finding provides local decisionmakers more useful information to formulate measures for prevention and control of air pollution with the greatest health benefits. Moreover, we found that adverse respiratory effects of air pollution were not homogeneous, so differences in effect size might be associated with different seasons, sex and age groups. This study also observed that females and younger groups (<65 years old) were more sensitive to air pollutants.

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## Declaration of competing interest

The authors declare that they have no competing interests.

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## Abbreviations

| | |
|---|---|
| GAM | Generalized Additive Model |
| ER | Excessive Risk |

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2020.113658.

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# Seasonality of drinking water sources and the impact of drinking water source on enteric infections among children in Limpopo, South Africa

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ABSTRACT

Enteric infections and water-related illnesses are more frequent during times of relative water abundance, especially in regions that experience bimodal rainfall patterns. However, it is unclear how seasonal changes in water availability and drinking water source types affect enteric infections in young children. This study investigated seasonal shifts in primary drinking water source type and the effect of water source type on enteric pathogen prevalence in stool samples from 404 children below age 5 in rural communities in Limpopo Province, South Africa. From wet to dry season, 4.6% (n = 16) of households switched from a source with a higher risk of contamination to a source with lower risk, with the majority switching to municipal water during the dry season. In contrast, 2.6% (n = 9) of households switched from a source with a lower risk of contamination to a source with higher risk. 74.5% (n = 301) of the total households experienced interruptions in their water supply, regardless of source type. There were no significant differences in enteric pathogen prevalence between drinking water sources. Intermittent municipal water distribution and household water use and storage practices may have a larger impact on enteric infections than water source type. The limited differences in enteric pathogen prevalence in children by water source could also be due to other exposure pathways in addition to drinking water, for example through direct contact and food-borne transmission.

## 1. Introduction

Water quality varies greatly across South Africa, with rural areas being the most vulnerable to poor water access and/or quality (Meissner et al., 2018; Mellor et al., 2012). Health outcomes of poor drinking water quality include enteric infections, diarrhea, childhood stunting, anxiety and depression, and death (Abia et al., 2017; Liu et al., 2014; Pickering et al., 2019; Workman and Ureksoy, 2017). In South Africa, diarrhea is one of the leading causes of death among young children (Dorrington et al., 2014). Water, sanitation, and hygiene measures remain critically important to reduce exposure to pathogens causing water-borne illness, especially among children in low-resource settings, who are at the greatest risk from enteric infections and their associated symptoms and complications (Brown et al., 2013; Cumming et al., 2019; Rogawski McQuade et al., 2019).

Households may change their water source due to seasonal variations in rainfall and water availability or maintenance and governance issues (Bisung et al., 2015; Dos Santos et al., 2017; Heleba, 2011; Majuru et al., 2012). Therefore, access to water can vary across seasons and throughout the year. Differences in water source types used in the wet versus dry seasons may have different health implications and contribute to the seasonality of enteric infections observed in many contexts (Lal et al., 2012), such as the seasonality of *Shigella* in Limpopo, South Africa (Rogawski McQuade et al., 2020). For example, there may be an increase in infections in times of relative water abundance or the wet season (Ashbolt, 2015). This can be due to reductions in water quality, such as increased chemical or microbial contamination from runoff (Ngoye and Machiwa, 2004; Ouyang et al., 2006). In contrast,

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during times of water scarcity or the dry season, lack of water may negatively influence health due to unsanitary conditions, inadequate water availability for consumption and hygiene, and the reliance on alternative sources of water. Alternative water sources can increase the risk of water-related diseases if they are of lower water quality (Bradley and Bartram, 2013). Low-income households are the most vulnerable during periods of low water abundance (Majuru et al., 2016). Additionally, seasonality can affect resource mobilization and external support availability in communities (Holmatov et al., 2017; Kelly et al., 2018).

The risk of fecal contamination varies by water source type (Kostyla et al., 2015). Improved water sources include piped water, public tap or standpipe, borehole, protected dug well or spring, and rainwater. In contrast, unimproved water sources include unprotected dug well or spring, tanker truck, and surface water from rivers, dams, lakes, ponds, streams, canals, or irrigation channels (WHO & UNICEF, 2016). Unimproved water sources have significantly higher risk of being contaminated compared to improved water sources (Shields et al., 2015; Kumpel and Nelson, 2016). The use of water sources with higher contamination during times of lower water abundance is one reason why water-related disease prevalence may differ across seasons (Pearson et al., 2016).

We previously assessed total coliform bacteria and *E. coli* in local water sources (Edokpayi et al., 2018) and samples of drinking water from households (manuscript under review) within a community in Limpopo, South Africa. We documented high levels of contamination of water sources in this area, which changed seasonally. Here, we further analyze data collected during this study to describe changes in primary water sources of households across wet and dry seasons and investigate how water source type affects the risk of enteric infections in children under the age of 5 in this community.

## 2. Material and methods

### 2.1. Procedures

We analyzed data from a randomized controlled trial of the impact of low-cost point-of-use water treatment technologies on enteric infections and linear growth among children in Limpopo, South Africa. The study design and primary results have been previously reported (Hill et al., 2020). Briefly, the study enrolled one child under the age of 3 from 404 households in rural villages from the Dzimauli community in Limpopo, South Africa. Participant households were recruited from 18 villages and completed a baseline questionnaire between June–November 2016. Smaller villages were combined into two larger groups (n = 40 in Group 1 and n = 64 in Group 2) for data analysis. The remainder of villages were analyzed by village (Fig. 1).

The baseline questionnaire included questions relating to demographics, primary drinking water sources, and water use practices of the child and their household. Primary drinking water sources were classified into 5 categories: municipal, surface water from tap/pipe, directly from surface, groundwater, and other/unknown. The five water source types are defined in Table 1. Mutale Water Works is the municipal water treatment plant for the communities in this study, as shown in Fig. 1. However, not all households necessarily receive and use municipal water due to inadequate infrastructure and intermittent supply. Demographics recorded for each child included age, sex, and socioeconomic status, defined by the WAMI index, a score based on water and sanitation access, asset ownership, maternal education, and average monthly household income (Psaki et al., 2014). Additionally, the primary caregivers' demographics were collected, including their mother's age and education level. Water use practices included drinking water storage type and treatment. Drinking water storage types included metal and plastic buckets, jerry cans, and plastic bottles, while typical drinking water treatment included letting the water stand and settle, adding chlorine, boiling, and no treatment.

**Table 1**
Primary drinking water source type classification.

| Water Source Type | Definition |
|---|---|
| Municipal | Piped from municipal water treatment plant |
| Surface water from tap/pipe | Piped from local surface water sources, such as the Mutale River, a lined canal, pond, or a shallow/hand-dug well |
| Directly from surface | Directly obtained from local surface water sources |
| Groundwater | Obtained from a spring or borehole |
| Other/unknown | Other or unknown water source |

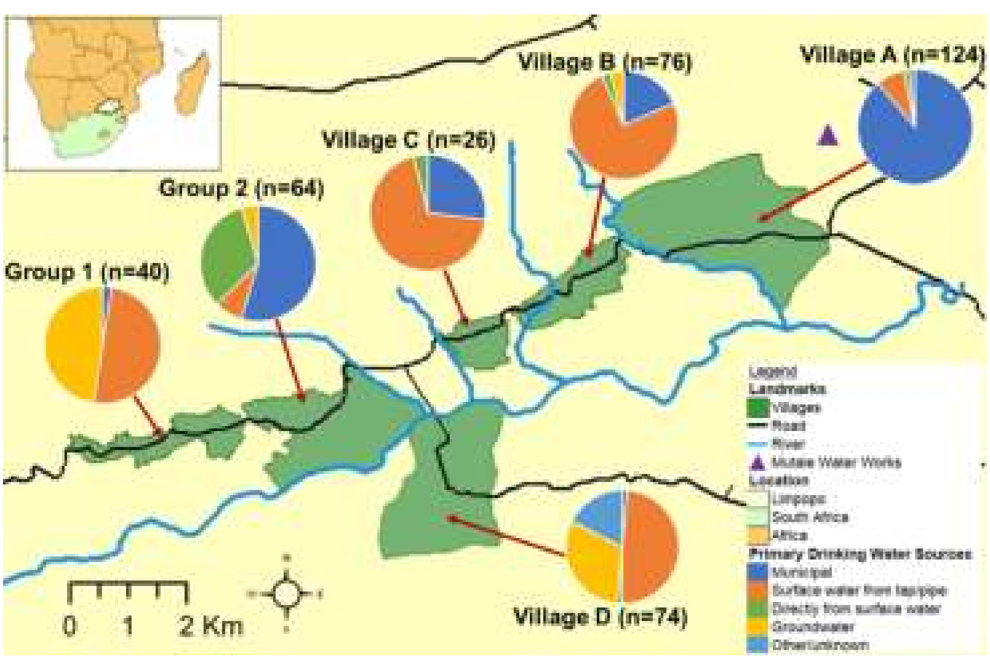


**Fig. 1.** Map of study villages with prevalence of primary drinking water sources. Top left inset map highlights the Dzimauli community (study area; black point), in Limpopo province (light tan), South Africa (green). The location of the municipal water treatment plant, Mutale Water Works, is highlighted with a purple triangle. Source: ArcMap GIS. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Field researchers conducted follow-up questionnaires and collected a stool sample from the youngest child under 3 years of age every 3 months. During each visit, a questionnaire was given to the child's caregiver to collect information on primary water sources, water use practices, and recent health information about the child, including diarrhea in the past 7 days. Stool samples were stored at −70 °C before testing, which was conducted in batches every 6 months. DNA was extracted from stool using the Qiagen QIAamp Fast DNA Stool MiniKit (Qiagen, Valencia, VA, USA) and tested for enteropathogens using multiplex real-time PCR, as previously described (Hill et al., 2020). Targeted genes for amplification identified enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli*/enteropathogenic *E. coli* (EHEC/E-PEC), *Giardia, Campylobacter, Cryptosporidium,* enterotoxigenic *E. coli* (ETEC), *Shigella*, and adenovirus (Liu et al., 2014). Detections at a PCR cycle threshold of <35 were considered positive as previously (Hill et al., 2020).

### 2.2. Data analysis

All analyses were conducted using SAS software (version 9.4). We categorized the wet season from November to March, and the dry season from April to October. We categorized drinking water source for each household by season and assessed changes in primary water source between wet and dry seasons. In a minority of cases, households used more than 2 types of water sources during one wet/dry season, in which case, the water source for that season was categorized as "mixed." If a change in water source occurred between seasons, the change was noted if it was to a water source with a higher risk or lower risk of contamination. "Municipal" and "groundwater" were classified as low risk, improved water sources while "surface water from tap/pipe," directly from surface water," and "Other/unknown" was classified as high risk, unimproved water sources (WHO & UNICEF, 2016).

We estimated prevalence ratios for the effects of primary water

**Table 2**
Baseline characteristics by primary drinking water source among 404 children.

| | Primary Drinking Water Source | | | | |
|---|---|---|---|---|---|
| | Municipal (n = 168) | Surface water from tap/pipe (n = 146) | Directly from surface water (n = 27) | Groundwater (n = 48) | Other/unknown (n = 15) |
| *Child Characteristics* | | | | | |
| Sex, n (%) | | | | | |
| Male | 82 (48.8%) | 72 (49.3%) | 13 (48.2%) | 21 (43.8%) | 7 (46.7%) |
| Female | 86 (51.2%) | 74 (50.7%) | 14 (51.8%) | 27 (56.2%) | 8 (53.3%) |
| Average Age of Child (years), n (%) | | | | | |
| <1 | 60 (35.7%) | 60 (41.1%) | 11 (40.7%) | 15 (31.3%) | 9 (60.0%) |
| 1–2 | 62 (36.9%) | 52 (35.6%) | 8 (29.6%) | 20 (41.7%) | 4 (26.7%) |
| 2–3 | 46 (27.4%) | 34 (23.3%) | 8 (29.6%) | 13 (27.0%) | 2 (13.3%) |
| Socioeconomic Status Score (WAMI)[a] | | | | | |
| Mean (SD) | 0.80 (±0.10) | 0.78 (±0.10) | 0.63 (±0.10) | 0.80 (±0.13) | 0.83 (±0.09) |
| Length/height-for-age z-score at baseline | | | | | |
| Mean (SD)[b] | −1.41 (±1.22) | −1.38 (±1.28) | −1.43 (±1.38) | −1.18 (±1.23) | −1.38 (±1.98) |
| Weight-for-age z-score at baseline | | | | | |
| Mean (SD)[b] | −0.35 (±1.19) | −0.28 (±1.21) | −0.58 (±1.46) | −0.34 (±1.22) | −0.21 (±1.31) |
| Height-for-weight z-score at baseline | | | | | |
| Mean (SD)[b] | 0.55 (±1.38) | 0.64 (±1.39) | 0.25 (±1.45) | 0.39 (±1.44) | 1.25 (±1.60) |
| Stunted at baseline, n (%)[b] | 54 (32.5%) | 45 (31.9%) | 9 (33.3%) | 10 (21.3%) | 7 (53.9%) |
| Underweight at baseline, n (%)[b] | 15 (9.0%) | 10 (6.9%) | 4 (14.8%) | 4 (8.3%) | 1 (6.7%) |
| Wasted at baseline, n (%)[b] | 6 (3.6%) | 4 (2.8%) | 1 (3.7%) | 4 (8.5%) | 0 |
| *Child's Mother's Demographics* | | | | | |
| Mother's age, mean (SD; years) | 27.7 (±6.7) | 28.0 (±6.5) | 29.0 (±7.8) | 28.0 (±6.4) | 25.6 (±5.6) |
| Highest school grade level of mother, n (%) | | | | | |
| None | 1 (0.6%) | 0 | 0 | 0 | 0 |
| Primary | 7 (4.2%) | 4 (2.8%) | 2 (7.4%) | 1 (2.1%) | 0 |
| Secondary | 99 (58.9%) | 90 (62.9%) | 17 (63.0%) | 23 (47.9%) | 8 (57.1%) |
| Matriculation | 36 (21.4%) | 41 (28.7%) | 7 (25.9%) | 11 (22.9%) | 2 (14.3%) |
| Undergraduate | 18 (10.7%) | 6 (4.2%) | 1 (3.7%) | 7 (14.6%) | 4 (28.6%) |
| Post-graduate | 7 (4.2%) | 2 (1.4%) | 0 | 6 (12.5%) | 0 |
| Missing | 0 | 0 | 3 | 0 | 1 |
| *Water Use Practices* | | | | | |
| Typical drinking water treatment, n (%) | | | | | |
| Let stand and settle | 8 (4.8%) | 2 (1.3%) | 0 | 1 (2.1%) | 0 |
| Add bleach/chlorine | 5 (3.0%) | 8 (5.5%) | 1 (3.7%) | 1 (2.1%) | 0 |
| Boil | 12 (7.1%) | 14 (9.6%) | 1 (3.7%) | 6 (12.5%) | 0 |
| Other | 3 (1.8%) | 0 | 0 | 0 | 0 |
| None | 140 (83.3%) | 122 (83.6%) | 25 (92.6%) | 40 (83.3%) | 15 (100.0%) |
| Covered water storage vessels, n (%) | 138 (82.1%) | 122 (83.6%) | 14 (51.9%) | 41 (85.4%) | 13 (86.7%) |
| Drinking water storage type, n (%) | | | | | |
| Metal buckets | 5 (3.0%) | 6 (4.1%) | 0 | 0 | 0 |
| Plastic buckets | 71 (42.3%) | 57 (39.0%) | 10 (37.0%) | 21 (43.8%) | 3 (20.0%) |
| Jerrycan | 81 (48.2%) | 74 (50.7%) | 16 (59.3%) | 20 (41.7%) | 9 (60.0%) |
| Plastic bottles | 1 (0.6%) | 2 (1.4%) | 1 (3.7%) | 1 (2.1%) | 0 |
| Other | 10 (6.0%) | 7 (4.8%) | 0 | 6 (12.5%) | 3 (20.0%) |
| Continuous main water supply, n (%) | | | | | |
| Continuous | 3 (1.8%) | 36 (24.7%) | 23 (85.2%) | 27 (56.3%) | 14 (93.3%) |
| Sometimes interrupted | 165 (98.2%) | 110 (75.3%) | 4 (14.8%) | 21 (43.8%) | 1 (6.7%) |

[a] Psaki et al., 2014.
[b] Baseline length/height unavailable for 10 (2.5%) children; baseline weight unavailable for 1 (0.2%) child.

source on the prevalence of enteric infections using log-binomial regression. Poisson regression was used for a combined outcome of the total count of positive pathogen detections. Generalized estimating equations were used in both models to account for correlation between stool samples from the same child, and models were adjusted for age, socioeconomic status, season (wet/dry), and randomization group for the water treatment intervention.

## 3. Results

Study participants were geographically distributed within the Dzimauli community in Limpopo, South Africa. 30.7% (n = 124) of study participants were from Village A, 18.8% (n = 76) from Village B, 18.3% (n = 74) from Village D, 15.8% (n = 64) from Group 2, 9.9% (n = 40) from Group 1, and 6.4% (n = 26) from Village C (Fig. 1). Surface water from tap/pipe was the most common source of drinking water in 4 out of the 6 village groups. In the remaining 2 village groupings, the most common source of drinking water was municipal. Village A had the largest number of participants (n = 124) with 89% (n = 110) relying on municipal water and most of the rest (8%, n = 10) obtaining surface water from tap/pipe as their primary source of drinking water. Approximately a third (n = 21, 32.8%) of participants in Group 2 obtained their drinking water directly from surface water (Fig. 1).

The study population consisted of 404 children under the age of 3, with the highest percentage of children (38.4%) being under the age of 1 year old. 48.3% of the study children were male and 51.7% were female. Across both study years, 41.6% (n = 168) households used municipal water as their primary source of drinking water, 36.1% (n = 146) used surface water from tap/pipe, 6.7% (n = 27) used direct collection from surface water, 11.9% (n = 48) used groundwater, and 3.7% (n = 15) used another or unknown source (Table 2). No form of drinking water treatment was used in most households across drinking water source groups (n = 342, 84.7%). While water treatment was rare prior to consumption, boiling water was the most used form of treatment (n = 33, 8.2%). In terms of drinking water storage, the majority of participants used covered storage vessels (n = 328, 81.2%), with jerrycans (n = 200, 49.5%) being the most common, followed by plastic buckets (n = 162, 40.1%), regardless of their water source. Water supply was not continuously available (was interrupted) for 14.8% (n = 4) of those who relied on direct collection from surface water, but interruptions were much more common for those who relied on municipal (n = 165, 98.2%), surface water from tap/pipe (n = 110, 75.3%), and groundwater (n = 21, 43.8%; Table 2).

In terms of childhood growth, the highest prevalence of stunting at baseline was found among children who used other/unknown water sources (n = 7, 53.9%), while the highest prevalence of underweight was found among children who used direct collection from surface water (n = 4, 14.8%). Lastly, wasting was most common among children who used groundwater (n = 4, 8.5%). Across all groups, the average age of the child's mother was 28 years old, with the most common school grade completed by the mother being secondary school. The average socioeconomic status score was the lowest for those who used directly from surface water (mean WAMI score: 0.63) compared to the other groups (mean WAMI score: 0.80).

### 3.1. Seasonality of water sources

Changes in primary drinking water sources across wet and dry seasons over the 2 years of the study were rare. A majority of the households (n = 275, 78.3%) used the same water source year-round (Table 3). Of those that changed water sources, 2.6% (n = 9) switched to a higher risk source. The majority of those who changed to a higher risk source (n = 6, 66.7%) went from using groundwater during the wet season to surface water during the dry season. Contrastingly, 4.6% (n = 16) of households switched to a lower risk source, with the majority switching to municipal water during the dry season (Table 3).

### 3.2. Enteric infections

Overall, EAEC, EHEC/EPEC, *Giardia, and adenovirus* infections were the most prevalent across the different water source groups (Fig. 2). While the prevalence ratios for some enteric infections suggested small differences in infection prevalence across drinking water source types, the estimates were imprecise given the small numbers of infections for some water sources (Table 4). There were no significant differences in enteric infections between drinking water sources. Additionally, there were no differences in the total number of pathogens detected among children drinking surface water from tap/pipe (adjusted prevalence ratio (aPR): 0.99, 95% CI: 0.89, 1.10), directly from surface (aPR: 0.92, 95% CI: 0.70, 1.20), groundwater (aPR: 0.98, 95% CI: 0.87, 1.11), and other/unknown source (aPR: 0.95, 95% CI: 0.71, 1.27) compared to municipal water. The prevalence of *Giardia* and ETEC infections among children who drank groundwater were 1.21 (95% CI: 0.94, 1.55) and 1.19 (95% CI: 0.84, 1.69) times the prevalence of those infections among those who drank municipal water over the 2-year follow-up period (Table 4). EHEC/EPEC infections among children who relied directly from surface water was 1.19 (95% CI: 0.87, 1.62) times as likely as those infections among those who relied on municipal water.

## 4. Discussion

We investigated seasonal shifts in primary drinking water source type and the effect of water source type on pathogen prevalence in children in rural communities in Limpopo Province, South Africa. Overall, changes in primary drinking water sources across seasons were small. Higher risk sources were more common during the wet season. Both of these findings were similarly found in a study by Pearson et al. (2016) in Uganda and Tanzania. Our study area and those in Pearson et al. (2016) in rural communities exhibit a unimodal dry/wet seasonality with an extended dry season in a semi-arid climate. Pearson et al. (2016) found that more households used lower risk sources during the dry season, compared to the wet season, as we did in South Africa. Lower risk source usage in the dry season would suggest lowest risk for enteric infections in the dry season according to Kostyla et al. (2015). This is additionally supported from earlier microbiological analyses of water samples from our study area that found that bacterial levels of surface

**Table 3**
Number of participants who changed their primary drinking water source from the wet to dry season over 2 years.

| | | Dry Season Water Source | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Municipal | Surface Water from Tap/Pipe | Directly from Surface | Groundwater | Other/ Unknown | Mixed | Wet Season Total |
| Wet Season Water Source | Municipal | 121 | 2 | 1 | 1 | 0 | 7 | 132 |
| | Surface Water from Tap/Pipe | 8 | 102 | 2 | 3 | 0 | 15 | 130 |
| | Directly from Surface | 0 | 0 | 9 | 1 | 0 | 5 | 15 |
| | Groundwater | 1 | 5 | 1 | 43 | 0 | 11 | 61 |
| | Other/Unknown | 2 | 1 | 0 | 2 | 0 | 1 | 6 |
| | Mixed | 3 | 2 | 0 | 0 | 0 | 2 | 7 |
| | Dry Season Total | 135 | 112 | 13 | 50 | 0 | 41 | 351 |

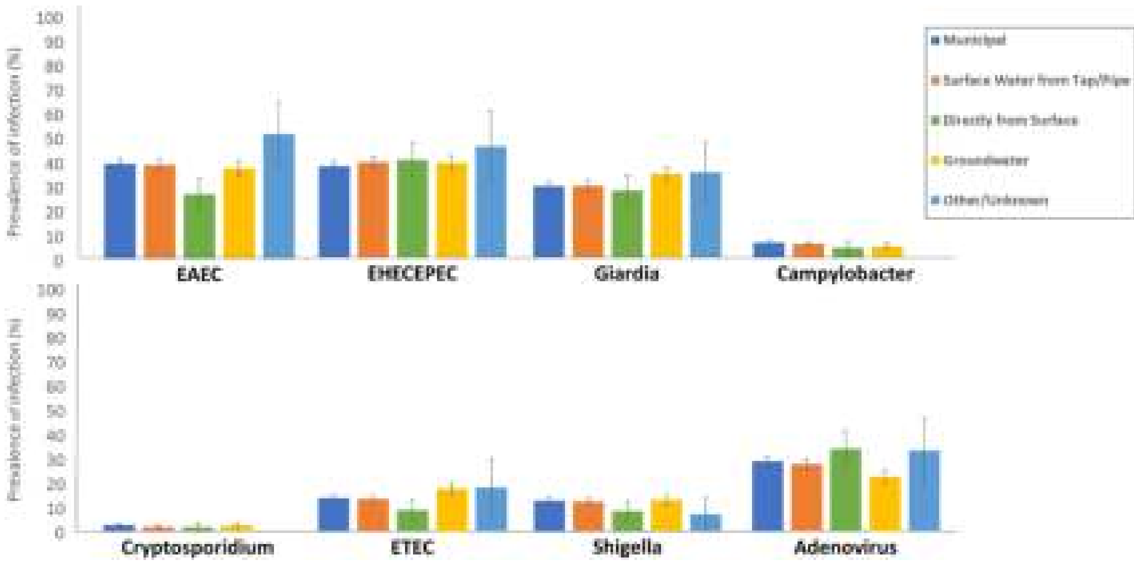


**Fig. 2.** Prevalence of enteric infections over 2 years of follow-up by primary drinking water source among 404 children. Error bars denote standard errors. EAEC: enteroaggregative *E. coli*; EHEC/EPEC: enterohemorrhagic *E. coli*/enteropathogenic *E. coli*; ETEC: enterotoxigenic *E. coli*.

water sources were higher in the wet season than in the dry season (Edokpayi et al., 2018). The increase in bacteria during the wet season could be caused by increased runoff carrying bacteria from contaminated sources to these bodies of water.

There were no significant differences in enteric pathogen prevalence between drinking water sources. Earlier microbiological analysis from this study (Edokpayi et al., 2018) found that while municipal water never tested positive for fecal contamination from *E. coli* from 2016 to 2017, intermittent water distribution and prolonged storage made municipal water an inconsistent water source with high risk of recontamination during storage. There was a high prevalence of total coliform bacteria in household-stored water in those who used municipal water, even though the municipal water had no fecal contaminants when sampled directly from the tap (Edokpayi et al., 2018). Intermittent availability could therefore result in similar levels of contamination between "high risk" and "low risk" water sources and could explain why households might switch from lower risk municipal water to a higher risk source regardless of season. Prior research suggests that water contamination often occurs during storage through the use of water transfer devices, using a hand or bowl to transfer from the storage container for consumption (Heitzinger et al., 2015; Packiyam et al., 2016; Wright et al., 2018). These factors may have a larger effect on enteric infection than water source type, since a majority of households experienced interrupted water supply and practiced no form of drinking water treatment regardless of water source type. Water quantity and consistency of availability for consumption and hygiene may ultimately be more important for preventing transmission of certain enteric pathogens than contamination of the water source.

The limited effects of water source on enteric pathogen prevalence in children could also be due to the fact that children were exposed through other pathways in addition to drinking water. All of the pathogens observed are transmitted via the fecal-oral route, where direct person-to-person contact and indirect contact through contaminated food are examples of other potential transmission pathways through which children could be exposed (Anim-Baidoo et al., 2016; Penakalapati et al., 2017). Specifically, *Campylobacter* is a leading foodborne bacterial pathogen, generally through undercooked meat and raw milk, while *Shigella* tends to be transmitted from person-to-person (Lampel et al., 2018; Luangtongkum et al., 2009). Additionally, this study was limited by only analyzing primary drinking water source data. We did not consider that households may use more than one drinking water source or use separate sources for different household purposes, like cooking and bathing. Children could be exposed to enteric pathogens through water or contact with infected persons from outside sources, for example neighbors, non-household family members, or daycare centers, or through eating contaminated foods.

Access to safe and clean drinking water is a critical determinant of health, with social, economic, and environmental consequences. Especially among young children who are at the greatest risk for enteric infections, contaminated drinking water can contribute to environmental enteropathy, a syndrome marked by increased intestinal permeability, impaired immune function in the gut, and malabsorption, which may lead to child growth stunting (Korpe and Petri, 2012). Because households' primary drinking water source can vary across seasons, water sources and the potential for seasonal differences are key components that need to be evaluated to ensure equitable access to safe drinking water year-round. Future research could involve qualitatively investigating the decision-making process behind drinking water source choice and reasons for changes in water sources. Understanding these dynamics as well as how water is used and stored are important to reduce enteric pathogen exposure from high risk sources and could be useful in community planning, distribution, and management of clean water.

Increasing access to, and the consistency of, the municipal water supply are clear opportunities to improve water quality in Limpopo. Access to municipal water varied by village in the same community, but was not related to distance from the municipal water treatment plant. This may reflect varied ability of the traditional leadership across villages to negotiate with the municipal leadership to improve water access based on the requests of community members. Previous research in Dzimauli has documented strong citizen engagement through traditional authority structures to advocate for improved water services (Bulled, 2017). However, these engagement efforts may be more successful among individuals with higher socioeconomic or political status, ultimately resulting in inequitable access. The South African constitution documents that water is a human right (Bill of Rights, 1996); continued advocacy to ensure continuous supply equitably throughout communities is a key step towards achieving this right for all. Increasing access to and consistency of municipal water is one of the many opportunities to improve health outcomes in this community.

**Table 4**
Effect of primary drinking water source on prevalence of enteric pathogens over 2 years.

| Pathogen | Drinking Water Source | Samples Tested N | Positive Cases N *(%)* | Prevalence Ratio[a] (95% CI) |
|---|---|---|---|---|
| EAEC | Municipal | 461 | 189 (41.0) | 1 |
| | Surface Water from Tap/Pipe | 373 | 151 (40.5) | 1.02 (0.88, 1.18) |
| | Directly from Surface | 43 | 12 (27.9) | 0.78 (0.43, 1.40) |
| | Groundwater | 225 | 88 (39.1) | 0.96 (0.81, 1.13) |
| | Other/Unknown | 13 | 7 (53.9) | 1.00 (0.53, 1.88) |
| EHEC/EPEC | Municipal | 440 | 181 (41.1) | 1 |
| | Surface Water from Tap/Pipe | 369 | 158 (42.8) | 1.05 (0.89, 1.23) |
| | Directly from Surface | 41 | 18 (43.9) | 1.19 (0.87, 1.62) |
| | Groundwater | 214 | 91 (42.5) | 1.04 (0.87, 1.24) |
| | Other/Unknown | 10 | 5 (50.0) | 1.22 (0.68, 2.18) |
| *Giardia* | Municipal | 462 | 149 (32.3) | 1.00 |
| | Surface Water from Tap/Pipe | 375 | 121 (32.4) | 1.03 (0.82, 1.29) |
| | Directly from Surface | 43 | 13 (30.2) | 0.91 (0.60, 1.39) |
| | Groundwater | 225 | 85 (37.8) | 1.21 (0.94, 1.55) |
| | Other/Unknown | 13 | 5 (38.5) | 1.44 (0.77, 2.69) |
| *Campylobacter* | Municipal | 459 | 33 (7.2) | 1 |
| | Surface Water from Tap/Pipe | 371 | 24 (6.5) | 0.92 (0.56, 1.51) |
| | Directly from Surface | 43 | 2 (4.7) | 0.91 (0.22, 3.71) |
| | Groundwater | 224 | 12 (5.4) | 0.72 (0.38, 1.37) |
| | Other/Unknown | 12 | 0 (0.0) | - |
| *Cryptosporidium* | Municipal | 461 | 15 (3.4) | 1 |
| | Surface Water from Tap/Pipe | 373 | 9 (2.4) | 0.68 (0.32, 1.47) |
| | Directly from Surface | 43 | 1 (2.3) | 0.68 (0.08, 5.64) |
| | Groundwater | 225 | 7 (3.1) | 1.03 (0.44, 2.38) |
| | Other/Unknown | 13 | 0 (0.0) | - |
| ETEC | Municipal | 431 | 66 (15.3) | 1 |
| | Surface Water from Tap/Pipe | 358 | 53 (14.8) | 0.99 (0.71, 1.38) |
| | Directly from Surface | 41 | 4 (9.8) | 0.78 (0.32, 1.93) |
| | Groundwater | 210 | 41 (19.5) | 1.19 (0.84, 1.69) |
| | Other/Unknown | 10 | 2 (20.0) | 1.42 (0.51, 3.94) |
| *Shigella* | Municipal | 458 | 64 (14.0) | 1 |
| | Surface Water from Tap/Pipe | 374 | 51 (13.6) | 0.96 (0.69, 1.33) |
| | Directly from Surface | 43 | 4 (9.3) | 0.72 (0.32, 1.64) |
| | Groundwater | 224 | 32 (14.3) | 1.02 (0.71, 1.46) |
| | Other/Unknown | 13 | 1 (7.7) | 0.49 (0.08, 3.18) |
| Adenovirus | Municipal | 441 | 139 (31.5) | 1 |
| | Surface Water from Tap/Pipe | 342 | 103 (30.1) | 0.95 (0.77, 1.17) |
| | Directly from Surface | 35 | 13 (37.1) | 1.25 (0.82, 1.92) |
| | Groundwater | 205 | 50 (24.4) | 0.78 (0.59, 1.03) |
| | Other/Unknown | 11 | 4 (36.7) | 0.99 (0.48, 2.07) |
| Total Pathogens Detected[b] | Municipal | 462 | 1.81 (±1.41) | 1 |
| | Surface Water from Tap/Pipe | 375 | 1.79 (±1.41) | 0.99 (0.89-1.10) |
| | Directly from Surface | 43 | 1.56 (±1.20) | 0.92 (0.70-1.20) |
| | Groundwater | 225 | 1.80 (±1.41) | 0.98 (0.87-1.11) |
| | Other/Unknown | 13 | 1.85 (±0.90) | 0.95 (0.71-1.27) |

[a] Adjusted for age, SES, season, and water treatment randomization group.
[b] Effects estimated are mean (standard deviation) number of pathogens detected and detection rate ratios.

## Declaration of conflicts of interest

Dr. Dillingham provides consulting services to Warm Health Technology, Inc and has received a grant for an investigator-initiated project from Gilead Sciences, Inc. All other authors declare no conflict of interests.

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# Short term effects of air pollutants on hospital admissions for respiratory diseases among children: A multi-city time-series study in China

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A B S T R A C T

Evidence concerning short-term acute association between air pollutants and hospital admissions for respiratory diseases among children in a multi-city setting was quite limited. We conducted a time-series analysis to evaluate the association of six common air pollutants with hospital admissions for respiratory diseases among children aged 0–14 years in 4 cities (Guangzhou, Shanghai, Wuhan and Xining), China during 2013–2018. We used generalized additive models incorporating penalized smoothing splines and random-effect meta-analysis to calculate city-specific and pooled estimates, respectively. The exposure-response relationship curves were fitted using the cubic spline regression. Subgroup analyses by gender, age, season and disease subtype were also performed. A total of 183,036 respiratory diseases hospitalizations were recorded during the study period, and 94.1% of the cases were acute respiratory infections. Overall, we observed that increased levels of air pollutants except $O_3$, were significantly associated with increased hospital admissions for respiratory disease. Each 10 μg/m$^3$ increase in $PM_{2.5}$, $SO_2$ and $NO_2$ at lag 07, $PM_{10}$ at lag 03 and per 1 mg/m$^3$ increase in CO at lag 01 corresponded to increments of 1.19%, 3.58%, 2.23%, 0.51% and 6.10% in total hospitalizations, respectively. Generally, exposure-response relationships of $PM_{2.5}$ and $SO_2$ in Guangzhou, $SO_2$, $NO_2$ and CO in Wuhan, as well as $SO_2$ and $NO_2$ in Xining with respiratory disease hospitalizations were also found. Moreover, the adverse effects of these pollutants apart from $PM_{2.5}$ in certain cities remained significant even at exposure levels below the current Chinese Ambient Air Quality Standards (CAAQS) Grade II. Children aged 4–14 years appeared to be more vulnerable to the adverse effects of $PM_{2.5}$, $SO_2$ and $NO_2$. Furthermore, with the exception of $O_3$, the associations were stronger in cold season than in warm season. Short-term exposure to $PM_{2.5}$, $SO_2$, $NO_2$ and CO were associated, in dose-responsive manners, with increased risks of hospitalizations for childhood respiratory diseases, and adverse effects of air pollutants except $PM_{2.5}$ held even at exposure levels below the current CAAQS Grade II in certain cities.

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## 1. Introduction

The WHO estimates that approximately 90% of people worldwide breathe polluted air by 2018, particularly in low- and middle-income countries where ambient air pollution levels remain high and almost stable (Osseiran, 2018). Children are more vulnerable to the detrimental effect of air pollution due to their narrower airways, developing lung functions, immature immune system and longer stay-time outdoors (Schraufnagel et al., 2019). It is estimated that 0.41 million children deaths per year are resulted from outdoor air pollution (Landrigan et al., 2019). Increasing evidence has shown the acute and chronic adverse effects of ambient air pollutants on increased risks of respiratory morbidity and mortality among children (Darrow et al., 2014; Gouveia et al., 2018; Lelieveld et al., 2018; Luong et al., 2020).

Previous single city studies have observed the relationships between short-term or long-term particulate matter (PM) exposure and childhood hospital admissions due to respiratory diseases (Horne et al., 2018; Liu et al., 2015; Luong et al., 2020; Nhung et al., 2017), especially for asthma (Hua et al., 2014; Iskandar et al., 2012) and pneumonia (César et al., 2016; Li et al., 2018; Nhung et al., 2017; Wang et al., 2019). Meanwhile, short-term health effects of gaseous pollutants ($SO_2$, $NO_2$, CO and $O_3$), far less than that of PM, have also been revealed (Carvalho et al., 2018; Li et al., 2018; Nhung et al., 2019; Zhang et al., 2019). Nevertheless, some studies have observed that the adverse effects of gaseous pollutants were higher than PM (Zheng et al., 2017; Zhu et al., 2017), which may suggest more attention should be paid to gaseous pollutants. Although most of previous studies have showed a positive effect of these pollutants on childhood respiratory health, negative or null results were also found (Liu et al., 2015; Parker et al., 2009; Zhu et al., 2017). The inconsistency might arise from different study regions and periods, healthy outcomes, population characteristics, pollution level and composition, and meteorological conditions, etc. Notably, publication bias may be present, thus particular caution should be taken in comparing and generalizing risk estimates between single-city studies (Anderson et al., 2005). As a result, it is imperative to explore health risks in a multicity or national setting to generate persuasive and precise effect estimates.

Some multicity studies have reported positive associations of air pollutants with respiratory disease hospitalizations (Agudelo-Castaneda et al., 2019; Atkinson et al., 2001; Liu et al., 2018; Qiu et al., 2018; Son et al., 2013; Tian et al., 2018, 2020, 2019), but majority of them focused on adults or all ages populations. Up to date, only one study reported strong and consistent associations of $PM_{10}$, $PM_{2.5}$, $SO_2$ and $NO_2$ with short-term increases in hospital admissions among children less than 14 years old in Australia and New Zealand (Barnett et al., 2005), but it didn't explore the exposure-response relationships. China is the biggest developing country experiencing severe air pollution. However, no multi-city study has been conducted to evaluate air pollutant-related hospitalizations due to respiratory disease among children in China. Besides, it is still unclear about the characteristics of susceptibility to the air pollution-related childhood respiratory effects.

Therefore, we aimed to investigate the short-term effect of six common air pollutants on pediatric hospitalizations for respiratory disease in four cities (Wuhan, Shanghai, Guangzhou and Xining) of China during 2013–2018, and explore possible expose-response relationships and susceptible characteristics.

## 2. Materials and methods

### 2.1. Study area

The study was conducted in four Chinese cities including Guangzhou, Shanghai, Wuhan and Xining, which varied in pollution levels and compositions, geographical characters and meteorological conditions. The major air pollution sources in Guangzhou and Shanghai are vehicle exhaust emission, traffic, and industrial activities. Xining is mainly marked by open pollution sources such as soil, construction cement and road dust. However, Wuhan has a typical mixed pollution mode covering dust, process sources (coal-fired power plants, industrial kilns), stationary combustion sources (coal burning) and motor vehicles (Zhao et al., 2016). On the other hand, Guangzhou, Shanghai and Wuhan have a sub-tropical monsoon climate (hot summer, warm winter, and abundant rainfall) with simultaneous rain and heat over the same period. Xining has a typical mainland plateau climate that featured with relatively longer period of cold season, and prevailing strong wind and sandstorm during spring.

### 2.2. Hospital admissions

Daily data of hospital admission for respiratory diseases of children aged $\leq$14 years was collected from eight hospitals in four cities from January 1, 2013 to December 31, 2018. Daily counts of hospitalizations respiratory diseases were coded in terms of the *International Classification of Diseases, the 10*th *Revision (ICD-10)* as follows: total respiratory disease (J00-J99), acute upper respiratory infections (AURIs, J00-J06) including common cold, nasosinusitis, pharyngitis, tonsillitis, laryngitis, laryngopharyngitis, tracheitis and other acute multisite upper respiratory tract infections; acute lower respiratory infections (ALRIs) including pneumonia (J12-J18) and bronchitis (J20-J22), asthma (J45, J46) and other respiratory diseases.

### 2.3. Air pollutants and meteorological conditions

Real-time hourly data of fine particles with aerodynamic diameter $\leq$2.5 μm ($PM_{2.5}$), inhalable particles with aerodynamic diameter $\leq$10 μm ($PM_{10}$), sulfur dioxide ($SO_2$), nitrogen dioxide ($NO_2$), carbon monoxide (CO), and 8-h maximum moving average for ozone ($O_3$) were obtained from the National Urban Air Quality monitoring network (http://106.37.208.233:20035/), which is running by the China National Environmental Monitoring Center. There are 35 air-monitoring stations in four cities, including 11 in Guangzhou, 10 in Shanghai, 10 in Wuhan and 4 in Xining. Daily mean air pollutants concentrations were averaged from the above monitoring stations in each city. For the calculation of 24-h average concentrations, at least 75% of the hourly values had to be available in a particular day. We excluded the entire monitoring station as long as there were more than 25% missing values during the whole period. Detailed information of measurement methods has been reported previously (Chen et al., 2011). In each city, parallel time-series of daily mean temperature and relative humidity were extracted from the China Meteorological Data Sharing Service System (http://data.cma.cn/).

### 2.4. Statistical analysis

The data of daily hospital admissions, air pollution levels and weather conditions were linked by date for each city. We applied quasi-Poisson regression in generalized additive models (GAMs) to evaluate the effects of air pollutants on hospital admissions for respiratory disease. To control for the long-term trend and seasonality, a natural spline smooth function with 7 degree of freedom (*df*) per year for time was brought into the model as empirical (Zheng et al., 2017). We also included natural smooth functions of current day mean temperature with 6*df* and relative humidity with 3*df* to control the effect of meteorological factors (Chen et al., 2012a, 2012b; Li et al., 2015) and indicator variables of public holidays and day of the week. In addition, we explored the association of air pollutants with different lag structures from the current day (lag 0) up to previous 7 days (lag 7) and moving average of current and previous 1–7 day concentrations (lag 01-lag 07). Since single-day lag models could underestimate the risk effects (Bell et al., 2004; Wang et al., 2018), we introduced moving average concentrations of air pollutants exhibited the strongest effects in our main analysis. Moreover, given that linear assumption between air pollutants and respiratory diseases may not be rational, we included a natural cubic spline function of air pollutants with 3 degrees of freedom to test the exposure-response (E-R) associations in each city.

Homogeneity test was performed to check the difference between city-specific estimates by means of Cochran's Q test. Then, a random-effect meta-analysis was applied to pool the effect estimates in four cities (Yin et al., 2017). The percent change (%) was calculated by the equation: percent change (%) = [exp $^{(\beta * \Delta con)}$ −1]*100, where $\beta$ is the coefficient obtained from time-series models and $\Delta$con is the variation in the concentrations of the pollutants under analysis.

We also explored the effect estimates by including exposure day of air pollutant below the Chinese Ambient Air Quality Standards (CAAQS) Grade II in each city using the above model. We further conducted stratified analysis by gender, age (≤1 year, 1–4 years and 4–14 years), season (warm season from April to September and cold season from October to March), and diseases subtype (AURIs and ALRIs). We classified age subgroups according to the characteristics for children of different age groups, such as physiological development, living environment, scope and intensity of outdoor activity. We tested the statistical significance of differences between effect estimates of the strata by calculating the 95% confidence interval (95%CI) as the below formula: $(Q1\text{-}Q2) \pm 1.96\sqrt{(SE1)^2 + (SE2)^2}$, where *Q1* and *Q2* are the effect estimates for each stratum; *SE1* and *SE2* are their corresponding standard errors. Regardless statistical significance, modification effect by a factor of two or more deserved more attention (Zeka et al., 2006).

### 2.5. Sensitivity analyses

In addition, we applied co-pollutant models to investigate the confounding effects of other pollutants at the same lag day once at a time. Given that $PM_{2.5}$ and $PM_{10}$ highly correlated (r = 0.88), they could not be added to the co-pollutant model. Furthermore, we altered the *df* (s) of long-term and seasonal trends (5-8*df* per year) to examine the robustness of the results. All analyses were conducted using the R statistical environment (version3.5.0.) with 'mgcv' and 'metafor' packages. The pooled estimates were presented as the percent change in hospitalizations for respiratory disease per 1 mg/m$^3$ increase in CO concentrations and each 10 μg/m$^3$ increment in the other air pollutants. All statistical tests were two-sided and values of $p < 0.05$ were considered statistically significant.

## 3. Results

A total of 183,036 respiratory diseases cases were included in the analysis from 2013 to 2018. The daily mean numbers of hospital admissions ranged from 6 (Guangzhou) to 60 (Wuhan). Among all records, there were more than 1.5 times cases in boys than in girls. The percentages of age-specific subgroups were 35.8% for ≤1 year, 40.5% for 1–4 years, and 23.7% for 4–14 years of age. As for subgroups of disease subtypes, AURIs and ALRIs accounted for 14.9% and 79.2%, respectively; while asthma and other respiratory diseases only accounted for 0.4% and 5.5%, respectively (Table S1).

**Table 1**
Descriptive summary of air pollutant concentrations and meteorological factors in four Chinese cities, 2013–2018.

| | Guangzhou | Shanghai | Wuhan | Xining |
|---|---|---|---|---|
| $PM_{2.5}$ (μg/m$^3$) | 35.8 (27.5) | 41.3 (35.8) | 67.0 (26.1) | 44.4 (23.8) |
| $PM_{10}$ (μg/m$^3$) | 55.8 (38.5) | 60.0 (45.3) | 99.0 (30.8) | 96.5 (52.6) |
| $SO_2$ (μg/m$^3$) | 13.4 (8.0) | 13.3 (8.0) | 12.0 (13.3) | 22.8 (15.1) |
| $NO_2$ (μg/m$^3$) | 43.6 (23.2) | 40.1 (25.3) | 49.1 (15.0) | 32.1 (12.5) |
| $O_3$ (μg/m$^3$) | 80.0 (67.7) | 90.4 (27.7) | 94.0 (60.5) | 75.0 (42.0) |
| CO (mg/m$^3$) | 0.9 (0.3) | 0.7 (0.3) | 1.0 (0.2) | 1.2 (0.6) |
| Temperature (°C) | 24.1 (9.7) | 19.1 (14.0) | 18.4 (15.9) | 7.8 (15.8) |
| Humidity (%) | 79.9 (14.0) | 72.8 (18.5) | 80.0 (15.8) | 57.1 (22.3) |

Data was expressed as Median (IQR).

Table 1 shows the descriptive statistics of daily air pollution and meteorological variables in the four cities. As compared to the other cities, the highest daily average levels of $PM_{2.5}$, $PM_{10}$, $NO_2$ and $O_3$ were observed in Wuhan, while $SO_2$ and CO were ranked top in Xining. Guangzhou had the lowest levels of $PM_{2.5}$ (35.8 μg/m$^3$) and $PM_{10}$ (55.8 μg/m$^3$), but relatively higher average temperature and humidity values. The lowest average daily $NO_2$ and CO were observed in Xining (32.1 μg/m$^3$) and Shanghai (0.7 mg/m$^3$), respectively. In addition, the daily median levels of $SO_2$ and CO did not exceed the CAAQS Grade II in four cities. The pairwise Spearman's correlation coefficients between ambient air pollutants and meteorological conditions are presented in Table S2. Except for the high correlation between $PM_{2.5}$ and $PM_{10}$ (correlation coefficient r = 0.88), the daily levels of air pollutants (except $O_3$) were positively and moderately correlated with each other (correlation coefficient r = 0.38–0.55). $PM_{2.5}$ and $PM_{10}$ in Guangzhou, Shanghai and Xining showed less spatial variability than gaseous pollutants among monitoring stations in each city. In contrary, spatial heterogeneity for $SO_2$ in Wuhan was smaller than the other pollutants (Table S3.1- Table S3.4).

The cumulative lag effects of air pollutants showed higher estimates than that of single-day exposure (Fig. S1). The city-specific and overall effects of six pollutants on total hospital admissions for respiratory diseases, at the lag days with the strongest effect estimates using single pollutant models, are shown in Fig. 1. Overall, we observed that air pollutants, except $O_3$, were significantly associated with increased total daily hospital admissions. Correspondingly, an increase of 10 μg/m$^3$ in $PM_{2.5}$, $SO_2$, $NO_2$ and $O_3$ at lag 07, and $PM_{10}$ at lag 03, related to a 1.19% (95% CI: 0.20%, 2.19%), 3.58% (0.56%, 6.69%), 2.23% (1.20%, 3.26%), 0.26% (−0.78%, 1.31%) and 0.51% (0.24%, 0.77%) increase in total hospitalizations. Each 1 mg/m$^3$ increase of CO at lag 01 was significantly associated with a 6.10% (2.59%, 9.72%) increase in hospital admissions. Nevertheless, the effect of air pollutants on hospitalization associations across cities showed a notable heterogeneity. The city-specific estimated $PM_{2.5}$, $SO_2$ and $O_3$ were the highest in Guangzhou, and with the maximum increment of 11.35% (4.17%, 19.03%) in respiratory diseases for each 10 μg/m$^3$ increase in $SO_2$. The largest effects of $NO_2$ and CO were displayed in Xining and Shanghai, respectively, while the estimates of $PM_{10}$ in Wuhan was comparable with that in Xining.

Fig. 2 shows the smoothing E-R curves between air pollutants, with the exception of $O_3$, and total hospitalizations for respiratory disease in four cities. In general, we observed significant exposure-responsive associations of pollutant-related respiratory hospitalizations, such as $PM_{2.5}$ and $SO_2$ in Guangzhou, $SO_2$, $NO_2$ and CO in Wuhan, as well as $SO_2$ and $NO_2$ in Xining. Curves for above pollutants showed almost linear associations, with the exception of parabolic shapes for $SO_2$ and CO in Wuhan that showing a linear increase at concentrations over 20 μg/m$^3$ and 1.0 mg/m$^3$, respectively. The curve for $NO_2$ in Xining became flat at higher concentrations. Notably, we were able to observe significantly increased risks of respiratory hospitalizations in some cities when the majority of air pollutants except $PM_{2.5}$ at levels below the CAAQS Grade II (Fig. 1, Table 2). For example, $SO_2$, $NO_2$ and $O_3$ in Guangzhou, $SO_2$ and CO in Shanghai, $PM_{10}$, $NO_2$ and CO in Wuhan and $NO_2$ in Xining. In addition, deleterious effects on ALRIs were almost similar with that in total hospitalizations, but no significant city-specific effects for AURIs existed, except for $NO_2$ with a 3.87% increase in pooled estimates (95% CI: 0.80%, 7.03%).

Table 3 illustrates the overall risk estimates of stratified analyses by gender, age, seasons and disease subtypes. For $PM_{2.5}$, $SO_2$ and $NO_2$, stronger effects were observed among children aged 4–14 years. The adverse effects of $PM_{2.5}$, $PM_{10}$, $SO_2$, $NO_2$ and CO in cold season were stronger than that in warm season. The associations between air pollutants and hospitalizations did not vary by gender, though the estimates were more pronounced in girls than that in boys. Additionally, the effects of air pollutants except CO tended to be higher on AURIs than that

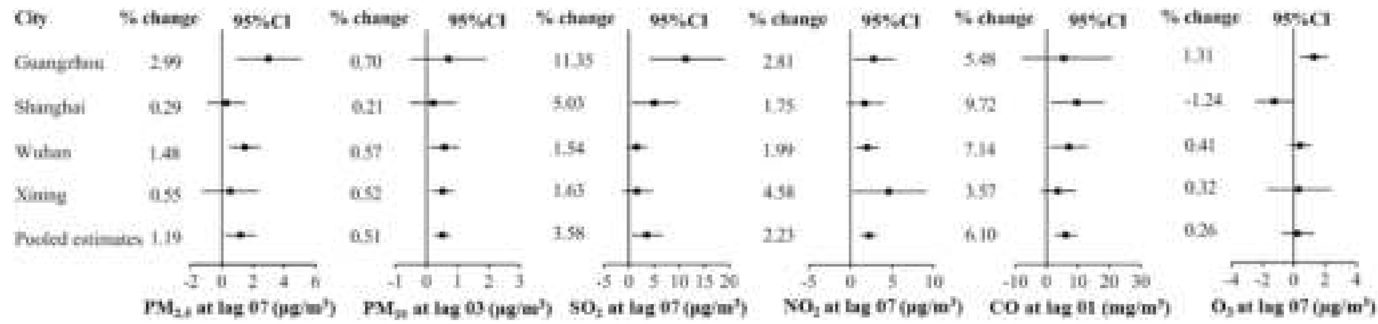


**Fig. 1.** City-specific and pooled percentage changes (% change) with 95% CI in total hospital admissions for respiratory disease. CI: confidence interval; lag 07, moving averaging concentrations of the present and the previous 7 days; lag 03, moving averaging concentrations of the present and the previous 3 days; lag 01, average concentration of the current day and the previous day.
We presented city-specific and overall effects of six criteria pollutants on total hospitalizations due to respiratory disease at the lag days with strongest effect estimates using single pollutant models. Overall, air pollutants (except $O_3$) were significantly associated with increased hospitalizations. The effects of $PM_{2.5}$, $SO_2$ and $O_3$ were the highest in Guangzhou, whereas the largest effect of $NO_2$ and CO were displayed in Xining and Shanghai, respectively (Fig. 1).

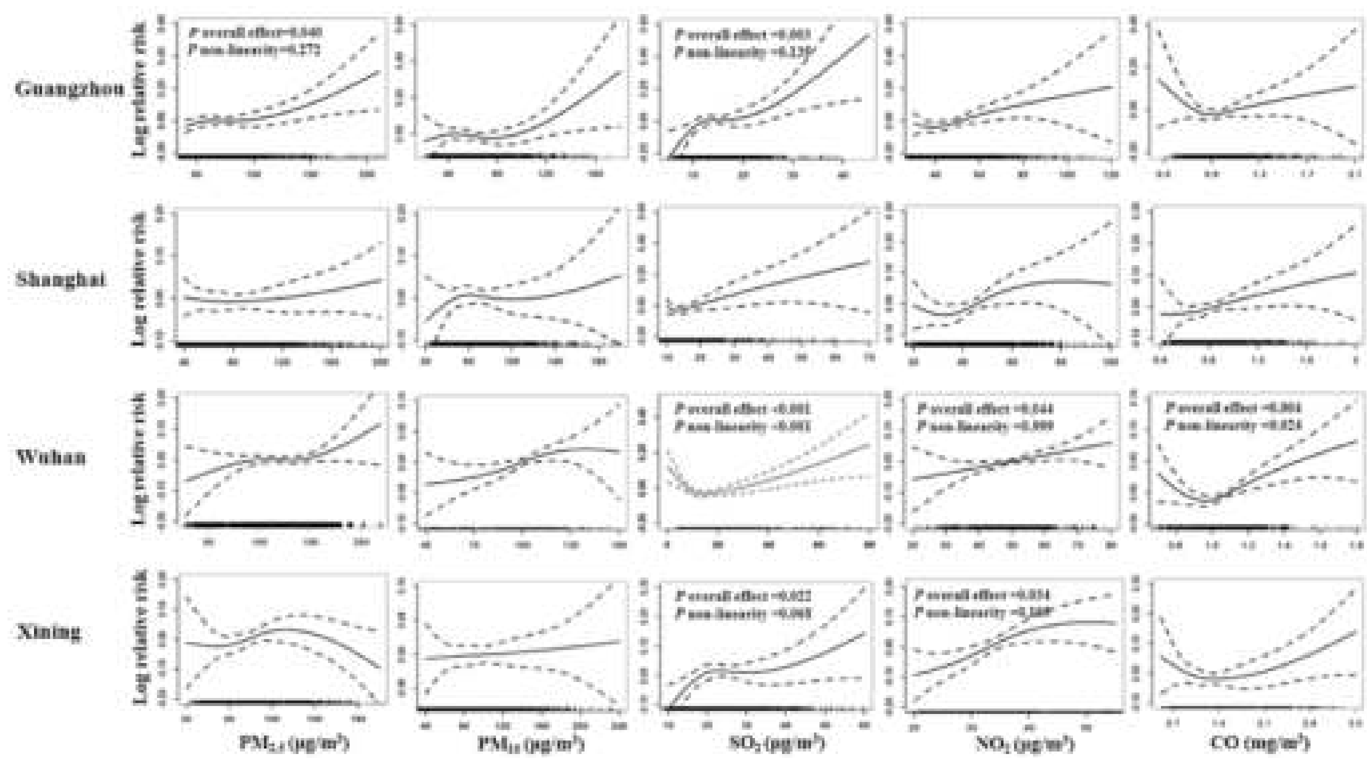


**Fig. 2.** City-specific smoothing exposure-response curves of air pollutants and daily total hospital admissions for respiratory disease ($df = 3$). X-axis is the moving averaging concentrations for $PM_{2.5}$, $SO_2$ and $NO_2$ at lag 07, for $PM_{10}$ at lag 03 and for CO at lag 01. The solid lines represent the mean estimates, and the dotted lines represent 95% confidence interval. Lag 07, moving averaging concentrations of the present and the previous 7 days; lag 03, moving averaging concentrations of the present and the previous 3 days; lag 01, average concentration of the current day and the previous day.
As shown in Fig. 2, the exposure-responsive curves were significant for $PM_{2.5}$ and $SO_2$ in Guangzhou, $SO_2$, $NO_2$ and CO in Wuhan, as well as $SO_2$ and $NO_2$ in Xining. Above curves except $SO_2$ and CO in Wuhan indicated almost linear relationships with respiratory diseases. Besides, the curve for $NO_2$ in Xining became flat at higher concentrations.

on ALRIs. The estimates of air pollutants-related hospital admissions in stratified analyses showed heterogeneity by cities (Table S4).

Additionally, almost identical estimates were observed by changing degree of freedom (5-8*df*/year) for time trend (Fig. S2), which suggesting that our primary estimated effects were relatively robust. On the other hand, the co-pollutant models showed that pooled effect estimates of some air pollutants substantially altered (Table S5.). For example, the effects of $SO_2$ and $NO_2$ remained statistically significant, after controlling for other pollutants ($PM_{2.5}$, $PM_{10}$, CO and $O_3$) in the models, while the effect estimates for $PM_{2.5}$ attenuated dramatically and further lost its statistical significance after adjustment for gaseous pollutants.

## 4. Discussion

To our knowledge, this is the first multi-city study in China to explore the short-term effects and E-R relationships between six air pollutants and hospitalizations due to respiratory disease among children. Our study suggested that short-term exposure to air pollutants with the exception of $O_3$ increased the risks of respiratory disease hospitalizations, and E-R relationships were found for $PM_{2.5}$ and $SO_2$ in Guangzhou, $SO_2$, $NO_2$ and CO in Wuhan, as well as $SO_2$ and $NO_2$ in Xining. In addition, we observed significant adverse effects of air pollutants except for $PM_{2.5}$ even below the current CAAQS Grade II in certain cites.

**Table 2**
City-specific and pooled percentage change (% change) with 95% CI in hospital admissions for respiratory disease linked with air pollutant concentrations below the current Chinese Ambient Air Quality Standards (CAAQS) Grade II.

| | $PM_{2.5}$ at lag 07 | $PM_{10}$ at lag 03 | $NO_2$ at lag 07 | $O_3$ at lag 07 |
|---|---|---|---|---|
| Total | | | | |
| Guangzhou | 2.85 (−0.34, 6.14) | 0.60 (−0.74, 1.96) | 4.05 (0.20, 8.05)* | 1.86 (0.68, 3.06)** |
| Shanghai | −2.28 (−4.93, 0.45) | 0.10 (−0.89, 1.09) | −0.08 (−2.92, 2.84) | −1.34 (−3.03, 0.38) |
| Wuhan | 0.21 (−2.25, 2.73) | 0.69 (0.05, 1.34)* | 2.12 (0.39, 3.87)* | 0.91 (−0.03, 1.86) |
| Xining | 2.09 (−1.31, 5.61) | −0.05 (−1.14, 1.05) | 4.58 (0.22, 9.13)* | −0.11 (−2.21, 2.03) |
| Pooled estimates | 0.56 (−1.64, 2.81) | 0.43 (−0.03, 0.88) | 2.22 (0.46, 4.01)* | 0.49 (−0.77, 1.77) |
| AURIs | | | | |
| Guangzhou | 6.05 (−0.86, 13.44) | 0.99 (−1.83, 3.90) | 5.44 (−2.76, 14.32) | 1.32 (−1.21, 3.91) |
| Shanghai | −6.15 (−14.06, 2.48) | −3.02 (−6.14, 0.21) | 6.59 (−2.68, 16.74) | −2.19 (−7.48, 3.40) |
| Wuhan | 2.57 (−2.11, 7.48) | 0.76 (−0.57, 2.11) | 3.37 (−0.25, 7.12) | 0.47 (−1.41, 2.40) |
| Xining | 2.85 (−7.87, 14.81) | −0.66 (−4.0, 2.81) | 0.79 (−12.32, 15.85) | 1.19 (−4.97, 7.75) |
| Pooled estimates | 1.79 (−2.77, 6.56) | −0.11 (−1.73, 1.54) | 3.87 (0.80, 7.03)* | 0.60 (−0.82, 2.05) |
| ALRIs | | | | |
| Guangzhou | 1.96 (−1.68, 5.74) | 0.77 (−0.79, 2.34) | 3.72 (−0.68, 8.32) | 2.03 (0.66, 3.41)** |
| Shanghai | −1.4 (−4.38, 1.67) | 0.37 (−0.74, 1.49) | −0.88 (−4.02, 2.36) | −0.57 (−2.46, 1.35) |
| Wuhan | −1.82 (−4.61, 1.05) | 0.87 (0.15, 1.59)* | 2.47 (0.54, 4.44)* | 0.86 (−0.19, 1.93) |
| Xining | 2.32 (−1.22, 5.98) | 0.11 (−1.03, 1.26) | 4.72 (0.21, 9.44)* | −0.02 (−2.22, 2.23) |
| Pooled estimates | 0.04 (−2.06, 2.18) | 0.61 (0.10, 1.11)* | 2.20 (0.04, 4.40)* | 0.78 (−0.27, 1.84) |

*$p < 0.05$; **$p < 0.01$; CI: confidence interval; Total, total respiratory hospitalizations; ALRIs, acute lower respiratory infections; AURIs, acute upper respiratory infections; lag 07, moving averaging concentrations of the present and the previous 7 days; lag 03, moving averaging concentrations of the present and the previous 3 days; The 24-h average CAAQS Grade II references for $PM_{2.5}$, $PM_{10}$, $NO_2$ and $O_3$ were set to 75 μg/m$^3$, 150 μg/m$^3$, 80 μg/m$^3$ and 160 μg/m$^3$, respectively. The concentrations of $SO_2$ and CO in all cities did not exceed the limits of CAAQS Grade II.

### 4.1. Heterogeneity of city-specific and overall effect estimates

In this study, we observed heterogeneity in both the peak effects lag days and the magnitude with effects estimates across cities, which were in accord with previous reports based on all ages population (Chen et al., 2017; Qiu et al., 2018; Scarinzi et al., 2013; Tian et al., 2018). A study in 17 cities of Sichuan Basin, China during 2015–2016 reported that the strongest effects of $PM_{2.5}$, $PM_{10}$, $NO_2$ and $SO_2$ on overall respiratory hospital admissions occurred in lag 01, lag 01, lag 0 and lag 02, respectively (Qiu et al., 2018). Similarly, a nation-wide study in 31 cities of China found $PM_{2.5}$ and $SO_2$ at lag 0–2 days, $PM_{10}$ and $NO_2$ at lag 0–1 days were most strongly associated total emergency room visits (Chen et al., 2017). Another study involving 25 Italian cities during 2006–2010 demonstrated that $PM_{2.5}$, $PM_{10}$ and $NO_2$ at lag 05 concentrations consistently contributed to the maximum increment in urgent admissions for respiratory disease (Scarinzi et al., 2013). However, the strongest effects of major pollutants in our study prolonged over one week. The underlying reason may be relatively higher proportion of ALRIs across cities in our research (79.2%). Worthy of note was that children having mild respiratory symptoms are likely to take medicine at home or in a small clinic, rather than to visit hospital until their symptoms worsen, which could cause several days lag and underestimates of the effects (Zeger et al., 2000).

We also found stronger determinants of gaseous pollutants ($SO_2$, $NO_2$ and CO) than $PM_{2.5}$ and $PM_{10}$ in city-specific and pooled estimates. Moreover, significant effects remained for gaseous pollutants after adjustment for $PM_{2.5}$ or $PM_{10}$, which indicated that gaseous pollution especially $NO_2$ might be strong predictors for respiratory diseases. This was in line with several prior multicity studies in China (Chen et al., 2017; Qiu et al., 2018; Tian et al., 2018). For example, Qiu et al. (2018) found that per 10 μg/m$^3$ increment in $SO_2$ and $NO_2$ was associated with 3.30% and 3.91% increased overall respiratory admission in children subgroups, while $PM_{2.5}$ and $PM_{10}$ only corresponded to 0.63% and 0.47%, respectively. The possible reason for higher health risks in gaseous pollutants has not been revealed previously. As known gaseous pollutants always originated from a common source and might be involved in the formation of PM. It was possible that the adverse effects of $SO_2$ and $NO_2$ might greatly contribute to fine particles (Tolbert et al., 2007; Zheng et al., 2017), but cautious should be taken because the larger spatial or within-day variability of particles may resulting in a great measurement error (Fusco et al., 2001).

In city-specific analysis, traffic-related air pollution such as $PM_{2.5}$, $SO_2$, $NO_2$ and $O_3$ contributed to a majority of hospitalizations due to respiratory disease in Guangzhou. Particularly, the health risks remained at $SO_2$, $NO_2$ and $O_3$ levels not exceeding the current regulation limits in Guangzhou. We found significant effects of $PM_{10}$, $PM_{2.5}$, $NO_2$ and CO in Wuhan, even $PM_{10}$, $NO_2$ and CO below the CAAQS Grade II, which is in accord with a mixed construction dust-motor vehicles pollution pattern. Apart from different pollutant sources and compositions, the heterogeneity might due to variety in geographic and meteorological patterns, socio-economic levels, and reinforced public health policies (Kong et al., 2012; Kuerban et al., 2020; Tian et al., 2018; Zhao et al., 2016; Zhu et al., 2018). Additionally, it was noteworthy that stronger associations between air pollutants ($PM_{2.5}$ in Guangzhou, $NO_2$ in Xining and CO in Shanghai) and respiratory diseases among certain cities with the lowest daily mean concentrations, which was supported by recent multicity studies in China (Tian et al., 2019; Zhu et al., 2017). Possible explanation was that the tolerance and susceptibility of children might vary by cities. A prior "harvesting effects" hypothesis might partly interpret our findings (Costa et al., 2017), that was to say respiratory diseases especially respiratory infections among children developed quickly so that health seeking behavior always occur early before air pollution reached a more sever levels.

### 4.2. E-R relationships and the effect below the current CAAQS

Evidence for the E-R relationships between air pollutants and hospital admissions for respiratory disease was limited. Curves for $PM_{2.5}$ and $SO_2$ in Guangzhou, $NO_2$ in Wuhan, as well as $SO_2$ and $NO_2$ in Xining indicated almost linear association with respiratory diseases, whereas parabolic curves for $SO_2$ and CO in Wuhan. Previous literature based on a particular single city showed inconsistent shapes concerning the relationships of air pollutants-induced childhood hospitalizations due to asthma (Ma et al., 2019; Zhang et al., 2019) and lower respiratory diseases (Zhu et al., 2017). Nevertheless, possible reason for the stable effects at higher concentration is partly that biochemical and cellular processes may be saturated with small doses of toxic components (Pope

**Table 3**
Gender, age, seasons and disease subtypes specific percentage change (% change) with 95% CI in hospital admissions for respiratory disease.

| Subgroups | $PM_{2.5}$ at lag 07 | $PM_{10}$ at lag 03 | $SO_2$ at lag 07 | $NO_2$ at lag 07 | CO at lag 01 | $O_3$ at lag 07 |
|---|---|---|---|---|---|---|
| Gender | | | | | | |
| Boys | 1.21 (0.32, 2.10) | 0.47 (0.15, 0.79) | 2.61 (−0.64, 5.95) | 1.92 (0.68, 3.17) | 5.01 (−0.68, 11.04) | 0.25 (−0.49, 0.99) |
| Girls | 1.32 (−0.22, 2.89) | 0.57 (0.19, 0.95) | 3.88 (1.36, 6.47) | 2.82 (0.92, 4.76) | 7.49 (2.17, 13.10) | 0.33 (−1.14, 1.81) |
| Age (years) | | | | | | |
| ≤1 | 0.56 (−0.19, 1.32) | 0.45 (0.08, 0.82) | 1.46 (−1.45, 4.46) | 0.77 (−0.66, 2.22) | 2.76 (−3.51, 9.44) | 0.34 (−0.28, 0.96) |
| 1–4 | 0.95 (−0.17, 2.08) | 0.48 (−0.26, 1.23) | 1.76 (0.17, 3.37) | 0.78 (−0.30, 1.86) | 5.96 (−1.21, 13.65) | 0.36 (−0.77, 1.51) |
| 4–14 | 1.45 (0.55, 2.35)[b] | 0.46 (−0.14, 1.06) | 3.53 (0.97, 6.16)[b] | 2.61 (1.23, 4.01)[b] | 10.66 (−0.10, 22.58) | −0.05 (−1.21, 1.12) |
| Season | | | | | | |
| Cold | 1.53 (0.61, 2.45)[a] | 0.72 (0.39, 1.05) | 6.30 (2.05, 10.72) | 3.98 (2.09, 5.89) | 9.13 (3.36, 15.21)[a] | −0.60 (−1.48, 0.28) |
| Warm | −0.12 (−1.39, 1.17) | 0.38 (−0.08, 0.84) | 4.46 (0.57, 8.50) | 2.43 (−2.53, 7.64) | −0.27 (−12.78, 14.04) | 0.46 (−0.57, 1.50) |
| Disease Subtype [c] | | | | | | |
| ALRIs | 1.06 (0.10, 2.04) | 0.50 (0.22, 0.78) | 2.41 (0.13, 4.73) | 2.19 (1.05, 3.35) | 6.84 (3.05, 10.77) | 0.25 (−0.70, 1.21) |
| AURIs | 2.26 (0.58, 3.96)[a] | 0.81 (0.11, 1.53) | 3.96 (−2.99, 11.41) | 2.35 (0.01, 4.75) | 2.43 (−6.27, 11.94) | 0.90 (−0.10, 1.92) |

CI: confidence interval; ALRIs, acute lower respiratory infections; AURIs, acute upper respiratory infections; Cold season, from October to March; warm season, from April to September; lag 07, moving averaging concentrations of the present and the previous 7 days; lag 03, moving averaging concentrations of the present and the previous 3 days; lag 01, moving average of the current day and the previous day.

[a] The difference between gender (boys vs. girls), seasons (cold vs. warm) and disease subtypes (ALRIs vs. AURIs) was significant ($p < 0.05$).

[b] The difference of two ages (compared with the lowest age groups) were significant ($p < 0.05$).

[c] Asthma and other respiratory disease were not included in the analysis because the number of cases were too small in certain cities.

et al., 2009). Besides, the heterogeneity in dose-responsive curves of air pollutants across cities may also be explained by variety in pollutant levels, constitutes and susceptibility of children to some extent.

Notably, harmful effects of air pollutants exposure except $PM_{2.5}$ existed even below CAAQS Grade II in certain cities. A recent multicity study among adults in China also reported the adverse effects of $PM_{10}$, $NO_2$, $SO_2$ and CO below the current regulatory limits on hospital admissions (Tian et al., 2018), which partially support our findings. Respiratory diseases especially respiratory infections are very common among children, but the adverse effects of air pollution below the current CAAQS on respiratory hospitalizations among Chinese children have not been explored before. Thus, further research based on children in a national setting is necessary to clarify the associations. The current limitation standard is still at WHO-recommended transition level, which might not be sufficient to protect Chinese children against respiratory disease because that racial and ethnic disparities might serve as potential modification factors for the associations (Grineski et al., 2010). Furthermore, given the heterogeneous city-specific effects, public policy revision or emission reduction actions (e.g. clean energy application and traffic control) should be determined carefully not only meet the country comprehensive tradeoff, but also adapt for the local conditions.

### 4.3. Stratified analyses for air pollutant-related respiratory hospitalizations

#### 4.3.1. Stratified analyses by age

Currently, there was no consensus regarding the susceptible age subgroup in children. Herein, we observed a higher effect of $PM_{2.5}$, $SO_2$ and $NO_2$ on respiratory diseases in children aged 4–14 years. Similarly, a multi-city study in Colombia reported that effects of $PM_{2.5}$, $NO_2$ and CO on emergency department visits for respiratory disease were the highest in children aged 5–10 years (Rodriguez-Villamizar et al., 2018). Another multi-city study in South Brazil observed stronger associations for $SO_2$ and $NO_2$ with respiratory hospitalizations among children 6–15 years old (Agudelo-Castaneda et al., 2019). One possible explanation could be that kindergarten or school children (4–6 years or 7–14 years), compared with children < 4 years, are more likely to increase their frequency and duration of outside activities, thus they are prone to inhale more volume of air pollutants (Gehring et al., 2013). On the contrary, breast-feeding for infants may provide natural protection from respiratory disease in some extent (Duijts et al., 2010). Our findings provided evidence to identify susceptible populations, and guidance to bring forward effective behavior intervention measures.

#### 4.3.2. Stratified analyses by season

Previous studies found that season could modify the health effects of air pollution. We found that the effects of $PM_{2.5}$, $PM_{10}$ and CO on total respiratory diseases were significantly stronger in cold season. It was supported by several reports (Bai et al., 2018; Li et al., 2018; Zheng et al., 2017), but in conflict with other studies reporting greater effects in warm or transition season (Alessandrini et al., 2016; Song et al., 2018; Wang et al., 2019). A possible explanation would be the higher exposure levels and variation of $PM_{2.5}$, $PM_{10}$ and CO in cold season than in warm season in our study. In addition, cold climate would attenuate respiratory defenses and induce contraction of tracheal smooth muscle, then further weaken pulmonary circulation and lung perfusion (Kan et al., 2008). Besides, individual behavior characteristics may account for exposure pattern in a great extent. Guangzhou, Shanghai and Wuhan characterized with simultaneous rain and heat over the same period in warm season. Children are prone to stay indoors due to high temperature and frequent rainy days. Moreover, the widespread usage of air conditionings in summer alters ventilation and compels children to stay indoors as well (Cao et al., 2009; Medina-Ramón et al., 2006).

#### 4.3.3. Stratified analyses by disease subtype

Furthermore, greater effects of air pollutants (except CO) on hospitalizations for AURIs than ALRIs were consistent with previous studies among children in Spain (Amarillo and Carreras, 2012) and China (Liu et al., 2019; Zheng et al., 2017). Taken easily, due to the nasal ciliary mucus barrier, the concentration of pollutants decrease sharply during transporting to the deeper respiratory tract. However, a single-city study in Shijiazhuang among 0-14-year-old children observed that the associations of $NO_2$ and $PM_{2.5}$ with pneumonia were stronger than AURIs (Song et al., 2018). With respect to CO, we found that the risk of hospitalizations for ALRIs [6.84% (3.05%, 10.77%)] was statistically evident. In line with a multi-study in Australia and New Zealand, reported a positive association of CO exposure with pneumonia and acute bronchitis in infants and children 1–4 years of age. Similarly, Santus et al. (2012) estimated a 5.9% and 9.4% increase of asthma and pneumonia hospitalizations in children under 16 years when exposed to each 1 $\mu g/m^3$ increase of CO at lag 02, respectively. On the contrary, another study in Hong Kong showed that per 1 ppm increase in CO was related to a 3.9% decrease in emergency hospital admissions for respiratory tract infections (Tian et al., 2013). It could be explained by plausible biological experimental evidence that revealed the anti-inflammatory role of CO (Chin and Otterbein, 2009; Nobre et al., 2007). The risk effects of ambient pollutants on cause-specific respiratory diseases and the underlining mechanism regarding the CO-induced ALRI-related admissions need further exploration.

### 4.4. Limitations

Our study has several limitations mentioned as follows. First, it should be note that admission data were only from 1 to 3 hospitals in each of the four designated cities. It might decrease statistical power and affect generalizability of the findings. Nevertheless, the associations were prone to be robust either changing the degree of freedom or choosing different lag structures. Second, an inherent property of ecological studies (Lin et al., 2016), averaging data from monitoring stations as a surrogate for individual exposure might cause measurement errors. Thus, specific individual-level exposure assessments such as distance weighting incorporating population density, land use regression models and other approaches were imperative in further study. Third, a moderate or high collinearity among air pollutants limited ability to distinguish the independent effect of each pollutant. Fourth, other possible factors (such as pollutants sources, social-economic, using air conditional or purifier, etc.) were not take into consideration owing to data unavailability. Finally, the small proportion of daily counts for AURIs hospitalizations may reduce the statistically power and further affect the reliability of the estimates.

## 5. Conclusions

In summary, the multi-city study firstly indicated that short-term exposure to $PM_{2.5}$, $SO_2$, $NO_2$ and CO, were dose-responsive associated with increased respiratory diseases hospitalization among children. Adverse effects remained even when exposure levels of air pollutants except $PM_{2.5}$ below the current CAAQS Grade II in certain cities. For $PM_{2.5}$, $SO_2$ and $NO_2$, stronger effects were found in children aged 4–14 years. Moreover, air pollutants-related hospitalizations apart from $O_3$ appeared to be stronger in the cold season. Further studies in a national setting are needed to elucidate the associations.

## Declaration of competing interest

The authors declare that no actual or potential competing financial interests exist in this paper.

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## Appendix A. Supplementary data

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