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Association of silica dust exposure with mortality among never smokers: A 44-year cohort study

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ABSTRACT

The association of silica dust exposure with mortality among never smokers has not been well established. We aimed to evaluate the association of silica dust exposure with mortality among never smokers. We studied 17,130 workers employed for at least 1 year between January 1, 1960 and December 31, 1974, with follow-up until the end of 2013. Cumulative respirable silica dust exposure (CDE) was estimated by linking a job-exposure matrix to personal work history. We observed 3937 deaths during 589,357.26 person-years of follow-up. Significant positive exposure-response relationships were found between CDE and mortality from all cause (HR = 1.01, 95% CI = 1.01–1.02), respiratory tuberculosis (HR = 1.04, 95%CI = 1.02–1.06), CVDs (HR = 1.03, 95%CI = 1.02–1.04), and diseases of the respiratory system (HR = 1.06, 95%CI = 1.04–1.07). We found higher standardized mortality ratios for respiratory tuberculosis (2.62, 2.32–2.95), CVDs (1.43, 1.32–1.54), and pneumoconiosis (77.75, 68.21–88.25) among silica dust exposed workers. In addition, we estimated that 4.19%, 20.69%, 7.48% and 34.06% of deaths for all cause, respiratory tuberculosis, CVDs, and diseases of the respiratory system among Chinese workers were attributed to silica, after adjusting for other covariates. With regard to lung cancer, compared with unexposed group, the HRs and 95% CI were 0.94 (0.52–1.71), 1.86 (1.15–3.00), 1.65 (0.95–2.86) for low, medium, and high exposed workers, respectively. Long-term silica dust exposure is associated with increased mortality in the absence of cigarette smoking.

1. Introduction

Crystalline silica is one of the commonest minerals on earth, and silica dust has been reported to be one of the most serious occupational hazards in the workplace (Steenland and Ward, 2014). It is estimated that tens of millions of workers worldwide (Leung et al., 2012) and 23 million Chinese workers are exposed to silica dust. The adverse health effects of silica exposure are an increasing public health concern for decades. Long-term exposure to silica dust has been established to be associated with higher mortality of silicosis (Mundt et al., 2011), cardiovascular diseases (CVDs) (Liu et al., 2014), lung cancer (Liu et al., 2013) and other respiratory diseases (Chiazze et al., 2002).

In our previous study, we have explored the association between silica dust exposure and total and specific mortality among 74,040 workers (Chen et al., 2012). And we found positive association between silica dust exposure and mortality of respiratory diseases, lung cancer and CVDs. Meanwhile, studies revealed that smoking also play an important role in the occurrence and progress of these diseases (Duncan et al., 2019; Hirsch et al., 2017). As we know, cigarette smoking has been confirmed to be the main cause of lung cancer and respiratory diseases mortality. According to the report of centers for disease control and prevention in US, smoking causes about 90% of all lung cancer deaths and about 80% of all death from chronic obstructive pulmonary disease (COPD). Meanwhile, stroke and coronary heart disease caused

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by smoking are among the leading causes of death in the United States. In addition, the interaction between silica dust exposure and cigarette smoking on the risk of total and cause-specific mortality has also been reported previously (Brown, 2009; Wang et al., 2020). However, evidence on the association between silica dust exposure and diseases mortality among never smokers is limited.

Thus, we conducted a large cohort study of 17,130 never smokers from 20 metal mines and 9 pottery factories followed from January 1, 1960 to December 31, 2003. We aimed to assess the association of long-term exposure to silica dust with total and cause-specific mortality among never smokers and to determine population attribute risks (PAR) of mortality associated with the exposure among never smokers.

2. Material and methods

2.1. Study population

The silica cohort has been reported elsewhere (Chen et al., 1992, 2012). Briefly, the cohort was established in the late 1980s, it included 74,040 workers from 20 metal mines and 9 pottery factories worked for 1 year or more between January 1, 1960 and December 31, 1974. The cohort was retrospectively followed to 1960 and prospectively followed to the end of 2003. In the present study, we conducted analyses only among 17,130 never smokers.

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline. The study was approved by the Tongji Medical College Institutional Review Board (No. 0990–0279), written informed consent was obtained before interviews.

2.2. Silica exposure assessment

Silica exposure assessment was reported in detail in previous published papers (Dosemeci et al., 1993). In brief, quantitative assessment of silica exposure was conducted using a job-exposure matrix. In the matrix, the total dust concentration for each job title in one specific year in all workplaces of this job title and this specific year and such exposure matrix has been calculated for each mine or factory in the cohort since 1950. It was estimated using data from similar jobs or the same job at different years for missing data for years or jobs (less than 20%).

Work histories for each worker were obtained from personal employment records in mines/factory files. They include job titles and duration years of each worker's full employment. By linking the job exposure matrix, cumulative respirable silica dust exposure (CDE, mg/m^3-year) for each worker was estimated as follows:

$$CDE = \sum_{i=1}^{n} (Ci \times Ti) \quad (1)$$

where n is the total number of job titles; Ci is the 8 h time-weighted mean concentration of dust for the ith job title; and Ti is the working years for the ith job title.

We calculated CDE from the start date of silica-exposed work to the earliest one of the following: end of employment, lost to follow-up, died or the end of 2003.

2.3. Cause of death

All workers were tracked for their vital status by local hygienists during the follow-up period. Information on causes of death was collected from various ways: medical records in the hospital (60.5%); employment registers, accident records, or death certificates (35.2%); or oral reports from relatives (4.3%) (Chen et al., 2012). The 10th International Classification of Diseases (ICD-10) was used to code causes of death.

2.4. Statistical analysis

The basic characteristic of the participants was reported as mean (SD) for continuous variables and as number (percentages) for categorical variables. Cox proportional hazard models were used to calculate hazards ratios (HRs) and 95% confidence intervals (CIs) for CDE and the risk of selected causes of death, with adjustment for gender, year of hire (four categories: 1950 or earlier, 1951–1960, 1961–1970, 1971 or later), age at hire (continuous), and type of facility (four categories: tungsten mines, iron and copper mines, tin mines, and pottery factories). CDE was categorized into four groups based on the percentiles from the exposure distribution. The linear trend tests were conducted by including the median value for each level of dust as a continuous variable in the models. In addition, we evaluated the nonlinear relationship between CDE and total and cause-specific mortality by using restricted cubic splines with 4 knots at percentiles 5, 35, 65 and 95% of the distribution.

The population attributable risk percent (PAR%) was calculated as follows:

$$PAR\% = [P \times (RR\text{-}1)]/[P \times (RR\text{-}1)+1] \times 100\% \quad (2)$$

Where P is the percentage of silica-exposed workers among all industrial workers (16.3%) (China, 2009), and RR is the relative risk, which is estimated from HR in our study.

Standardized mortality ratios (SMR) were also used to reflect the death information. SMRs were calculated among 16,918 workers with excluding 212 deaths before 1970, as national death rate data in China were not available before 1970. The expected number of cause-specific deaths were calculated by multiplying the gender-, age-, periods-, and cause-specific person-years at risk with 5-year intervals for age and period by the corresponding mortality rates in China. All statistical analyses were performed using SAS 9.4 software (SAS Institute, Cary, NC), and a two-sided p-value < 0.05 was regarded as statistically significant.

3. Results

A total of 17,130 participants (mean [SD] age at hire, 23.99 [7.12] years; 10,992 [64.17%] men) were included in this study. The basic characteristics of participants based on CDE are summarized in Table 1. Finally, there were 1179 (6.88%) workers still working at the end of the follow up. A total of 8942 (52.20%) workers were exposed to silica dust. The average CDE and duration of silica dust exposure was 3.50 mg/m^3-y and 18.67 years, respectively. During a median follow-up of 34.40 years (589,357.26 persons-years), a total of 3937 deaths were reported. Mortality rate was 668.02 per 100,000 person-years for all participants, with 848.68 per 100,000 person-years for silica exposed workers and 460.65 per 100,000 person-years for no silica exposed workers.

The HRs and 95% CI for total and cause-specific mortality associated with CDE are revealed in Table 2. CVDs, malignant neoplasms, cerebrovascular diseases, and diseases of the respiratory system were the top 4 causes of death. Compared with unexposed workers, the mortality was significantly higher among silica-exposed workers from all cause (HR = 1.27, 95%CI = 1.18–1.36), respiratory tuberculosis (HR = 2.60, 95%CI = 1.95–3.46), CVDs (HR = 1.50, 95%CI = 1.28–1.74), and diseases of the respiratory system (HR = 4.17, 95%CI = 3.17–5.48). Among CVDs, it is more evident for pulmonary heart diseases (HR = 3.37, 95%CI = 2.53–4.50). Categorical CDE were significantly associated with higher risk of mortality from all cause, respiratory tuberculosis, CVDs (including pulmonary heart diseases), and diseases of the respiratory system (including pneumoconiosis). We also observed possible association of silica exposure with the mortality of lung cancer. Compared with unexposed group, we observed higher risks of lung cancer for medium (HR = 1.86, 95%CI = 1.15–3.00) and high exposed group (HR = 1.65, 95%CI = 0.95–2.86).

The nonlinear relationship between CDE and total and cause-specific

Table 1
Basic characteristics of participants based on CDE.

Characteristic	Total	Level of CDE[c]			
		Unexposed	Low	Medium	High
	(N = 17,130)	(N = 8188)	(N = 2980)	(N = 2981)	(N = 2981)
Status at the end of follow-up (n,%)					
Working	1179 (6.88)	825 (10.08)	159 (5.34)	167 (5.60)	28 (0.94)
Left	2831 (16.53)	2102 (25.67)	415 (13.93)	222 (7.45)	92 (3.09)
Retired	9183 (53.61)	3997 (48.82)	1867 (62.65)	1750 (58.71)	1569 (52.63)
Died	3937 (22.98)	1264 (15.44)	539 (18.09)	842 (28.25)	1292 (43.34)
Male (n,%)	10,992 (64.17)	3947 (48.20)	2441 (81.91)	2380 (79.84)	2224 (74.61)
Year of birth, mean ± SD	1938.52 ± 10.76	1940.57 ± 10.12	1942.29 ± 9.45	1937.03 ± 10.21	1930.63 ± 10.01
Year of hire, mean ± SD	1962.52 ± 7.28	1963.77 ± 6.94	1965.53 ± 6.53	1961.45 ± 6.86	1957.15 ± 6.29
Year of hire, (n, %)					
1915–1950	595 (3.47)	151 (1.84)	19 (0.64)	72 (2.42)	353 (11.84)
1951–1960	7904 (46.14)	3236 (39.52)	940 (31.54)	1684 (56.49)	2044 (68.57)
1961–1970	5527 (32.27)	3099 (37.85)	1198 (40.20)	765 (25.66)	465 (15.60)
1971–1975	3104 (18.12)	1702 (20.79)	823 (27.62)	460 (15.43)	119 (3.99)
Age at hire, mean ± SD	23.99 ± 7.12	23.20 ± 6.73	23.24 ± 5.98	24.41 ± 6.75	26.52 ± 8.74
Age at first exposure, mean ± SD[b]	23.25 ± 7.01	NA	24.83 ± 7.47	23.49 ± 6.46	21.43 ± 6.62
Year of first exposure, mean ± SD[b]	1959.90 ± 10.32	NA	1967.12 ± 8.08	1960.53 ± 7.86	1952.06 ± 8.86
Cumulative total exposure, mean ± SD, mg/m^3-y[b]	145.54 ± 171.83	NA	40.79 ± 38.76	80.48 ± 54.19	315.30 ± 200.28
CDE, mean ± SD, mg/m^3-y[b]	3.50 ± 4.11	NA	0.48 ± 0.25	1.95 ± 0.76	8.08 ± 4.20
Duration of silica dust exposure, mean ± SD[b]	18.67 ± 10.32	NA	13.85 ± 9.74	18.98 ± 9.44	23.18 ± 9.58
Pneumoconiosis cases (n,%)[a]	1442 (8.42)	NA	85 (2.85)	357 (11.98)	1000 (33.55)
Age at first diagnosis of pneumoconiosis, mean ± SD[a]	45.99 ± 10.29	NA	48.69 ± 8.23	47.62 ± 10.51	45.18 ± 10.27
Age at last exposure, mean ± SD[b]	43.01 ± 10.57	NA	39.51 ± 11.14	43.86 ± 10.12	45.65 ± 9.44

[a] Results was just among pneumoconiosis.
[b] Results was just among silica dust-exposed workers.
[c] Levels of CDE was tertiles of CDE of all workers exposed to silica dust: low, 0–0.967mg/m^3-y; medium, 0.968–3.693 mg/m^3-y; high, >3.693 mg/m^3-y.

Table 2
Estimated HRs for total and cause-specific mortality associated with CDE in the cohort.

Cause of Death (ICD-10 Codes)	Number of Death	HRs for Levels of CDE versus Unexposed			
		Low	Medium	High	P_{trend}
Malignant neoplasms (C00–C97)	701	1.04 (0.83–1.31)	1.14 (0.92–1.40)	0.98 (0.78–1.24)	0.72
Malignant neoplasm of nasopharynx (C11)	36	0.76 (0.27–2.19)	1.34 (0.59–3.03)	0.28 (0.07–1.08)	0.06
Malignant neoplasm of liver and intrahepatic bile ducts (C22)	202	1.05 (0.69–1.60)	1.02 (0.69–1.52)	1.03 (0.67–1.58)	0.93
Lung cancer (C33–C34)	129	0.94 (0.52–1.71)	1.86 (1.15–3.00)	1.65 (0.95–2.86)	0.11
Certain infectious and parasitic diseases (A00–B99, J65)	457	1.39 (0.93–2.09)	1.88 (1.37–2.57)	2.83 (2.14–3.73)	<0.001
Respiratory tuberculosis (A15–A16, J65)	398	1.42 (0.88–2.30)	2.22 (1.57–3.14)	3.19 (2.35–4.34)	<0.001
Cardiovascular diseases (I00–I52, I70–I99)	973	1.08 (0.84–1.39)	1.33 (1.09–1.61)	1.80 (1.51–2.13)	<0.001
Pulmonary heart diseases (I26–I27)	485	1.28 (0.76–2.13)	2.40 (1.70–3.40)	4.56 (3.36–6.18)	<0.001
Hypertensive heart disease (I11)	131	1.42 (0.81–2.48)	0.92 (0.56–1.51)	0.78 (0.50–1.23)	0.16
Ischemic heart disease (I20–I25)	179	1.12 (0.70–1.77)	1.44 (0.98–2.13)	0.75 (0.47–1.19)	0.10
Chronic rheumatic heart disease (I05–I09)	41	0.15 (0.02–1.18)	0.62 (0.26–1.49)	0.79 (0.36–1.71)	0.97
Cerebrovascular diseases (I60–I69)	643	1.06 (0.83–1.36)	1.06 (0.86–1.32)	0.91 (0.73–1.13)	0.28
Diseases of the respiratory system (J00–J99)	575	2.39 (1.63–3.49)	3.55 (2.58–4.88)	5.96 (4.40–8.06)	<0.001
Pneumoconiosis (J60–J65)*	293	1 (ref)	3.67 (1.86–7.26)	8.98 (4.46–18.08)	<0.001
Diseases of the digestive system (K00–K93)	198	1.14 (0.74–1.76)	1.12 (0.76–1.65)	0.77 (0.51–1.16)	0.12
External causes of morbidity and mortality (V01–Y98)	304	1.60 (1.20–2.15)	1.30 (0.93–1.80)	0.68 (0.44–1.06)	0.02
All diseases (A00–Y98)	3937	1.17 (1.05–1.30)	1.22 (1.11–1.34)	1.39 (1.27–1.52)	<0.001

Adjusted for gender, year at hire (five categories: 1950 or earlier, 1951–1960, 1961–1970, 1971 or later), age at hire (continuous), and type of facilities (four categories: tungsten mines, iron/copper mines, tin mines and pottery factories).

mortality was further evaluated by the spline curve in Fig. 1. The results confirmed that the mortality was significantly higher among silica-exposed workers from all cause, respiratory tuberculosis, pulmonary heart diseases, and diseases of the respiratory system (pneumoconiosis).

The PAR% of silica exposure was 4.19% for mortality from all causes, 20.69% from respiratory tuberculosis, 7.48% for mortality from CVDs (including 27.89% from pulmonary heart diseases), and 34.06% for mortality from diseases of the respiratory system, after adjusting for other covariates.

The SMRs for deaths from all and specific cause from 1970 to 2003 are reported in Table 3. Compared with the mortality from 1970 to 2003 in China, the mortality among silica-exposed workers significantly elevated for certain infectious and parasitic diseases (SMR = 4.19), respiratory tuberculosis (2.62), CVDs (1.43), and diseases of the respiratory system (1.40). Among CVDs, it is significantly higher for pulmonary heart diseases (1.92), hypertensive heart disease (2.46), and ischemic heart disease (1.26). In addition, significantly elevated mortality was also found for nasopharynx cancer (1.76) and liver cancer (1.28).

4. Discussion

In the present cohort study with a large sample and long-term follow-up, we found a significant expose-response relationship between long-term silica dust exposure and mortality among never smokers, including all cause, respiratory tuberculosis, non-malignant respiratory diseases, and CVDs. Meanwhile, the SMRs of the latter three diseases from 1970 to 2003 among silica exposed workers were still higher than

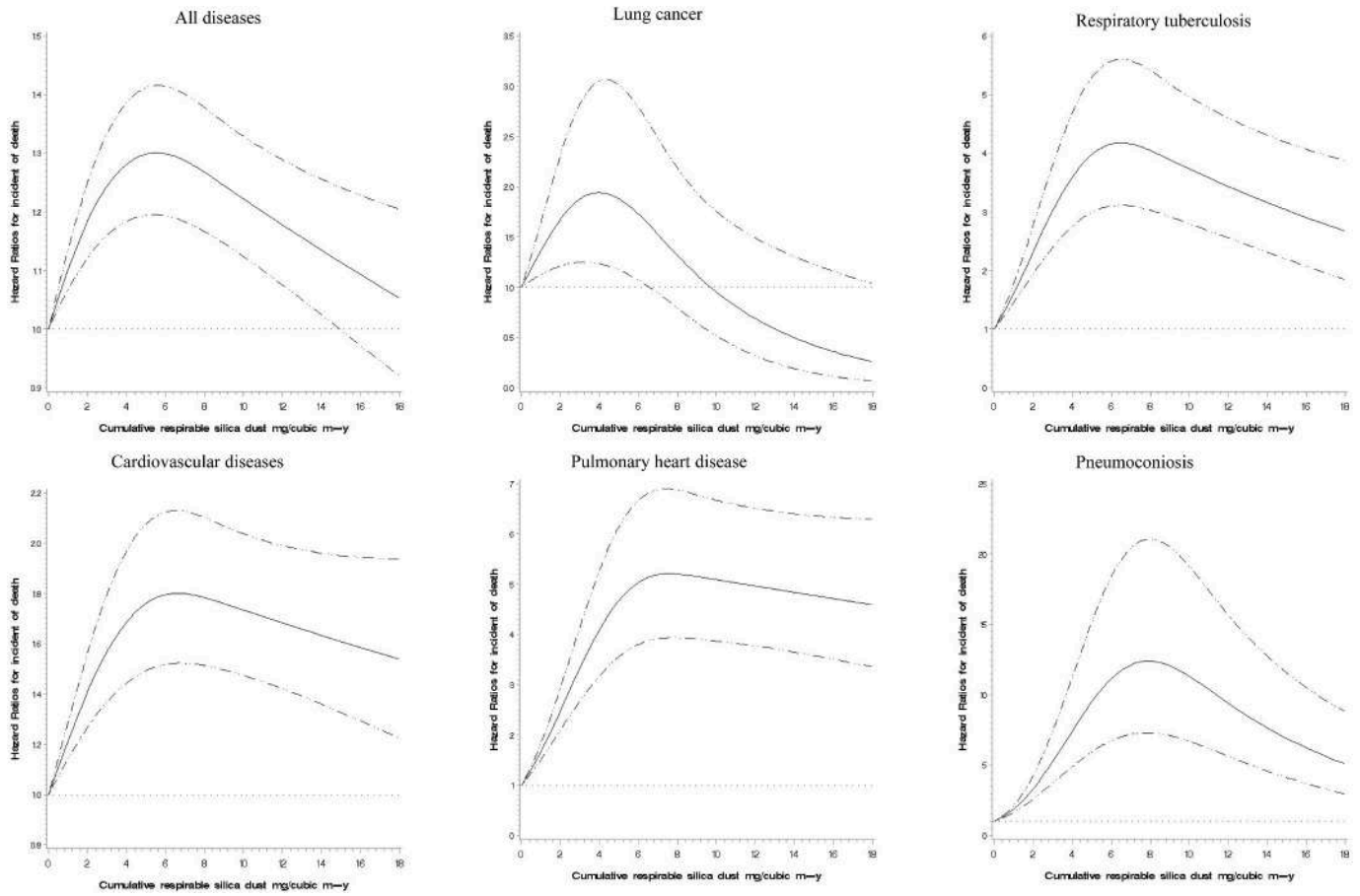

Fig. 1. Multivariable adjusted spline curves for association between cumulative respirable silica dust and mortality. Adjusted for sex, year of hire (four categories: 1950 or earlier, 1951–1960, 1961–1970, 1971 or later), age at hire (continuous), and type of facility (four categories: tungsten mines, iron and copper mines, tin mines, and pottery factories).

the general population.

The exposure-response relationships between CDE and mortality from all cause, respiratory tuberculosis, CVDs and diseases of the respiratory system have been reported in our previous study conducted among 74,040 workers (Chen et al., 2012). The present study reconfirmed the results among never smokers, which strongly indicated the relationship between silica dust exposure and mortality from these diseases. The findings may have important public health implications. Silica exposure is common in the workplace around the world and has been reported to be associated with several diseases, our study identified the significant independent attribution of silica exposure on all cause, respiratory tuberculosis, CVDs and diseases of the respiratory system in the absence of cigarette smoking.

The results for the association between silica exposure and above-mentioned diseases were consistent with other published cohort studies. Mannetje et al. verified the elevated silicosis mortality among silica-exposed workers in a pooled analysis of six cohorts (t Mannetje et al., 2002). Tse et al. confirmed the exposure-response relationship between silica dust exposure and mortality from non-malignant respiratory diseases (Tse et al., 2007). Few studies have reported the association between silica dust exposure and pulmonary heart diseases mortality. A retrospective cohort conducted among 6266 male workers found an increased standardized rate ratio (SRR) of 1.79 for pulmonary heart disease mortality (Dong et al., 1995), but the SRR was calculated by comparing the mortality rates with 11,470 male steel workers, the HRs were not reported either. Previous analysis of this cohort with 42, 572 workers revealed a positive exposure-response relationship between silica exposure and pulmonary heart diseases mortality (Liu et al., 2014). The exposure-response relationship could also be found in the current study, but the HRs were lower than those when cigarette smoking workers were excluded. Our present study conducted the exposure-response analyses with sufficient data on silica exposure, larger study population and longer follow-up time. In addition, we also found the exposure-response relationship between silica dust exposure and respiratory tuberculosis, which has been less studied before.

The association between silica dust exposure and lung cancer has been debated for several years (Keil et al., 2018). Most studies indicated the positive association between silica dust exposure and lung cancer, but others did not get the similar conclusion. A pooled analysis of 10 large silica-exposed cohorts with good-quality exposure data found a significant positive exposure-response relationship between silica exposure and lung cancer mortality (Steenland et al., 2001). In addition, a meta-analysis study revealed similar results, and showed studies with and without controlling for cigarette smoking yield similar relative risks (Lacasse et al., 2009). However, some experts disagreed with this conclusion, as the positive association between silica dust exposure and lung cancer could not be found in all industrial circumstances (Hessel et al., 2000). The main point for the debate is that it is difficult to rule out the risk caused by confounders, especially for tobacco smoking and occupational hazards other than silica. Sogl M et al. have confirmed a positive exposure-response relationship between silica dust and lung caner after adjusting for radon and arsenic with effect modifiers (Sogl et al., 2012); however, the individual information on the potential confounder smoking was not included in the analysis. Cigarette smoking is also a causative factor for lung cancer and the proportion of smokers is high among silica exposed workers (Zhang et al., 2009). Therefore,

Table 3
Estimated SMRs for death of workers in the cohort (N = 16,918), 1970–2003.

Cause of Death (ICD-10 Codes)	SMR (95%CI), 1970–2003				
	Total	Unexposed	Low	Medium	High
Malignant neoplasms (C00–C97)	0.72 (0.66–0.77)	0.68 (0.60–0.77)	0.85 (0.70–1.02)	0.83 (0.71–0.98)	0.62 (0.52–0.73)
Malignant neoplasm of nasopharynx (C11)	1.65 (1.14–2.31)	1.50 (0.80–2.56)	1.67 (0.54–3.79)	3.14 (1.62–5.44)	0.66 (0.13–1.85)
Malignant neoplasm of liver and intrahepatic bile ducts (C22)	1.14 (0.98–1.30)	0.94 (0.74–1.19)	1.40 (0.98–1.93)	1.39 (1.02–1.85)	1.11 (0.82–1.48)
Lung cancer (C33–C34)	0.59 (0.49–0.70)	0.51 (0.37–0.68)	0.54 (0.30–0.88)	0.84 (0.58–1.17)	0.55 (0.38–0.78)
Certain infectious and parasitic diseases (A00–B99, J65)	2.98 (2.69–3.29)	1.35 (1.06–1.69)	1.73 (1.18–2.45)	3.41 (2.72–4.22)	6.17 (5.34–7.09)
Respiratory tuberculosis (A15–A16, J65)	1.80 (1.61–2.00)	0.71 (0.53–0.92)	0.78 (0.48–1.19)	2.13 (1.67–2.67)	4.18 (3.59–4.84)
Cardiovascular diseases (I00–I52, I70–I99)	1.13 (1.06–1.20)	0.70 (0.62–0.80)	0.83 (0.66–1.03)	1.27 (1.10–1.46)	1.81 (1.64–1.99)
Pulmonary heart diseases (I26–I27)	1.28 (1.17–1.41)	0.36 (0.27–0.47)	0.48 (0.29–0.73)	1.33 (1.07–1.64)	2.98 (2.65–3.35)
Hypertensive heart disease (I11)	2.23 (1.86–2.65)	1.89 (1.38–2.52)	2.78 (1.68–4.32)	2.46 (1.62–3.57)	2.34 (1.65–3.20)
Ischemic heart disease (I20–I25)	1.18 (1.01–1.37)	1.07 (0.83–1.36)	1.48 (0.97–2.16)	1.85 (1.38–2.41)	0.77 (0.53–1.08)
Chronic rheumatic heart disease (I05–I09)	0.45 (0.32–0.62)	0.51 (0.31–0.79)	0.08 (0.01–0.39)	0.43 (0.17–0.87)	0.60 (0.31–1.05)
Cerebrovascular diseases (I60–I69)	0.86 (0.79–0.93)	0.85 (0.75–0.96)	1.00 (0.80–1.23)	0.96 (0.80–1.13)	0.76 (0.64–0.88)
Diseases of the respiratory system (J00–J99)	0.94 (0.86–1.03)	0.28 (0.21–0.35)	0.84 (0.63–1.10)	1.15 (0.95–1.38)	1.78 (1.58–2.00)
Pneumoconiosis (J60–J65)	45.40 (39.83–51.53)	–	15.64 (7.82–27.72)	53.91 (40.64–70.11)	128.10 (109.75–148.67)
Diseases of the digestive system (K00–K93)	0.64 (0.55–0.74)	0.52 (0.40–0.66)	0.85 (0.59–1.19)	0.91 (0.68–1.20)	0.53 (0.38–0.73)
External causes of morbidity and mortality (V01–Y98)	4.26 (3.75–4.82)	3.67 (2.96–4.50)	10.49 (8.24–13.16)	5.07 (3.81–6.61)	1.85 (1.24–2.65)
All diseases (A00–Y98)	0.83 (0.81–0.86)	0.65 (0.61–0.69)	0.88 (0.81–0.97)	0.94 (0.88–1.01)	1.01 (0.96–1.07)

SMRs were estimated based on Chinese national mortality rates (not available before 1970).

smoking is considered to be an important interfering factor in evaluating the carcinogenicity of silica dust. In the present study, our study confirmed that silica dust exposure was associated with lung cancer in the absence of cigarette smoking, though the higher risk of lung cancer in the high exposed group was not statistically significant. There are several possible reasons, including the healthy worker survivor effect, which refers to a depletion of the number of susceptible people in the population at high exposure levels and less reliable estimates at those levels. This phenomenon was also observed in studies of other occupational populations (Stayner et al., 2003). Our study could provide some important evidence for the association between silica dust exposure and lung cancer, as it was found in the absence of cigarette smoking in the present study.

Our study has several strengths. First, it was a long follow-up cohort study with large sample size. Second, the detail information of silica dust exposure during the lifetime was collected. The limitation should also be acknowledged. First, long-term exposure to silica dust was evaluated carefully, but measurement errors were inevitable. Silica concentrations before 1950 were estimated by using those in 1950, which may have led to the underestimation of exposure for those who worked before 1950 (4.0%). However, the results were almost the same when we excluded the workers whose silica exposure occurred before 1950 (data not shown). Second, the use of personal protective equipment was not considered in our study, but they were rarely used (<5% of the workers) or used improperly, indicating that the use of personal protective equipment had little effect on the results. Third, although a number of confounders were adjusted in our study, there were still other occupational risk factors which were not included, such as radon and polycyclic aromatic hydrocarbon (PAHs), which were also reported to paly an important role in respiratory diseases. However, less than 10% workplace of job titles have a level of radon exceeding the limit (0.3WL), and no statistical difference was found in the incidence of silicosis or lung cancer between the high radon exposure group (≥0.3WL) and low radon exposure group (<0.3WL) (P>0.05). In addition, the level of polycyclic aromatic hydrocarbon (PAHs) were also far less than the limit (0.1 mg/m^3), which may have little effect on health outcomes. Finally, smoking information for all participants were collected in 1995 and 2004 (Liu et al., 2013; Wang et al., 2020), respectively, and ever smokers (current and former smokers) were defined as those who had smoked cigarettes regularly for at least 1 year at any point in their lifetimes in this study, others were never smokers. Since cigarette smoking for the deceased were derived from their colleagues or next-of-kin, there may be information bias. In order to evaluate the accuracy of information, a data reliability analysis was examined for 1990 randomly selected subjects through face-to-face questionnaires, and the agreement on smoking status (never or ever) between next-of-kin and colleagues of decedents was 89.1%, and agreement on smoking status between self-report and next-of-kin (or colleagues) for living subjects was 93.6% (Liu et al., 2013).

5. Conclusions

Our study shows a significant exposure-response relationship between silica dust exposure and mortality from all cause, respiratory tuberculosis, non-malignant respiratory diseases, and CVDs among never smokers. In addition, elevated risk of lung cancer could also be found for silica exposed never smokers. Our findings could provide some information for the association between silica dust exposure and lung cancer.

Contributors

DW and WC designed research. DW conducted research, analyzed data, and wrote the paper. All authors contributed to the acquisition, analysis, or interpretation of the data, and revised the manuscript for important intellectual content. WC has primary responsibility for final content and is the study guarantor. All authors read and approved the final manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Declaration of competing interest

None declared.

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Associations of fine particulate matter and constituents with pediatric emergency room visits for respiratory diseases in Shanghai, China

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ABSTRACT

Background: Although ambient fine particulate matter ($PM_{2.5}$) has been associated with adverse respiratory outcomes in children, few studies have examined $PM_{2.5}$ constituents with respiratory diseases in children in China.

Objectives: To investigate the associations of short-term exposure to $PM_{2.5}$ and its constituents with pediatric emergency room visits (ERVs) for respiratory diseases in Shanghai, China.

Methods: We collected daily concentrations of $PM_{2.5}$ and its constituents in urban Shanghai from January 1, 2016, to December 31, 2018. Daily pediatric ERVs for four major respiratory diseases, including upper respiratory tract infection, bronchitis, pneumonia, and asthma, were obtained from 66 hospitals in Shanghai during the same period. Associations of exposure to daily $PM_{2.5}$ and constituents with respiratory ERVs were estimated using the over-dispersed generalized additive models.

Result: Short-term exposure to $PM_{2.5}$ and its constituents were associated with increased pediatric ERVs for respiratory diseases. Specifically, an interquartile range increase in the 3-day average $PM_{2.5}$ level (31 $\mu g/m^3$) was associated with 1.86% (95%CI: 0.52, 3.22), 1.53% (95%CI: 0.01, 3.08), 1.90% (95%CI: 0.30, 3.52), and 2.67% (95%CI: 0.70, 4.68) increase of upper respiratory tract infection, bronchitis, pneumonia, and asthma ERVs, respectively. As for $PM_{2.5}$ constituents, we found organic carbon, ammonium, nitrate, selenium, and zinc were associated with higher risk of respiratory ERVs in the single constituent and the constituent-$PM_{2.5}$ models.

Conclusion: Short-term exposure to $PM_{2.5}$ was associated with increased pediatric ERVs for respiratory diseases. Constituents related to anthropogenic combustion and traffic might be the dominant contributors of the observed associations.

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1. Introduction

The incidence of respiratory diseases, especially respiratory tract infection and asthma, has increased considerably in children in recent years and acute respiratory infection has become one of the major causes of hospitalization for young children around the world (Asher and Pearce, 2014; Shi et al., 2017). Previous studies suggested that hospital admission for pneumonia among children increased by 2.9 times from 2000 to 2015 globally with greater growth found in developing countries (Achilleos et al., 2017).

Several studies have suggested that ambient air pollution, especially fine particulate matter (particulate matter with an aerodynamic diameter $\leq$2.5 μm, $PM_{2.5}$) is a modifiable risk factor of respiratory diseases among children (Bouazza et al., 2018; Horne et al., 2018; Li et al., 2018; Liu et al., 2019; Nascimento et al., 2017). In China, increased pediatric visits for respiratory infection and asthma have been associated with $PM_{2.5}$ in previous studies, but the results were still inconsistent in magnitude (Liu et al., 2017; Zheng et al., 2015, 2017). One possible reason for such inconsistency is that as a mixture with complex constituents, ambient $PM_{2.5}$ may differ in respiratory toxicity as determined by chemical constituents from multiple sources (Liao et al., 2015). Although several studies have considered the associations of $PM_{2.5}$ chemical constituents and hospital visits for respiratory diseases, evidence for children were still limited compared to the general population (Kim et al., 2012; Ostro et al., 2009; Peng et al., 2009).

The aim of this time-series study is to examine the associations of short-term exposure to $PM_{2.5}$ and its constituents with EVRs for four common respiratory diseases, including upper respiratory tract infection, bronchitis, pneumonia, and asthma in children from Shanghai, China.

2. Materials and methods

2.1. Data collection

The study period was from January 1, 2016 to December 31, 2018. Daily pediatric ERVs during this period were extracted from electronic medical records of 66 hospitals in Shanghai, China (Fig. 1). We used the International Classification of Disease, Revision 10 (ICD10) to identify ERVs for upper respiratory tract infection (J06), bronchitis (J20/J21/J40), pneumonia (J12~J18), and asthma (J45~J46). Patients who were not residents of Shanghai were excluded. Details on data collection can be found in our previous publication (Liu et al., 2020).

Daily mass concentrations of $PM_{2.5}$ during the study period were calculated by averaging the daily means across ten fixed-site monitoring stations in the Shanghai National Air Quality Monitoring Network (Fig. 1). Daily concentrations of $PM_{2.5}$ constituents, including organic and elemental carbon, water soluble ions, and trace elements, were only available in the Shanghai Pudong Atmospheric Monitoring Supersite and thus were used to represent the $PM_{2.5}$ constituent levels in Shanghai (Fig. 1). Concentrations of organic (OC) and elemental carbon (EC) were measured by a semi-continuous OC/EC analyzer (model RT-4, Sunset Laboratory Inc.) with an upstream parallel-plate organic denuder and a $PM_{2.5}$ cyclone. Three major water-soluble inorganic ions, including nitrate (NO_3^-), sulfate (SO_4^{2-}) and ammonium (NH_4^+), were measured using the Monitor for AeRosols and Gases in ambient Air system (MARGA) (ADI, 2080; Applikon, Netherlands). Nine trace elements, including arsenic (As), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se), vanadium (V), and zinc (Zn), were measured by a nondestructive energy dispersive X-ray fluorescence spectrometry (Model XACT 625, Cooper Environmental, Services, LLC). Detailed descriptions of air pollution monitoring and relevant parameters can be found elsewhere (Niu et al., 2018).

Meteorological data, including daily mean temperature and relative humidity, were obtained from Shanghai Meteorological Bureau. Daily concentrations of other gaseous pollutants, including nitrogen dioxide (NO_2), sulfur dioxide (SO_2), carbon monoxide (CO), and ozone (O_3, 8-h maximum values) were also collected from the ten fixed-site monitoring stations for $PM_{2.5}$.

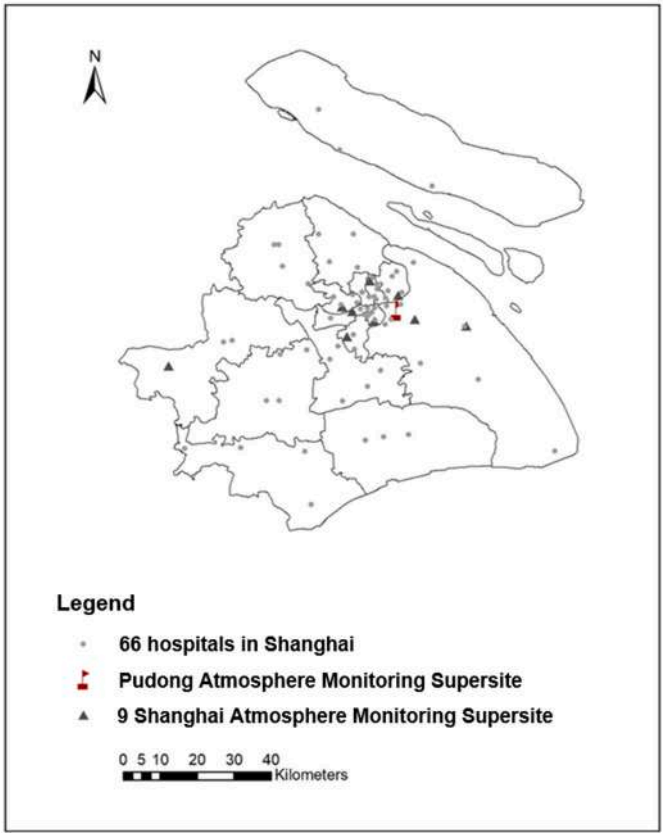

Fig. 1. The locations of 66 hospitals and atmospheric monitor stations in this study.

2.2. Statistical analysis

We used the generalized additive models (GAM) with a quasi-Poisson distribution to estimate the associations of daily $PM_{2.5}$ total mass and constituents with ERVs during the study period, respectively. Covariates considered in the models included: (1) a natural cubic spline with 7 degrees of freedom (df) per year for calendar days to control for long-term and seasonal trends; (2) natural cubic splines with 6 and 3 dfs for the current-day temperature and relative humidity; (3) a binary variable for public holidays; and (4) dummy variables of day of the week (DOW). To explore the potential lag effects of $PM_{2.5}$, we consider single-day exposures on the current (lag 0) and in 1, 2, and 3 days previously (lag 1–lag 3 days) and the cumulative average exposure for up to 3 days prior (i.e., lag 01, 02, and 03 days).

To further control for potential confounding by total $PM_{2.5}$ in the associations of constituents with respiratory ERVs, we then fitted constituents-$PM_{2.5}$ models by additionally adjusting for total $PM_{2.5}$ mass for each constituent, respectively. We also conducted sensitivity analyses to evaluate the robustness of our results. First, we replaced the current day temperature with moving averages from the current day to the previous 3, 7, and 14 days, respectively, to examine the possible lagged confounding of ambient temperature. Further, we considered a distributed lag nonlinear model (DLNM) for the 14-day average temperature to examine for residual confounding. Next, we used a natural spline of 10 df per year for calendar days to control for potential confounding by pollen and influenza seasons. Finally, we adjusted for daily SO_2, NO_2, CO, and O_3 in the models, separately, to control for confounding by co-exposure to these pollutants.

All analyses were conducted in R software (Version 3.3.5, R Foundation for Statistical Computing, Vienna, Austria) with the "mgcv" package. Results were presented as the percentage changes and 95% confidence intervals (CIs) in daily ERVs for each disease per interquartile range (IQR) increase of exposure to $PM_{2.5}$ and constituents. All statistical tests were two-sided and a $p < 0.05$ was considered statistically significant.

3. Results

3.1. Descriptive statistics

During the study period, we identified a total of 868,352 ERVs for upper respiratory tract infection (daily average = 792), 731,916 for bronchitis (daily average = 668), 126,802 for pneumonia (daily average = 116), and 108,817 for asthma (daily average = 99), respectively (Table 1). The daily ERVs for all four diseases distributed evenly by day of week (Supplementary Material Fig. 1). The daily average $PM_{2.5}$ was 40 μg/m^3 during the study period, which was higher than the World Health Organization Air Quality Guidelines (25 μg/m^3). Among the constituents, NO_3^- had the largest proportion (25.9%), followed by SO_4^{2-} (18.9%), NH_4^+ (16.4%), and OC (13.4%). The daily average temperature and relative humidity were 17.8 °C and 73%, respectively.

Spearman correlation coefficients among $PM_{2.5}$ constituents varied in both the magnitude and direction (Supplementary Material Table S1). Overall, there were moderate to high correlations (Spearman r = 0.47–0.90) with $PM_{2.5}$ for all constituents except for V, and the highest correlations were found between $PM_{2.5}$ and NH_4^+ (Spearman r = 0.90), OC and EC (Spearman r = 0.89), and NH_4^+ and NO_3^- (Spearman r = 0.89). Most of the $PM_{2.5}$ constituents had moderate and positive correlations with SO_2, NO_2, and CO, and weak and negative correlation with temperature and relative humidity.

3.2. Regression results

We observed positive associations of daily $PM_{2.5}$ levels and ERVs for all 4 respiratory diseases. The magnitude of the associations differed by lag days and by diseases and asthma had the strongest association with $PM_{2.5}$. As shown in Fig. 2, the strongest associations with $PM_{2.5}$ were found on lag02 days for upper respiratory tract infection, pneumonia, and asthma, and on lag01 for bronchitis. For example, an IQR increase in total $PM_{2.5}$ mass (31 μg/m^3) in lag 02 days was significantly associated with a 1.86% (95%CI: 0.52, 3.22), 1.53% (95%CI: 0.01, 3.08), 1.90% (95%CI: 0.30, 3.52), and 2.67% (95%CI: 0.70, 4.68) increase of ERVs in upper respiratory tract infection, bronchitis, pneumonia, and asthma, respectively. Generally, the model with exposures in lag 0–2 days had the strongest associations with the outcomes as well as the smallest quasi-Poisson Akaike Information Criterion (Supplementary Material Table S3), so we reported estimates in lag 0–2 days in the following analyses.

Fig. 3 showed the percentage change of daily ERVs for upper respiratory tract infection, bronchitis, pneumonia, and asthma with an IQR increase of $PM_{2.5}$ constituents in the single constituent models. Specifically, we found OC, SO_4^{2-}, NO_3^-, NH_4^+, Se, and Zn were associated with higher daily ERVs for all 4 respiratory diseases. Among all the constituents, NH_4^+ had the strongest associations with upper respiratory tract infection, bronchitis, and asthma, and Se had the strongest association with pneumonia. For instance, an IQR increase of NH_4^+ was association with 2.74% (95%CI: 1.55, 3.95), 2.40% (95%CI: 1.05, 3.77) and 1.86% (95%CI: 0.01, 3.75) increase of ERVs for upper respiratory tract infection, bronchitis, and asthma, respectively; and an IQR increase of Se was associated with 2.40% (95%CI: 0.79, 4.04) increase in pneumonia.

After adjusting for $PM_{2.5}$ total mass in constituent-$PM_{2.5}$ models, we found associations of SO_4^{2-} and NO_3^- with daily ERVs attenuated, while associations of OC, Se, and Zn were similar with the single constituent models (Fig. 4). Of note, estimates for NH_4^+ in the constituent-$PM_{2.5}$ models were higher than those in the single constituent models with an IQR increase of NH_4^+ was associated with 7.11% (95%CI: 4.21, 10.09),

Table 1
Daily emergency room visits for respiratory diseases, air pollution levels, and meteorological factors.[a].

variables	mean	SD	Minimum	P (25)	Median	P (75)	Maximum
Emergency Room visits							
Upper respiratory tract infection	792	284	260	609	754	916	2,390
Bronchitis	668	362	167	388	555	894	2,370
Pneumonia	116	58	35	77	101	141	824
Asthma	99	51	30	63	86	123	380
$PM_{2.5}$							
Total mass (μg/m^3)	40	26	6	21	33	51	184
OC (μg/m^3)	5	3	1	3	5	7	20
EC (μg/m^3)	2	1	0	1	2	3	10
SO_4^{2-} (μg/m^3)	8	4	0	4	6	10	37
NO_3^- (μg/m^3)	10	10	1	4	8	14	70
NH_4^+ (μg/m^3)	7	5	0	3	5	9	35
As (ng/m^3)	7	5	0	7	6	10	37
Cr (ng/m^3)	6	4	0	5	5	7	24
Cu (ng/m^3)	14	9	0	11	11	18	84
Mn (ng/m^3)	36	21	0	29	30	45	140
Ni (ng/m^3)	5	2	0	3	4	6	25
Pb (ng/m^3)	35	4	0	29	28	44	264
Se (ng/m^3)	3	2	0	3	3	5	4
V (ng/m^3)	7	5	0	8	5	10	30
Zn (ng/m^3)	155	96	0	141	126	201	712
Meteorological conditions							
Temperature (°C)	18	9	−6	10	19	25	35
Relative humidity (%)	73	12	29	64	74	82	100
Gaseous pollutants							
SO_2 (μg/m^3)	12	5	3	8	10	14	50
NO_2 (μg/m^3)	42	19	10	28	39	54	125
CO (mg/m^3)	0.7	0.2	0.4	0.6	0.7	0.8	1.9
O_3 (μg/m^3)	83	37	9	55	78	102	235

[a] Definition of abbreviations: SD = standard deviation, $PM_{2.5}$ = fine particulate matter, NO_2 = nitrogen dioxide, SO_2 = sulfur dioxides, CO = carbon monoxide, O_3 = ozone.

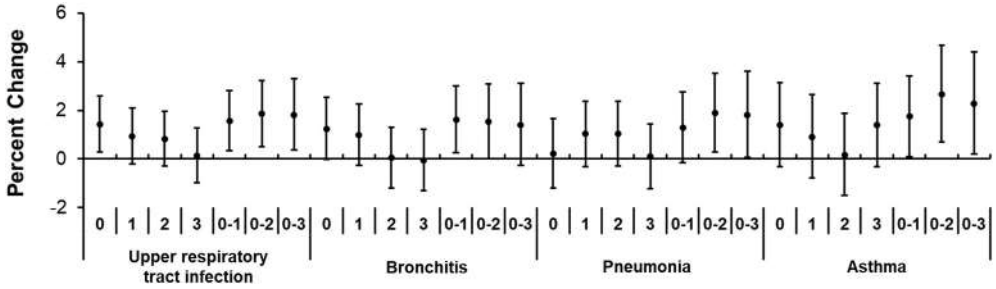

Fig. 2. Percentage changes (mean and 95% confidence intervals) in daily ERVs for 4 diseases per IQR (31 μg/m^3) increase in concentrations of $PM_{2.5}$ with different lag days.

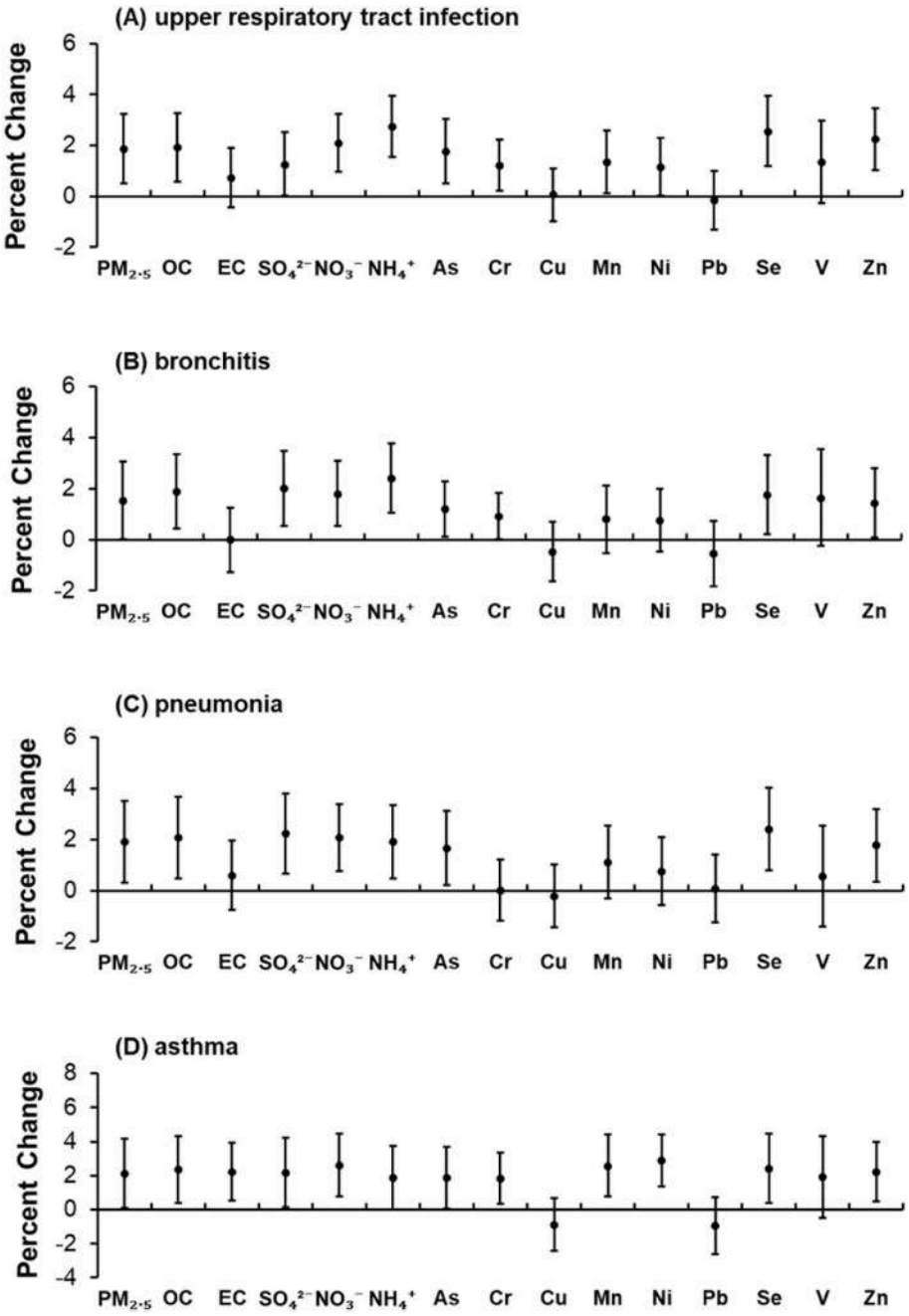

Fig. 3. Percentage changes (mean and 95% confidence intervals) in daily pediatric respiratory ERVs associated with an IQR increase of $PM_{2.5}$ and constituents (lag 0–2 days) in single-constituent models.

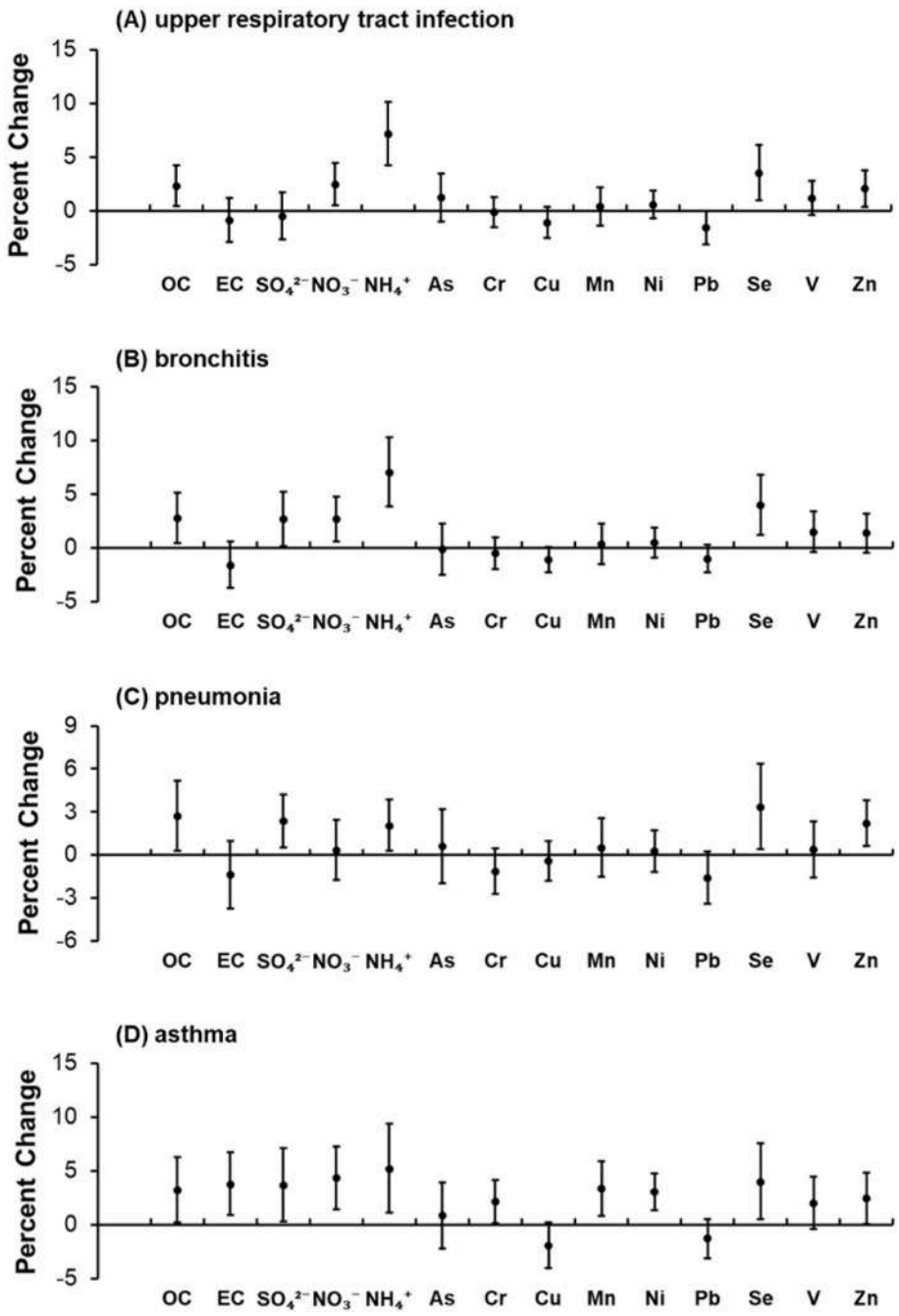

Fig. 4. Percentage changes (mean and 95% confidence intervals) in daily pediatric respiratory ERVs associated with an IQR increase of $PM_{2.5}$ constituents (lag 0–2 days) in constituent-$PM_{2.5}$ models.

7.00% (95%CI: 3.84, 10.25), 2.04% (95%CI: 0.26, 3.85) and 5.16% (95%CI: 1.15, 9.34) increase of ERVs for upper respiratory tract infection, bronchitis, pneumonia and asthma, respectively.

Estimates changed little for the associations of $PM_{2.5}$ constituents and respiratory ERVs when using longer lags or a DLNM model for temperature or using 10 df in the natural cubic spline for calendar days (Supplementary Material Table S2 and Figure S2 and S3). After adjusting for SO_2, NO_2, and CO in the models, the associations between $PM_{2.5}$ constituents and pediatric ERVs attenuated and the 95% CIs were wider (SSupplementary Material Figure S4~7), while the model estimates remained robust after adjusting for O_3.

4. Discussion

Results from this time-series study showed that ambient $PM_{2.5}$ and its chemical constituents such as OC, NH_4^+, Se, and Zn were associated with increased risk of pediatric emergency room visit for respiratory diseases in Shanghai, China. These associations were robust in sensitivity analyses. Our findings provided evidence to the associations of $PM_{2.5}$ constituents on children's respiratory diseases in China.

We found short-term exposure to $PM_{2.5}$ was associated with increased risk of pediatric respiratory ERVs. This result was consistent with previous evidences from China suggesting exposure to $PM_{2.5}$ would increase emergency room visits of respiratory diseases (Tian et al., 2017; Xu et al., 2016). Children are believed to be more vulnerable to ambient air pollution due to their higher breath rate, narrower airway,

undeveloped lung, and more time spent outdoors (Xing et al., 2020). However, so far, few studies have examined the associations between $PM_{2.5}$ level and pediatric ERVs for respiratory diseases in China (Liu et al., 2020). Several studies reported positive associations between $PM_{2.5}$ and children's respiratory hospital admissions in Asian countries but the magnitude of these associations differed due to the different outcomes of interest (e.g., hospitalization vs emergency visits), age, and lag periods (Hua et al., 2014; Lee et al., 2006). For instance, a time-series study in Vietnam suggested that a 39.4 μg/m^3 increase of 6-day average $PM_{2.5}$ was associated with 1.3% increase of hospital admission for bronchitis and asthma among children aged 1–5 (Nhung et al., 2018). Another study in Jinan, China showed children hospital admissions for upper respiratory infection increased by 1.57% per 10 μg/m^3 increase of $PM_{2.5}$ in lag 03 days (Liu et al., 2019). In addition, stronger associations of $PM_{2.5}$ with respiratory outcomes in children were reported by studies conducted in countries with lower $PM_{2.5}$ levels. For example, one study in US found that a 10 μg/m^3 increase of $PM_{2.5}$ exposure was associated with 15–32% increase in the odds of acute lower respiratory infection among children (Horne et al., 2018). Another study in Ontario, Canada showed each interquartile change (5.92 μg/m^3) in 3-day mean $PM_{2.5}$ was associated with a 7.2% increased risk of emergency room visits for asthma among children under 9 years old (Weichenthal et al., 2016). Possible explanations for such differences include variations of the exposure levels and chemical compositions of $PM_{2.5}$, and population characteristics by study location.

Among the carbonaceous constituents, we found OC was associated with ERVs for all the four major respiratory diseases and this association remained robust after adjusting for $PM_{2.5}$ total mass. Previous studies have linked OC with increased risk of adverse respiratory outcomes in different population (Kim et al., 2012; Peng et al., 2009; Wang and Lin, 2016). For example, a study in California suggested an IQR (4.5 μg/m^3) increase of OC was associated with an excess risk of 3.4% in respiratory hospital admission for children (Ostro et al., 2009). Another 18-year time-series study in Atlanta suggested an IQR (1.7 μg/m^3) increase of 3-day moving average concentration of OC predicted a 2% increase in emergency room visits for pneumonia and an 1.9% increase for upper respiratory infection among children aged 0–4 (Darrow et al., 2014). However, most current studies were conducted in developed countries, while evidence from developing countries such as China was scarce.

We also observed NH_4^+, SO_4^{2-}, and NO_3^-, which were the predominant compositions of $PM_{2.5}$, were associated with increased pediatric ERVs. In addition, the estimate of NH_4^+ was inflated after adjusting for total $PM_{2.5}$, which may suggest multi-collinearity in the model. Although the adverse health effects of these soluble ions in $PM_{2.5}$ have been documented in the literature, evidence in children were still sparse and inconsistent (Ferreira et al., 2016; Hwang et al., 2017; Ostro et al., 2009). A time-series study suggested that an IQR (3 μg/m^3) increase of sulfate was associated with a 1.3% increase in emergency room visits for upper respiratory infection among children aged 0–4 (Darrow et al., 2014). Another two studies from Taiwan found that NH_4^+, NO_3^-, and SO_4^{2-} were associated with increased respiratory mortality or asthma ERVs in the general population (Hwang et al., 2017; Wang et al., 2019).

Our results suggested trace element compositions, especially Se and Zn, were associated with increased pediatric ERVs for respiratory diseases. Among the limited epidemiological evidence on the trace element constituents of $PM_{2.5}$ and respiratory disease, Zn has been suggested to be associated with increased asthma risk in children. For example, Gehring et al. showed that exposure to PM constituents, in particular Fe, Cu, and Zn, may increase the risk of asthma and allergy in children (Gehring et al., 2015). Hirshon et al. found associations between Zn in particles and increased asthma morbidity among children (Hirshon et al., 2008). However, furhter evidence is warranted to confirm these findings.

There were several studies concerned with the potential biological mechanisms linking $PM_{2.5}$ and respiratory adverse events, among which oxidative stress was regarded as a crucial pathophysiological mechanism of $PM_{2.5}$-induced respiratory disease (Valavanidis et al., 2013; Zhang et al., 2016). Reactive oxygen species (ROS) could directly combine with particles or generate via cellular redox reaction through stimulation from specific particle components (Dellinger et al., 2001). For example, previous studies have hypothesized that the magnitude of ROS generation could be driven by transition metals (e.g., iron, copper, manganese, vanadium, nickel, chromium) and organic compounds (Cho et al., 2005; Gurgueira et al., 2002). $PM_{2.5}$ components can also impair respiratory system by inducing inflammatory response (Schaumann et al., 2004). NO_3^- has been reported to have the strongest toxic effect on the respiratory system in young mice among the three major $PM_{2.5}$ water-soluble inorganic components (NH_4^+, NO_3^- and SO_4^{2-}) (Zhang et al., 2021b). The supposed molecular mechanisms included inflammatory response, dysregulating lung gene expression, immune signaling, lysosome and circadian rhythms (Zhang et al., 2021a).

Our findings of $PM_{2.5}$ chemical constituents indicated that the observed adverse respiratory effects of $PM_{2.5}$ were contributed by particles from fuel combustion and industrial emission. The major sources of organic carbon include motor vehicles, heavy fuel oil burning, and industry in Shanghai. Soluble ions are mainly contributed by coal burning, industrial emission, and secondary sources and Se and Zn are mainly from coal burning and industrial emission (Zhou, 2020). Therefore, regulation on these emission sources should be given a higher priority for the purpose of health protection.

Our study has several limitations. First, we relied on $PM_{2.5}$ constituents measured in a single monitoring station to represent the overall exposure level in Shanghai. Therefore, exposure measurement errors are possible as this single station cannot fully capture the geographic variations of $PM_{2.5}$ constituents in an area of more than 6,000 km^2. However, such exposure measurement errors were likely non-differential and thus may bias the results towards the null (Zeger et al., 2000). Second, this study was conducted in one of the largest cities of China. Therefore, our results may not be applicable to children in other developing countries or even to the general children population in China. Moreover, we only considered ERVs in the analysis, while outpatient clinic visits and hospital admissions were not included. Children who ended up with hospital admissions usually had more severe condition and only account for a small proportion of the patients. Meanwhile, outpatient visits were sometimes scheduled and thus might introduce misclassification in the analysis. Therefore, ERVs can be more appropriate when investigating the short-term effects of air pollution on health (Winquist et al., 2012). Third, although we adjusted for potential confounders such as meteorological conditions, seasonality, and co-exposure to other gaseous pollutants, residual confounding by factors such as influenza and pollens was possible. Finally, the high correlations between $PM_{2.5}$ constituents and total $PM_{2.5}$ may limit our ability to estimate the associations of these constituents with the outcome that is independent of total $PM_{2.5}$. Therefore, our results should be interpreted with caution.

5. Conclusion

In this time-series analysis, we found short-term exposure to $PM_{2.5}$ and its constituents, mainly OC, NH_4^+, Se and Zn, were consistently associated with increased pediatric ERVs of respiratory diseases in Shanghai, China. Our results suggested that constituents related to anthropogenic combustion and traffic might dominate the adverse respiratory effects of $PM_{2.5}$ among children. This study added to the limited evidence on $PM_{2.5}$ and its constituents with children's respiratory health in developing countries.

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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.2021.113805.

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Attributes of drinking water, sanitation, and hygiene associated with microbiological water quality of stored drinking water in rural schools in Mozambique and Uganda

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ABSTRACT

Contaminated drinking water causes morbidity and mortality worldwide, especially in low- and middle-income countries. Drinking water quality has been studied extensively in household settings, but little research is available on drinking water quality in schools. School settings are of particular importance, because children are more susceptible than adults to a variety of diseases from contaminated drinking water. Many school water, sanitation and hygiene (WaSH) interventions have been studied for their efficacy to reduce diarrheal disease incidence, but few have evaluated drinking water quality, which reflects an important exposure pathway between WaSH services and health outcomes. Using school surveys developed from internationally established WaSH indicators and field microbiological water quality tests, we studied 374 rural schools in Mozambique and Uganda to understand the association between specific WaSH services and drinking water microbiological contamination, specifically testing most probable number (MPN) of *Escherichia coli*, an indicator of fecal contamination, per 100 mL. In Mozambique and Uganda, 71% and 83% respectively of rural schools had low risk drinking water quality (<1 *E. coli*/100 mL); thirteen percent and seven percent had very high-risk water quality (≥100 *E. coli*/100 mL). When accounting for all WaSH services studied, schools that used an improved-type water source had 0.22 times less *E. coli* in stored drinking water in Mozambique (95% CI: 0.07, 0.65) and 0.12 times less *E. coli* in Uganda (95% CI: 0.02, 0.80). In Mozambique, use of a water source within 30 minutes for travel and collection and the presence of water and soap/ash for handwashing were also significantly associated with less *E. coli* in drinking water. The findings of this study provide public health practitioners with implementable WaSH services to improve school drinking water quality, which has implications for the health, learning environment, and cognitive development of school children in rural Mozambique and Uganda.

1. Introduction

Contaminated drinking water continues to cause substantial morbidity and mortality worldwide (Clasen et al., 2007; Hunter et al., 2010; Wolf et al., 2018a). As of 2019, drinking water sources of an estimated two billion people were contaminated with feces and over 800,000 people die annually from diarrhea caused by poor water, sanitation, and hygiene, including nearly 300,000 children (World Health Organization, 2019). Community settings, like schools and health facilities, have become spotlights for water, sanitation, and hygiene (WaSH) programming in low-income countries (LICs), to reduce disease exposure in commonly frequented areas outside the home. WaSH services that protect users from pathogen exposure are sparse in these settings (Guo et al., 2017; Morgan et al., 2017) and poorly financed (Alexander et al., 2016; McGinnis et al., 2017), and monitoring for quality of services in countries is infrequent and inadequate (United Nations Children's Fund and World Health Organization, 2018b; World Health Organization and United Nations Children's Fund, 2019).

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Evidence from household studies for effective WaSH strategies informs interventions in school and other extra-household settings. Meta-analyses of household WaSH interventions show household water treatment, safe storage, and sanitation interventions reduce diarrheal risk (Hunter, 2009; Wolf et al., 2014) and are associated with lower odds of intestinal protozoa infections (Speich et al., 2016). In cross-sectional studies, the type of water source and residual chlorine treatment are consistent predictors of *Escherichia coli* (*E. coli*) contamination (Gupta et al., 2007; Jeandron et al., 2019; Trevett et al., 2004), but various water storage and usage practices show no association in Honduras (Trevett et al., 2004).

Schools offer a particular opportunity for improving health related to WaSH and water quality, as children spend a substantial portion of their daytime hours in school. While household water quality has improved, school-aged children are exposed to waterborne disease through poor school drinking water quality. Further, children are more susceptible than adults to diarrheal disease and other waterborne illnesses (Jasper et al., 2012). Water treatment with hygiene or sanitation in schools decreases respiratory illness (Patel et al., 2012) and parasitic infections (Erismann et al., 2017; Freeman et al., 2013), and increases health-related knowledge and behaviors (Chard and Freeman, 2018; Hetherington et al., 2017), enrollment, and gender parity (Garn et al., 2013). School water treatment and handwashing interventions also reduce absenteeism, especially for girls (Trinies et al., 2016). School hand hygiene interventions alone also reduce absences due to a variety of respiratory and enteral infections (Talaat et al., 2011). Lastly, schools are a learning environment and have the potential to be places where safe WaSH practices are learned by students (Anthonj et al., 2021), and these teachings can then be shared with their families and communities (Bresee et al., 2016; Dreibelbis et al., 2014).

Many researchers have tested the effect of school WaSH interventions on diarrheal disease incidence or missed school days (McMichael, 2019), but few have evaluated the quality of drinking water (microbiological or chemical) as an intermediary in this causal relationship. As improvement of drinking water quality is a mechanism for how school WaSH interventions improve health of school-aged children, we study microbiological stored drinking water quality in 374 rural schools in Mozambique and Uganda and analyze the association between school WaSH services and microbiological water quality.

2. Methods

2.1. Sampling, study population, data collection tools

The sampling methodology, survey instrument, and study population have been previously described (Morgan et al., 2017). Briefly, we conducted a stratified random sample of schools in specific rural regions of ten sub-Saharan African countries; here we describe results from the schools in Mozambique and Uganda that had stored drinking water and collected a water sample from it. These two countries were selected from the original study as the random sample of schools with water quality samples from stored drinking water was sufficiently large in each. Data collection consisted of two components: a standardized survey instrument to evaluate access, quantity, quality, continuity, and reliability of WaSH services (previously described (Morgan et al., 2017)); and microbiological water quality testing of stored drinking water. GPS coordinates of schools were also collected.

2.2. Ethics

Free and informed participant consent was obtained from all school officials surveyed. The Institutional Review Board of the University of North Carolina at Chapel Hill approved this study protocol on June 3, 2014 (IRB Reference ID: 14–0763). This study was approved by the corresponding national governing bodies: the Uganda National Council for Science & Technology (UNCST) and the Directorate of Water in the Ministry of Public Works and Housing in Mozambique.

2.3. WaSH factors analyzed

We analyzed descriptive statistics of WaSH services of the 374 rural schools in Mozambique and Uganda, including water source type, access (distance to source), storage, removal; sanitation facility type and condition; and hand hygiene access. Estimates were weighted based on stratified random sampling in selected rural regions, to account for different probabilities of selection of schools. We tested the association of these WaSH factors in schools on school microbiological water quality using estimated *E. coli.* We describe the variables and regression model here.

We used the WHO/UNICEF Joint Monitoring Programme categorizations of “improved” and “unimproved” types of water source and sanitation (World Health Organization, 2012). Improved-type drinking water sources decrease the risk of fecal contamination compared to unimproved-type drinking water sources, but do not guarantee microbial water safety (Bain et al., 2014; Shaheed et al., 2014). Improved-type water sources include piped water, boreholes, protected dug wells or springs, rainwater, and packaged water. Improved-type sanitation limits human contact with excrement, and include flush sewer systems, septic tanks, ventilated pit latrines, composting toilets, and pit latrines with slabs. We analyzed water storage by observing the use of a safe container (covered container, with a narrow opening, or with a wide opening and water treatment) and safe removal methods (pouring, spigot, tap, or long ladle for extracting water) (Centers for Disease Control, 2014). We analyzed hand hygiene facilities by assessing the presence of water and soap/ash for handwashing, a widely used indicator for hand-hygiene access (United Nations Children’s Fund and World Health Organization, 2018a). We also analyzed the presence of materials for hand-drying.

The conditions of improved-type sanitation in schools were observed by the presence of the following aspects of each sanitation facility: doors, doors that can be closed, doors with locks, holes in the structure, stability of latrine slab, caving walls of the structure, latrine pits that were too large, latrine pits that were caving in, used paper on slabs, and flies swarming.

2.4. Water quality testing

As this study was conducted within a larger multi-country, multi-site evaluation, we used *E. coli* as the microbial indicator as the organism is an indicator of fecal contamination, it does not grow naturally in the environment (Edberg et al., 2000), and field-based and laboratory testing are available, inexpensive, and are simple to conduct in rural areas of LICs. In each school, a 100 mL water sample was collected from the stored drinking water consumed by students, used by the school, in the same way members of the school extract water for drinking. We analyzed water quality using Aquagenx (Chapel Hill, NC) Compartment Bags to determine the most probable number (MPN) of *E. coli* (Stauber et al., 2014) according to the manufacturer’s instructions (Aquagenx, 2013).

2.5. Regression model

WaSH indicators measured in the survey were treated as ordinal predictor variables, and MPN of *E. coli* in 100 mL of stored drinking water as a discrete outcome variable. Predictor variables were selected for the model based on potential for contamination of stored drinking water and were indicator variables for the following WaSH services: improved-type water source; treatment of stored water; safe container for stored drinking water; safe removal method of stored drinking water; water source within 30 minutes roundtrip; improved-type sanitation; water and soap/ash present for handwashing. The association between each WaSH factor and water quality was tested in bivariate (unadjusted)

analysis using a negative binomial regression model (El-Shaarawi et al., 1981), because our outcome, concentration of *E. coli*, was discrete and overdispersed around zero. Unadjusted covariates that were significant at $p < 0.05$ or considered necessary to control for (e.g. treatment of water) were included in a multivariate (adjusted) model. Tests for collinearity of these factors were conducted before inclusion in an adjusted model, and interaction terms between predictors were evaluated. Model results are reported as incidence rate ratios.

Frequencies were calculated using PROC SURVEYMEANS and PROC SURVEYFREQ in SAS 9.4 (SAS Institute, Cary, NC, USA). The negative binomial regression model was computed using *nbreg* with the *irr* option in Stata 14 (StataCorp, College Station, TX, USA). Figures for descriptive statistics were generated using R 3.6.0. Maps were produced using the "sf" package in R 3.6.0. Schools with missing GPS points were mapped to their respective districts.

3. Results

3.1. School demographics

We studied 374 rural schools, 124 in Mozambique and 250 in Uganda, serving 206,487 total students (Table 1). Districts sampled were geographically disparate rural areas (Fig. 1), and sampled schools were predominantly primary schools. The median number of students enrolled was 374 in Mozambique and 510 in Uganda (Table 1). In Mozambique, the median numbers of boy and girl students were 191 (IQR: 35, 366) and 172 (IQR: 66, 322), respectively. In Uganda, the median numbers of boy and girl students were 238 (IQR: 180, 372) and 263 (IQR: 190, 375), respectively.

4. Descriptive statistics

4.1. Water quality

Seventy-one percent of rural schools in Mozambique and 83% in Uganda had <1 *E. coli* MPN/100 mL (Fig. 2A), the lowest health risk in the latest WHO classification (World Health Organization, 2017). Thirteen percent of rural schools in Mozambique and seven percent in Uganda had ≥100 *E. coli* MPN/100 mL, WHO's highest health risk category. Fewer schools in each country fell into the intermediate risk categories. Boreholes, an improved-type water source, were the most common water source used by rural schools in both countries (Fig. 2B). Stored drinking water of the highest health risk level (>100 *E. coli* MPN/100 mL) was found in rural schools with improved-type water sources in both countries (piped and purchased sources in Mozambique, boreholes and protected springs in both countries, and rainwater in Uganda).

4.2. Water source type and storage

Of schools with water sources, 89% percent of rural schools in Mozambique and 95% in Uganda had an improved-type water source; in 92% and 85%, respectively, the water source was within 30 minutes of the school, including collection time (Fig. 3A). Forty-eight percent of rural schools in Mozambique and 78% in Uganda had safe storage containers, and 19% and 62%, respectively, had means for safe removal of drinking water. Three percent of schools in Mozambique reported no storage of drinking water because they had on-plot water sources.

Ten percent and 17% of schools reported treatment of drinking water in Mozambique and Uganda, respectively. Treatment methods included boiling and chlorine in both countries, with one school in Uganda reporting filtration.

4.3. Sanitation type and quality

Sanitation facilities were predominantly of an improved type, though the conditions of sanitation facilities varied. Sixty-two percent in Mozambique and 91% in Uganda had improved-type sanitation facilities. Of schools with improved-type sanitation, the most frequent problem was a lack of doors: only 46% of rural schools in Mozambique and 58% in Uganda had doors on all latrines. (Fig. 3B).

4.4. Hand hygiene

The availability of handwashing facilities was notably absent: only 2% of rural schools in Mozambique and 14% in Uganda had water and soap or ash for handwashing present on the day of the survey. Only 2% of rural schools in each country had water, soap or ash, and drying materials for handwashing present.

4.5. Regression model

Several WaSH factors in rural Mozambique schools had unadjusted estimates that significantly correlated with *E. coli* MPN/100 mL in stored drinking water (Table 2). Schools with an improved-type water source had 0.29 (95% CI: 0.13, 0.64) times the incidence rate of *E. coli* in stored drinking water compared with schools with unimproved-type water sources. Schools with water sources within 30 minutes for collection had 0.28 (95% CI: 0.12, 0.68) times the incidence rate of *E. coli* as schools with more distant water sources. Schools with safe storage containers had 3.71 (95% CI: 1.38, 9.92) times the incidence rate of *E. coli* compared with schools without safe containers, and schools with means for safe removal of stored water (e.g. with a tap or ladle) had 2.47 (95% CI: 1.10, 5.54) times the incidence rate of *E. coli* as schools that did not. Schools with water and soap/ash for handwashing on the day of the survey had 0.04 (0.01, 0.19) times the incidence rate of *E. coli* than with schools without these materials for handwashing. Schools that had hygienic materials for hand-drying, in addition to water and soap/ash, on the day of the survey had 0.08 (0.06, 0.13) times the incidence rate of *E. coli* as schools without all three handwashing materials. In testing for collinearity, none of these variables had correlation coefficients above 0.8, and were all included in an adjusted model.

In an adjusted model with the selected predictors of water quality in Mozambique, an improved-type water source (IRR: 0.22, 95% CI: 0.07, 0.65), water sources within 30 minutes (IRR: 0.25, 95% CI: 0.08, 0.81) and water and soap/ash for handwashing present (IRR: 0.12, 95% CI: 0.02, 0.73) remained significant, each associated with less *E. coli*. A similar adjusted model is observed when the handwashing indicator includes materials for drying. An improved-type water source and water sources within 30 minutes have similar incidence rate ratios, and water, soap/ash, and drying materials are associated with 0.27 times the incidence rate of *E. coli* (95% CI: 0.10, 0.76).

Predictors of water quality in schools in Uganda showed a different picture. Schools with piped water sources or other improved-type water

Table 1
Demographics of rural schools studied.

Country	Districts sampled	Schools with water quality samples (n)	Median total students (IQR)	Median male students (IQR)	Median female students (IQR)	Median number of teachers (IQR)
Mozambique	13	124	374 (133, 698)	191 (35, 366)	172 (66, 322)	6 (3, 15)
Uganda	10	250	510 (372, 757)	238 (180, 372)	263 (190, 375)	10 (7, 13)

IQR: Interquartile range.

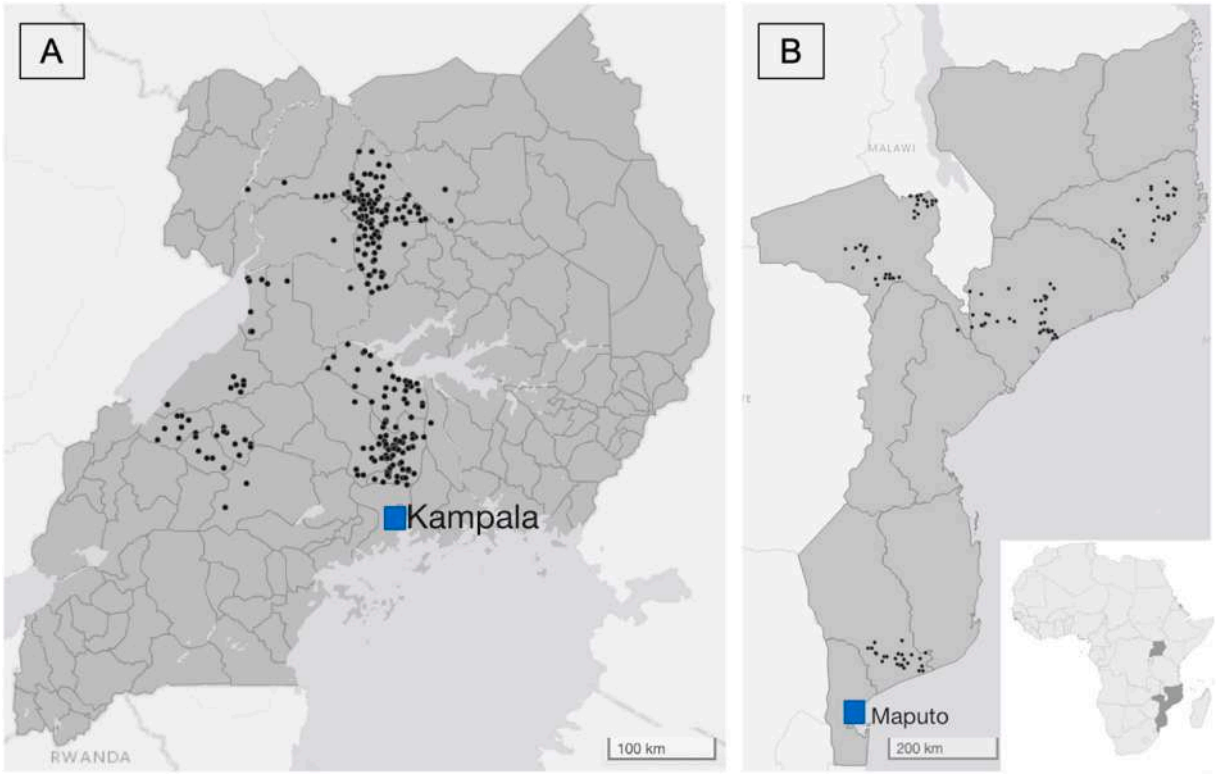

Fig. 1. Geographic locations of surveyed schools in Uganda (A, n = 250) and Mozambique (B, n = 124).

sources had 0.09 (95% CI: 0.02, 0.55) times the incidence rate of *E. coli* as schools with unimproved-type water sources. Schools that treated water had 2.36 (95% CI: 4.22, 11.22) times the incidence rate of *E. coli* as schools that didn't treat water. Lastly, schools with improved sanitation had 0.28 (95% CI: 0.10, 0.74) times the incidence rate of *E. coli* as schools with unimproved or no sanitation.

No collinearity was observed, and these three predictors were included in an adjusted model for Uganda. In the adjusted model, only an improved-type water source remained a significant predictor of water quality (IRR: 0.12, 95% CI: 0.02, 0.83).

In both countries, neither the method of water treatment (boiling or chlorination) nor specific conditions of latrines on visual inspection were associated with amount of *E. coli* contamination.

5. Discussion

We provide one of very few studies of rural school drinking water quality in LIC settings and that assesses the WaSH factors associated with safer microbiological drinking water quality in rural schools. Our large study (n = 374 rural schools) represents a sample from broad geographic areas in both countries studied, providing comparable and generalizable findings. Drinking water quality in both countries was good overall: 71% of schools in Mozambique and 83% in Uganda had <1 *E. coli* MPN/100 mL, with most schools drawing drinking water from boreholes. These water quality estimates are similar to previously published data from these countries (Agensi et al., 2019; Holcomb et al., 2020). In both Mozambique and Uganda, an improved-type water source was associated with less *E. coli* in unadjusted and adjusted models, with a piped water source in Ugandan schools additionally associated with less *E. coli*. This finding is not surprising, based on water quality evidence from household monitoring (Kirby et al., 2016; Shields et al., 2015). In Mozambique, additional significant predictors included a water source within 30 minutes and handwashing materials present on the survey day (water and soap/ash). Proximity to water source affects health in multiple ways. First, longstanding evidence from households suggests closer water sources leads to increased water quantity and better hygiene, regardless of water quality (Stelmach and Clasen, 2015). Second, and more specific to waterborne diarrheal disease, schools with closer water sources likely refill storage containers more often, which presents fewer potential opportunities for contamination of water during transport or longer storage periods. This is consistent with previous research in households that show an increase in disease incidence as distance from a water sources increases (Wang and Hunter, 2010).

In unadjusted models, rural schools in Mozambique with a safe storage container had 3.52 times the *E. coli* incidence rate in stored drinking water than schools that did not use a safe storage container; schools with means to remove drinking water safely had 2.47 times the *E. coli* incidence rate compared with schools that did not. The adjusted model for Mozambique controlled for these safe storage components, as well as water source type, source within 30 minutes for collection, treatment of stored water, and hand hygiene. In the adjusted model, use of a safe storage container and safe removal of water no longer significantly increased incidence rate of *E. coli.* Further, schools with handwashing facilities (water and soap/ash present for handwashing) had 0.12 times the *E. coli* incidence rate compared with schools without hand hygiene, while controlling for other significant WaSH predictors. This evidence suggests that water and soap/ash for handwashing reduces incidence rate of *E. coli* in school drinking water in Mozambique, regardless of safety of water storage. Handwashing with soap and water reduces the fecal contamination of hands of students and teachers accessing stored water, which can lead to less *E. coli* contamination of stored drinking water. Although studies of handwashing in households have established this relationship (Wolf et al., 2018b), our findings are the first to show that in schools, handwashing materials are associated with significantly less *E. coli* in stored water, regardless of stored water practices.

In unadjusted models of rural schools in Uganda, treatment of water and improved-type sanitation were significant predictors of *E. coli,* in addition to water source type. Reported water treatment was associated with increased incidence rate of *E. coli* in drinking water, contrary to

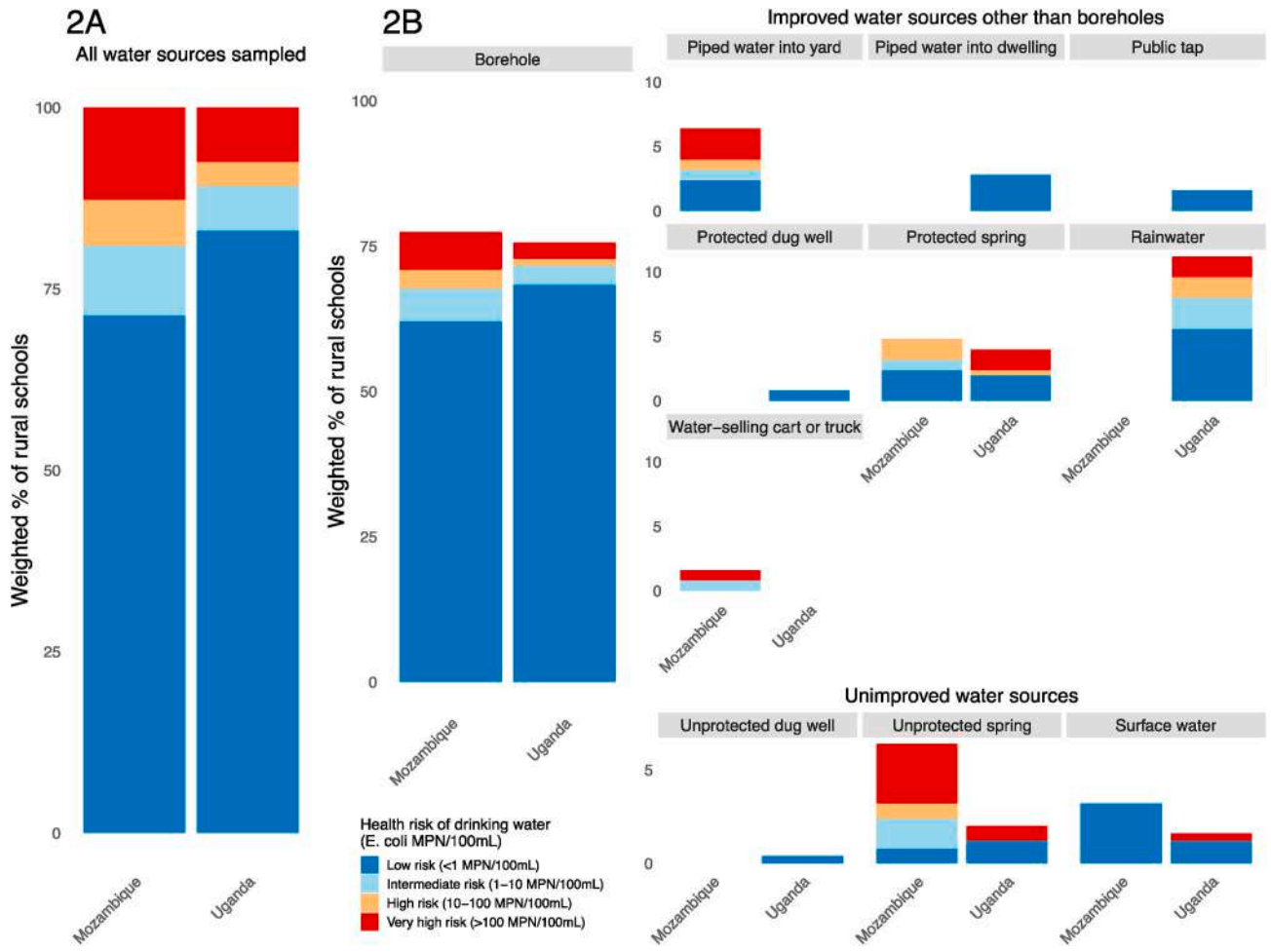

Fig. 2. A: Weighted percent of rural schools with drinking water in each WHO water quality risk level based on *E. coli* MPN/100 mL. B: Weighted percent of rural schools in each WHO water quality risk level by school water source type. Boreholes were the most common drinking water source type in schools. Other drinking water sources were less frequent; as such, the y-axis is shown on a smaller scale.

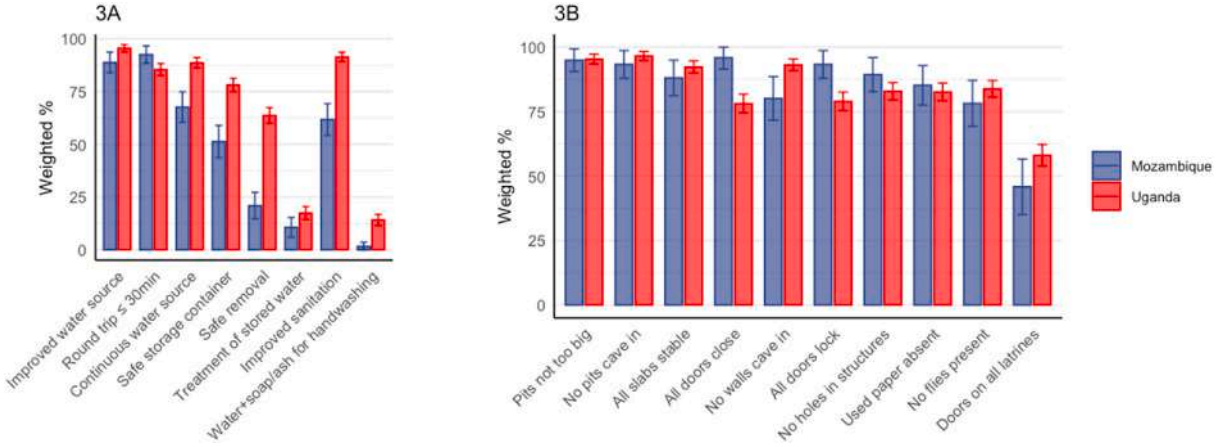

Fig. 3. A: Weighted percent of rural schools with each water, sanitation, and hygiene service studied in cross-sectional surveys, by country. B: Weighted percent of rural schools with improved-type sanitation facilities with all sanitation facilities meeting the conditions studied in cross-sectional surveys, by country. Error bars indicate 95% confidence intervals of population-level weighted percentages.

expectation. In the adjusted model, when controlling for use of a piped water source or other improved-type water source, improved-type sanitation, and treatment of water, water treatment was not associated with increased *E. coli;* only a piped water source or other improved-type remained significant. These findings suggest that of the WaSH services studied in Uganda, water source type most significantly predicts *E. coli* incidence rate in drinking water.

In both countries, specific conditions of latrines were not significantly associated with amount of *E. coli* contamination, a finding that has been similarly observed with sanitary inspections and water quality

Table 2
Incidence rate ratios (IRR) for *E. coli* most probably number in stored drinking water by water, sanitation, and hygiene service.

	Mozambique			Uganda		
	N	Unadjusted IRR (95% CI)	Adjusted IRR (95% CI)	N	Unadjusted IRR (95% CI)	Adjusted IRR (95% CI)
Improved-type water source	124	0.29 (0.13, 0.64)	0.22 (0.07, 0.65)	250	0.09 (0.02, 0.55)	0.12 (0.02, 0.80)
Water treatment	118	1.07 (0.30, 3.84)	0.39 (0.11, 1.33)	**250**	**2.36 (4.22, 11.22)**	1.75 (0.68, 4.52)
Continuous water source	118	5.53 (0.80, 38.15)		250	2.24 (0.33, 15.21)	
Safe Container	**124**	**3.52 (1.32, 9.40)**	3.58 (0.84, 15.19)	250	2.13 (0.71, 6.43)	
Safe Removal	**124**	**2.47 (1.10, 5.54)**	1.01 (0.29, 3.55)	249	1.98 (0.81, 4.86)	
Round trip < 30 min (self-reported)	**124**	**0.28 (0.12, 0.68)**	**0.25 (0.08, 0.76)**	250	0.61 (0.23, 1.65)	
Improved sanitation	123	0.94 (0.48, 1.87)		**250**	**0.28 (0.10, 0.74)**	0.44 (0.15, 1.29)
Feces absent on all latrines	123	3.26 (0.87, 12.22)		250	1.57 (0.58, 4.22)	
Water + soap/ash for handwashing observed	**122**	**0.04 (0.01, 0.19)**	**0.12 (0.02, 0.73)**	250	1.09 (0.36, 3.35)	
Water + soap/ash + drying materials observed for handwashing	**122**	**0.08 (0.06, 0.13)**	**0.27 (0.10, 0.76)**	250	2.30 (0.38, 13.93)	

§In Uganda, the variable for main water source included a category for piped sources. In Mozambique, this variable was binary, with only two options, improved- and unimproved-type water sources.
Bolded cells indicate null value (1.00) is not included in 95% confidence interval of estimate.
CI: confidence interval.

of water handpumps (Kelly et al., 2021).

5.1. Limitations

First, we did not sample microbial contaminants at the various points of study (water sources, door handles and other surfaces of sanitation facilities, hygiene facilities), nor at timepoints between storage and use, so we cannot isolate exact times or places of contamination. This is worth noting given the JMP "improved" and "unimproved" type classification does not involve sanitary inspection of water sources or sanitation facilities. Evidence from households suggests such contamination of drinking water after collection is common (Levy et al., 2008). We also did not sample water at different points in time throughout the year, which is important because seasonality can affect water quality. Second, while most indicators used were observations, we rely on self-reported time to water source due to insufficient GPS collection at the water source, which is less accurate than Euclidean distance measured with GPS (Ho et al., 2014). Finally, as this study was part of a larger multi-site, multi-country evaluation, we were unable to test other microbiological or physiochemical parameters associated with poor health outcomes through additional laboratory evaluation, but would recommend it in future studies.

This study concerns WaSH services associated with water quality in rural schools, in order to inform future school WaSH interventions that can be most successful in improving water quality, and thus, reducing disease incidence and absenteeism. Past studies that found school WaSH interventions had no effect on reducing disease incidence or absenteeism evaluated whole WaSH programs, not individual WaSH services, or relied on self-reported disease (Chard et al., 2019; Garn et al., 2017), while implementation of specific interventions, such as handwashing with soap and additional toilets in a recent study of schools in Nepal, has been shown to reduce intestinal parasitic infections (Shrestha et al., 2020). To avoid inaccuracies of subjective disease recall in the study of school WaSH interventions, future studies could focus on objective measures of disease, such as enteric pathogen antibodies (Chard et al., 2018) or on water quality (such as *E. coli* in stored water as a proxy for disease risk).

These results support the findings of water quality studies in other household and extra-household settings that show sanitation facilities and hand hygiene influence water quality, in addition to water source type and water treatment (Guo and Bartram, 2019; Holcomb et al., 2020). These findings have already been used by the funder to improve WaSH conditions and services in schools in respective settings. As we highlight WaSH services associated with school drinking water quality, our findings complement a recent study evaluating school system factors and water quality (Cronk et al., 2020), which found fewer schools in Mozambique and Uganda to have the lowest WHO risk water quality (61% and 56%, respectively, compared with our findings of 71% and 83%), despite sampling occurring after the present study. Our findings, as well as those of Cronk et al. (2020), are important for policymakers, school administrators, and public health practitioners in Mozambique and Uganda, as they provide specific WaSH services with potential to improve water quality and subsequently the health, learning environment, and cognitive development of their young people.

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Characterizing exposures to flame retardants, dioxins, and furans among firefighters responding to controlled residential fires

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ABSTRACT

Firefighters may encounter items containing flame retardants (FRs), including organophosphate flame retardants (OPFRs) and polybrominated diphenyl ethers (PBDEs), during structure fires. This study utilized biological monitoring to characterize FR exposures in 36 firefighters assigned to interior, exterior, and overhaul job assignments, before and after responding to controlled residential fire scenarios. Firefighters provided four urine samples (pre-fire and 3-h, 6-h, and 12-h post-fire) and two serum samples (pre-fire and approximately 23-h post-fire). Urine samples were analyzed for OPFR metabolites, while serum samples were analyzed for PBDEs, brominated and chlorinated furans, and chlorinated dioxins. Urinary concentrations of diphenyl phosphate (DPhP), a metabolite of triphenyl phosphate (TPhP), bis(1,3-dichloro-2-propyl) phosphate (BDCPP), a metabolite of tris(1,3-dichloro-2-propyl) phosphate (TDCPP), and bis(2-chloroethyl) phosphate (BCEtP), a metabolite of tris (2-chloroethyl) phosphate (TCEP), increased from pre-fire to 3-hr and 6-hr post-fire collection, but only the DPhP increase was statistically significant at a 0.05 level. The 3-hr and 6-hr post-fire concentrations of DPhP and BDCPP, as well as the pre-fire concentration of BDCPP, were statistically significantly higher than general population levels. BDCPP pre-fire concentrations were statistically significantly higher in firefighters who previously participated in a scenario (within the past 12 days) than those who were responding to their first scenario as part of the study. Similarly, firefighters previously assigned to interior job assignments had higher pre-fire concentrations of BDCPP than those previously assigned to exterior job assignments. Pre-fire serum concentrations of 2,3,4,7,8-pentachlorodibenzofuran (23478-PeCDF), a known human carcinogen, were also statistically significantly above the general population levels. Of the PBDEs quantified, only decabromodiphenyl ether (BDE-209) pre- and post-fire serum concentrations were statistically significantly higher than the general population. These results suggest firefighters absorbed certain FRs while responding to fire scenarios.

1. Introduction

Firefighters' exposures to flame retardants (FRs) including polybrominated diphenyl ethers (PBDEs), non-PBDE brominated flame retardants (NPBFRs), organophosphate flame retardants (OPFRs), and brominated and chlorinated dioxins and furans have increasingly become a topic of concern. PBDEs have been in use since the 1970s, are environmentally persistent, and can remain structurally unchanged on surfaces for long periods of time (e.g., years) (Alexander and Baxter, 2016; Easter et al., 2016). The increased interest in firefighters' exposures to FRs can largely be attributed to their presence in modern home furnishings (e.g., upholstered furniture, carpet padding, electronics),

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accumulation in humans, and association with adverse health effects (Herbstman et al., 2010; Linares et al., 2015).

Studies that have indicated an elevated risk of cancer for firefighters (Daniels et al., 2014; Jalilian et al., 2019; Lee et al., 2020; Pinkerton et al., 2020), the International Agency for Research on Cancer (IARC) designation of firefighting as a Group 2B possible human carcinogen (International Agency for Research on Cancer (IARC), 2010), and the complex mixture of combustion byproducts (e.g., polycyclic aromatic hydrocarbons (PAHs), formaldehyde, benzene, FRs) firefighters can be exposed to on the fireground have further raised concerns. IARC has not classified the potential carcinogenicity of PBDEs in humans to date. However, the National Toxicology Program (NTP) found evidence of PBDE carcinogenicity in rodent studies (National Toxicology Program , 2016). Other compounds firefighters are exposed to include dioxins, 2,3,7,8-tetrachlorodibenzo-para-dioxin (2378-TeCDD) and 2,3,4,7,8-pentachlorodibenzofuran (23478-PeCDF), which have been classified by IARC as Group 1 known human carcinogens, and a variety of other combustion byproducts that are known, probable, or possible human carcinogens (International Agency for Research on Cancer (IARC), 2010).

Over the past 10 years, the usage of penta-, octa-, and deca-PBDEs has been restricted globally by the Stockholm Convention (United Nations Environment, 2017). The use of organophosphate flame retardants (OPFRs) in furniture and other household items has increased as a result of PBDE's usage restriction following the classification of this compound class as a persistent organic pollutant (POPs) (Dishaw et al., 2011; National Institute of Environmental Health Sciences (NIEHS), 2018). The potential toxic effects of OPFRs are not fully understood. However, two OPFRs, tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and tris (2-chloroethyl) phosphate (TCEP), are listed in California Prop 65 as potentially carcinogenic (Environmental Protection Agency (EPA)U.S.E.P.A. and Cooke, 2017). Tris(1-chloro-2-propyl) phosphate (TCPP) has been found to be toxic to human cells at high concentrations (An et al., 2016), while triphenyl phosphate (TPhP or TPP) has been found to negatively affect development in zebrafish, mice, and rats (Du et al., 2016; Patisaul et al., 2013; Wang et al., 2018).

Studies have found a variety of FRs, dioxins, and furans on firefighter personal protective equipment (PPE) (Alexander and Baxter, 2016; Easter et al., 2016; Fent et al., 2020b; Mayer et al., 2019) and in air samples taken from a residential room-and-contents fire environment (Fent et al., 2020b). In addition, dust collected from fire stations has been found to contain higher FR levels (e.g., BDE-209 and TDCPP) than other occupational settings (Shen et al., 2015). A more recent study in Canada found fire station dust has high levels of BDE-209 (Gill et al., 2020). These studies suggest that firefighters have the potential to be exposed to these compounds while at the scene of a fire and may also bring the contamination back to their stations.

Biomonitoring and exposure assessment studies have also detected FRs in specimens collected from firefighters. Specifically, a study conducted by Shaw et al. reported elevated concentrations of PBDEs in firefighters' serum compared to the general population (Shaw et al., 2013). Park et al. (2015) reported similar findings, including relatively high serum levels of decabromodiphenyl ether (BDE-209) (Park et al., 2015). Another study reported higher levels of organophosphate flame retardants (OPFRs) metabolites in a sampling of firefighters' urine compared with the general population (Jayatilaka et al., 2017). In part because of these studies, a recent systematic review on occupational exposure to FRs listed firefighters as a workforce warranting further investigation (Gravel et al., 2019).

Exposure to combustion byproducts such as polycyclic aromatic hydrocarbons (PAHs) is also thought to be dependent on the job assignment for firefighters. Previous studies have reported that firefighters assigned to interior response activities (e.g., fire suppression or search and rescue) had higher biological levels of PAH metabolites compared to other job assignments (e.g., outside ventilation, incident command, pump operations, overhaul) on the fireground (Fent et al., 2020a). It is reasonable to assume that FR exposure may follow a similar pattern.

The purpose of this study was to characterize the biological levels of OPFR metabolites (in urine), and PBDEs, brominated and chlorinated furans, and chlorinated dioxins (in serum) in firefighters responding to controlled residential fire scenarios with modern home furnishings (containing FRs). This study design also allowed us to compare how exposures vary over time for firefighters assigned to different job assignments.

2. Methods

2.1. Study design

The study design is described in detail elsewhere (Fent et al., 2020b; Horn et al., 2018). Briefly, over a period of 2 weeks in the summer of 2015, 12 fires were ignited in a 111 m^2 wood-frame residential structure with gypsum board wall/ceiling linings and typical residential furnishings, containing a variety of FRs, including OPFRs, NPBFRs, and PBDEs (as reported in Fent et al., 2020b). The two bedrooms where the fires were ignited were furnished with a double bed (covered with a new foam mattress topper, comforter, and pillow), stuffed chair, side table, lamp, dresser, and flat screen television. The floors were covered with re-bonded polyurethane foam padding and new polyester carpet. Floor coverings in the fire rooms and nearby hallway were replaced after each fire. A fire was ignited and allowed to grow until the rooms approached flash-over conditions and became ventilation limited (typically 4–5 min) and then the firefighters were dispatched by apparatus from a nearby staging area and arrived on scene within 1 min. After each fire, the drywall and furniture were replaced. Study results reported here were collected from firefighters prior to and after three of the 12 fires.

A crew of twelve firefighters was paired up by job assignment to carry out a coordinated fireground response to a controlled residential fire, which was repeated the next day using a different fire suppression tactic. Approximately one to two weeks later, the returning firefighters were reassigned to new positions and repeated this experiment. This was done on a total of three crews (12 firefighters per crew, 4 burns per crew). Five firefighters dropped out of the study and were unable to return a week later and were replaced with new participants (resulting in a total of 41 participants). However, urine and serum specimens analyzed for FRs, dioxins and furans were only collected from one of the four fires for 36 firefighters. Crew A previously responded to a fire scenario as part of this study seven days prior to the fire where specimens were collected; Crew B responded to a fire scenario twelve days prior to the fire where specimens were collected; and Crew C provided specimens on the first fire they responded to as part of this study. The variability for each crew's recent fire exposure as part of this study allowed us to compare how time since last exposure impacted FR, dioxin, and furan urinary and serum concentrations. More information on the timing of the fire scenarios relative to the specimen collections is provided in Fig. 1. All firefighters participating in the fire scenarios wore a full PPE ensemble that included a protective hood, gloves, turnout gear, and self-contained breath apparatus (SCBA). Each firefighter was provided brand new turnout jackets, hoods, and gloves prior to the first scenario. Relevant demographic information for participating firefighters is provided in Table 1. Tobacco use was an exclusion criteria for this study.

Firefighters were assigned to one of three groups for each scenario. Firefighters assigned to interior response either pulled a primary hoseline and suppressed all active fire or entered the structure and searched for and rescued two simulated occupants (75 kg mannequins). Firefighters assigned to exterior response created openings in the windows and roof to ventilate the structure and/or completed typical exterior operations on the fireground (incident command (IC), pump operation). Importantly, these firefighters never entered the structure. Firefighters assigned to overhaul were outside the structure during active fire, either holding a secondary line or as a rapid intervention team (RIT). After the

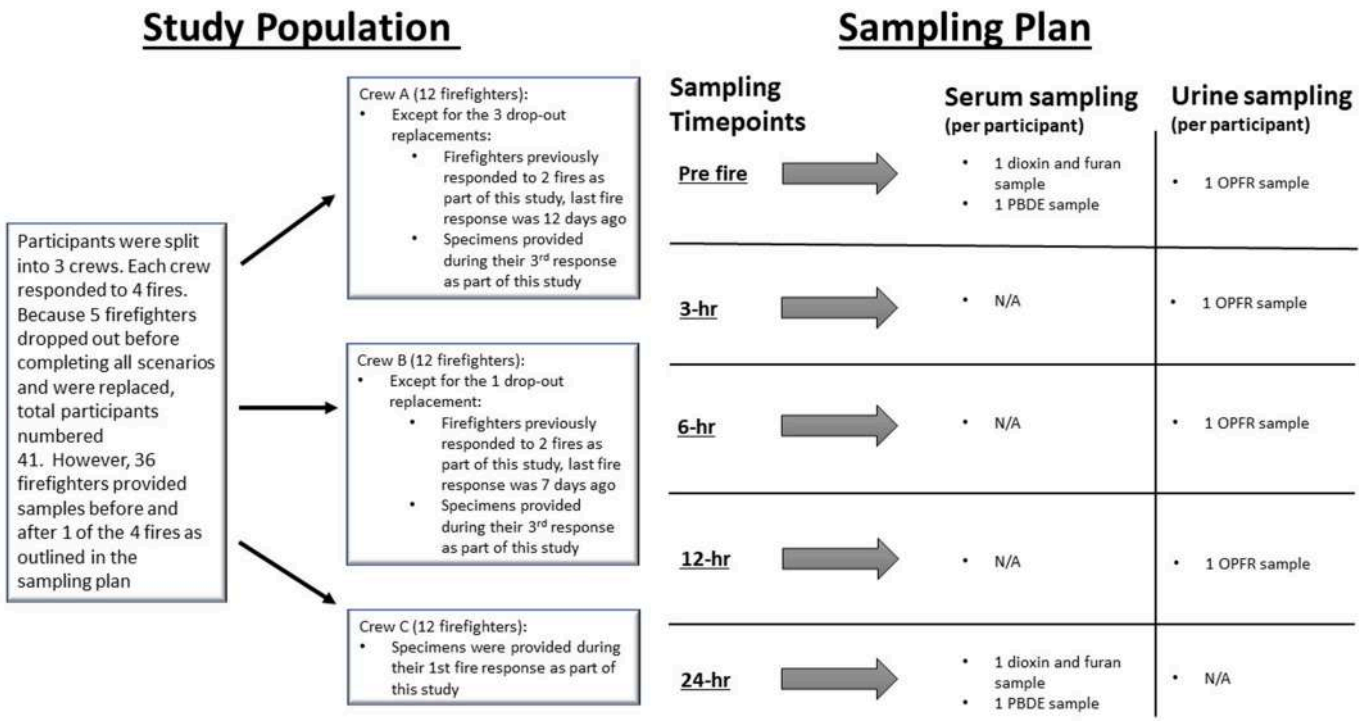

Fig. 1. Study population and sampling strategy for controlled residential fire responses with furnishings containing flame retardants.

Table 1
Characteristics of study participants.

Characteristic	Frequency
Sex	
Male (%)	32 (89)
Female (%)	4 (11)
Age	
Median (Range)	36 (21–52)
BMI	
Median (Range)	26.9 (20.5–34.2)
Home State	
Illinois (%)	22 (61)
Georgia (%)	4 (11)
Indiana (%)	4 (11)
South Dakota (%)	3 (8.3)
Wisconsin (%)	2 (5.5)
Ohio (%)	1 (2.8)

fire was suppressed by the interior firefighters, overhaul firefighters entered the structure to search for and suppress any smoldering items in the fire rooms, walls, and ceilings.

Immediately after completion of the assigned task, the firefighters walked to an open bay (approximately 40 m from the structure) where PPE was removed, turnout jackets hung in individual lockers and fire-fighting gloves placed on a shelf. Firefighters used skin cleansing wipes immediately post-fire and showered within an hour after the scenario. After doffing their gear, firefighters entered an adjacent bay where they provided biological samples. Firefighters provided a spot urine sample prior to the scenario (pre-fire) and 3 subsequent spot urine samples after the scenario (3-h, 6-h, 12-h post-fire). Firefighters also provided one serum sample prior to the fire (pre-fire serum) and one serum sample approximately 23 h after the scenario (post-fire serum).

2.2. Urine sampling

Prior to urine collection, participants were instructed to thoroughly rinse hands with water only and air dry their hands, avoiding the use of paper towels. Participants were also instructed to avoid touching the internal surface of the urine cup or the lid to avoid contaminating the sample. Participants were asked to provide a minimum 60 mL of urine for each void. Urine was put on ice and within 4 h, aliquoted into multiple tubes for analyses including 5 mL and 2 mL polypropylene vials for FR and creatinine quantification, respectively and then frozen at −20 °C. The samples were then shipped to the lab on dry ice and stored frozen until analysis.

2.3. Blood sampling

Blood was collected in multiple collecting tubes including two red top 10 mL glass blood collection tubes, and the samples were placed in a rack to clot for 2 h at room temperature. Blood samples were then centrifuged for 15 min at 1000-1300×*g*. Investigators pipetted serum from each participant's red-top tubes into separate 10 mL amber glass jars, one for PBDEs and serum lipids and one for dioxins and furans, and then froze the samples at −20 °C The samples were then shipped to the lab on dry ice and stored frozen until analysis.

2.4. Sample analyses

Urine samples (N = 144) were analyzed for eight OPFR metabolites and one NPBFR metabolite at the Centers for Disease Control and Prevention (CDC) as described by Jayatilaka et al. (2017) (Table 2). The OPFR metabolites measured were: diphenyl phosphate (DPhP), bis(1,3-dichloro-2-propyl) phosphate (BDCPP), bis(1-chloro-2-propyl) phosphate (BCPP), bis(2-chloroethyl) phosphate (BCEtP), di-p-cresylphosphate (DpCP), di-o-cresylphosphate (DoCP), dibutyl phosphate (DBuP), and dibenzyl-phosphate (DBzP); the NPBFR was 2,3,4,5-tetrabromobenzoic acid (TBBA). Specific gravity was measured in the field with a handheld refractometer (Atago, Uricon-Ne Product numbers 2722. Reading range 1.000–1.050 UG). Creatinine was measured at CDC using an enzymatic method with a Roche/Hitachi Cobas® c501 chemical analyzer (Roche Diagnostics, Inc., Indianapolis, IN). After enzymatic hydrolysis of 400-μL urine samples and off-line

Table 2
Flame retardant, dioxin, and furan biomarkers quantified in urine and serum.

Type of sample	Parent Chemical	Biomarker
Organophosphate Flame Retardants (OPFRs)		
Urinary	Triphenyl phosphate (TPP or TPhP), Isopropylphenyl diphenyl phosphate t-Butylphenyl diphenyl phosphate 2-Ethylhexyl diphenyl phosphate	Diphenyl phosphate (DPhP)
	Tris(1,3-dichloro-2-propyl) phosphate (TDCPP)	Bis(1,3-dichloro-2-propyl) phosphate (BDCPP)
	Tri-p-cresyl phosphate (TpCP)	Di-p-cresyl phosphate (DpCP)
	Tris(1-chloro-2-propyl) phosphate (TCPP or TClPP)	Bis(1-chloro-2-propyl) phosphate (BCPP)
	Tributyl phosphate (TBP or TBuP)	Dibutyl phosphate (DBP or DBuP)
	Tribenzyl phosphate (TBzP)	Dibenzyl phosphate (DBzP)
	Tris(2-chloroethyl) phosphate (TCEP)	Bis(2-chloroethyl) phosphate (BCEtP)
	Tri-o-cresyl phosphate (ToCP)	Di-o-cresyl phosphate (DoCP)
	Non-PBDE-brominated flame retardants (NPBFRs)	
	2-Ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB)	2,3,4,5-Tetrabromobenzoic acid (TBBA)
Polybromianted Diphenyl Ethers (PBDEs)		
Serum	2,2′,4-tribromodiphenyl ether (BDE-17)	BDE-17
	2,4,4′-tribromodiphenyl ether (BDE-28)	BDE-28
	2,2′,4,4′-tetrabromodiphenyl ether (BDE-47)	BDE-47
	2,3′,4,4′-tetrabromodiphenyl ether (BDE-66)	BDE-66
	2,2′,3,4,4′-pentabromodiphenyl ether (BDE-85)	BDE-85
	2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99)	BDE-99
	2,2′,4,4′,6-pentabromodiphenyl ether (BDE-100)	BDE-100
	2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153	BDE-153
	2,2′,4,4′,5,6′-hexabromodiphenyl ether (BDE-154)	BDE-154
	2,2′,3,4,4′,5′,6-heptabromodiphenyl ether (BDE-183)	BDE-183
	2,2′,3,3′,4,4′,5,5′,6-nonabromodiphenyl ether (BDE-206)	BDE-206
	decabromodiphenyl ether (BDE-209)	BDE-209
	Brominated furans	
	2,3,7,8-tetrabromodibenzofuran (2378-TeBDF)	2378-TeBDF
	2,3,4,7,8-pentabromodibenzofuran (23478-PeBDF)	23478-PeBDF
	1,2,3,4,7,8-hexabromodibenzofuran (123478-HxBDF)	123478-HxBDF
	Chlorinated dioxins	
	2,3,7,8-Tetrachlorodibenzodioxin (2378-TeCDD)	2378-TeCDD
	1,2,3,7,8-Pentachlorodibenzodioxin (12378-PeCDD)	12378-PeCDD
	1,2,3,4,7,8-Hexachlorodibenzodioxin (123478-HxCDD)	123478-HxCDD
	1,2,3,6,7,8-Hexachlorodibenzodioxin (123678-HxCDD)	123678-HxCDD
	1,2,3,7,8,9-Hexachlorodibenzodioxin (123789-HxCDD)	123789-HxCDD
	1234678-HpCDD	1234678-HpCDD
	Octachlorodibenzodioxin (OcCDD)	OcCDD
	Chlorinated furans	
	2,3,7,8-Tetrachlorodibenzofuran (2378-TeCDF)	2378-TeCDF
	1,2,3,7,8-Pentachlorodibenzofuran (12378-PeCDF)	12378-PeCDF
	(2,3,4,7,8-Pentachlorodibenzofuran) 23478-PeCDF	23478-PeCDF
	1,2,3,4,7,8-Hexachlorodibenzofuran (123478-HxCDF)	123478-HxCDF
	1,2,3,6,7,8-Hexachlorodibenzofuran (123678-HxCDF)	123678-HxCDF
	123789-HxCDF	123789-HxCDF
	2,3,4,6,7,8-Hexachlorodibenzofuran (234678-HxCDF)	234678-HxCDF
	1,2,3,4,6,7,8-Heptachlorodibenzofuran (1234678-HpCDF)	1234678-HpCDF
	1,2,3,4,7,8,9-Heptachlorodibenzofuran (1234789-HpCDF)	1234789-HpCDF
	Octachlorodibenzofuran (OcCDF)	OcCDF

solid phase extraction, target OPFR and NPBFR metabolites were separated via reversed phase high-performance liquid chromatography, and detected by isotope dilution-electrospray ionization tandem mass spectrometry.

Serum samples collected from firefighters were analyzed at CDC for a panel of PBDEs, brominated and chlorinated dioxins and furans performed by gas chromatography isotope dilution high resolution mass spectrometry (GC-IDHRMS) employing a DFS (Thermo DFS, Bremen, Germany) instrument, as previously detailed (Jones et al., 2012).

2.5. Data analysis

Descriptive statistics were displayed as frequency (%), mean ± standard deviation (SD), median, and range for firefighter characteristics. Number of samples, number of samples with concentrations below the limit of detection (LOD), geometric mean (GM), and geometric standard deviation (GSD) were provided for urine and serum concentrations by job assignment and by exposure time. LOD divided by square root of two was assigned to non-detectable concentrations (Hornung and Reed, 1990). Urinary concentrations were adjusted for creatinine (Boeniger et al., 1993).

A Welch's *t*-test or unequal variances *t*-test was used to determine concentration differences for all analytes between the U.S. general population aged 18 years and older and firefighters by job assignment and exposure time. The comparisons were also applied to each sex. A paired *t*-test was utilized to examine whether the change in serum concentrations from pre to post-fire was significantly different from zero. Concentrations for urinary and blood samples were log transformed because corresponding distributions were skewed to the right. For urinary samples, a mixed model with individual firefighter as a random effect was utilized to account for the statistical correlation among exposure time from the same firefighter. The model incorporated the use of maximum likelihood estimation method to reduce bias resulting from the data with non-detectable or left-censored concentrations (Jin et al., 2011). Univariable analyses of longitudinal urinary data were carried out using the log-transformed concentration as the dependent variable. Covariates treated as fixed effects, including exposure times (pre-fire, 3-h post, 6-h post, and 12-h post) and job assignments (exterior, interior, and overhaul), were evaluated. With respect to urine samples, an analysis of covariance (ANCOVA) was used to examine whether the means of a dependent variable, post urine concentration, were equal across job assignments, while statistically controlling for the effect of pre urine concentration. Statistical tests were two-sided at the 0.05 significance level. All analyses were performed in SAS version 9.4 (SAS Institute, Cary, NC).

3. Results

3.1. OPFR urinary results

Urinary concentrations of FRs measured among the majority of firefighters responding in three job assignment classifications during four urine collection times are summarized in Table 3. DPhP, BDCPP, and BCEtP were detected more frequently (detection rate > 60%) than the other metabolites measured in this study. Overall, GM concentrations of DPhP and BDCPP at multiple collection time points were higher than concentrations found in the general population. Specifically, 3-h and 6-h post-fire DPhP GM concentrations for all three job assignments (ranging from 1.38 μg/g creatinine to 1.75 μg/g creatinine) were statistically significantly greater than the GM of the general population (0.80 μg/g creatinine). Additionally, GM concentrations of BDCPP in the three job assignments during the four collection times ranged from 1.86 μg/g creatinine to 3.32 μg/g creatinine and were statistically significantly greater than the GM of general population (0.79 μg/g creatinine). We also stratified by sex and compared DPhP, BDCPP, and BCEtP concentrations with the general population in Supplemental Materials (Table S1). Results for the other urinary biomarkers detected less frequently (<60%) are provided in Supplemental Materials (Table S2).

Results of univariable analyses of repeated measures data with natural logarithm of urinary concentrations as the dependent variable are presented in Table 4. For DPhP and BDCPP, maximum urinary concentrations occurred 3-h post-firefighting, but this increase relative to the pre-fire concentrations was only statistically significant for DPhP (p-value is < 0.001). The mean urinary concentrations of DPhP and BDCPP decreased with each subsequent collection, however the 12-h post-fire DPhP concentrations were still higher than the pre-fire levels (p-value is < 0.05). For BCEtP, maximum urinary concentrations occurred 6-h post-firefighting (p-value is < 0.05 compared to the pre-fire concentrations), but then decreased to levels below the pre-fire concentrations (p-value is < 0.001) 12-h post-fire. There were no statistically significant differences in DPhP, BDCPP, and BCEtP for 3- and 6-h urinary mean concentrations among the three job assignments, adjusting for pre-fire concentrations. However, firefighters assigned to overhaul had statistically significantly higher 6-h BDCPP concentrations compared to those assigned to interior response in this analysis despite the requirement that firefighters wore SCBA during overhaul response.

Univariable results using pre-fire urinary concentrations as the dependent variable are provided in Table 5. Pre-fire BDCPP urinary concentrations were statistically significantly higher for firefighters who previously worked a scenario 7 days ago compared to those who were responding to their first scenario as part of this study (p-value is < 0.05). When comparing firefighters who last participated in a fire scenario 7 days and 10 or more days ago, firefighters who participated 10 days or more ago had statistically significantly lower BDCPP concentrations by comparison (p-value is < 0.05). When examining the job assignment for the previous scenario, firefighters who were previously assigned to interior response had statistically significantly higher pre-fire BDCPP concentrations than firefighters previously assigned to exterior response (p-value is 0.030).

Table 3
Firefighter urine biomarker concentrations[A] (μg/g creatinine) by job assignment compared to the general population (GP).

		Pre-fire Concentration			3-Hour Post-fire Concentration			6-Hour Post-fire Concentration			12-Hour Post-fire Concentration		
Biomarker	Job Assignment	N (N < LOD[B])	GM (GSD)	P-value (vs. GP)	N (N < LOD[B])	GM (GSD)	P-value (vs. GP)	N (N < LOD[B])	GM (GSD)	P-value (vs. GP)	N (N < LOD[B])	GM (GSD)	P-value (vs. GP)
DPhP	**All Firefighters**	36 (3)	0.97 (1.98)	0.103	36 (3)	1.67 (1.94)	**<0.001[E]**	36 (0)	1.58 (1.96)	**<0.001[E]**	36 (1)	1.20 (2.13)	**0.003[E]**
	Exterior	12 (2)	0.95 (2.37)	0.489	12 (1)	1.55 (2.05)	**0.009[E]**	12 (0)	1.38 (2.15)	**0.032[E]**	12 (0)	1.22 (2.18)	0.088
	Interior	12 (1)	1.04 (1.92)	0.196	12 (1)	1.72 (2.11)	**0.005[E]**	12 (0)	1.66 (2.31)	**0.012[E]**	12 (0)	1.28 (2.53)	0.105
	Overhaul	12 (0)	0.92 (1.74)	0.403	12 (1)	1.75 (1.75)	**<0.001[E]**	12 (0)	1.72 (1.43)	**<0.001[E]**	12 (1)	1.10 (1.78)	0.080
	General Population[C]	1901 (187)	0.80 (2.59)	Reference	**	**	Reference	**	**	Reference	**	**	Reference
BDCPP	**All Firefighters**	36 (0)	2.38 (2.12)	**<0.001[E]**	36 (0)	2.70 (1.97)	**<0.001[E]**	36 (0)	2.57 (2.01)	**<0.001[E]**	36 (0)	2.13 (1.99)	**0 < .001[E]**
	Exterior	12 (0)	2.73 (2.22)	**<0.001[E]**	12 (0)	3.32 (2.11)	**<0.001[E]**	12 (0)	2.63 (2.07)	**<0.001[E]**	12 (0)	2.23 (1.97)	**0 < .001[E]**
	Interior	12 (0)	2.09 (2.10)	**0.003[E]**	12 (0)	2.25 (1.83)	**<0.001[E]**	12 (0)	2.07 (1.98)	**<0.001[E]**	12 (0)	1.86 (2.06)	**0.002[E]**
	Overhaul	12 (0)	2.38 (2.13)	**<0.001[E]**	12 (0)	2.64 (1.95)	**<0.001[E]**	12 (0)	3.11 (1.96)	**<0.001[E]**	12 (0)	2.33 (2.01)	**0 < .001[E]**
	General Population[C]	1886 (174)	0.79 (2.83)	Reference	**	**	Reference	**	**	Reference	**	**	Reference
BCEtP	**All Firefighters**	36 (6)	0.28 (3.01)	**0.048[D]**	36 (8)	0.34 (2.09)	0.117	36 (1)	0.36 (1.83)	0.170	36 (5)	0.20 (2.03)	**<0.001[D]**
	Exterior	12 (2)	0.47 (2.43)	0.630	12 (2)	0.38 (1.94)	0.701	12 (1)	0.37 (1.75)	0.538	12 (2)	0.23 (1.81)	**0.005[D]**
	Interior	12 (3)	0.24 (2.92)	0.119	12 (4)	0.33 (2.10)	0.302	12 (0)	0.33 (1.78)	0.195	12 (1)	0.17 (2.51)	**0.006[D]**
	Overhaul	12 (1)	0.20 (3.37)	0.058	12 (2)	0.31 (2.34)	0.256	12 (0)	0.37 (2.02)	0.641	12 (2)	0.21 (1.82)	**0.002[D]**
	General Population[C]	1897 (240)	0.41 (3.10)	Reference	**	**	Reference	**	**[Ga]	Reference	**	**	Reference

A. Metabolites with less than 60% detection rate are summarized in Supplemental Materials (Table S2).
B. Limit of detection (LOD) for each analyte in μg/L: DPhP = 0.16, BDCPP = 0.11, BCEtP = 0.08.
C. Ospina, M., Jayatilaka, N., Wong, L.-Y., Restrepo, P., Calafat AM., 2018 Exposure to organophosphate flame retardant chemicals in the U.S. general population: Data from the 2013–2014 National Health and Nutrition Examination Survey. *Environmental International*. 110, 32–41. Participants aged 18 and older are included.
D. Results were significantly lower than the general population.
E. Results were significantly higher than the general population.
** GM and GSD of general population were listed in the pre-fire columns.

Table 4
Univariable analysis using urine metabolite concentrationsA (μg/g creatinine) as the dependent variable.

Outcome	Logarithm of DPhP Concentration			Logarithm of BDCPP Concentration			Logarithm of BCEtP Concentration		
Covariate	Estimate (SE)	Factor	P-value	Estimate (SE)	Factor	P-value	Estimate (SE)	Factor	P-value
Exposure Time									
Pre-Fire	Reference			Reference			Reference		
3-Hour Post	0.54 (0.10)	1.72	**<0.001**	0.13 (0.08)	1.13	0.141	0.15 (0.12)	1.16	0.243
6-Hour Post	0.52 (0.10)	1.68	**<0.001**	0.08 (0.08)	1.08	0.374	0.31 (0.12)	1.37	**0.013**
12-Hour Post	0.23 (0.10)	1.26	**0.022**	−0.11 (0.08)	0.89	0.191	−0.34 (0.12)	0.71	**0.009**
3-Hour Post	Reference			Reference			Reference		
6-Hour Post	−0.03 (0.10)	0.97	0.792	−0.05 (0.08)	0.95	0.548	0.17 (0.12)	1.18	0.176
12-Hour Post	−0.31 (0.10)	0.73	**0.003**	−0.24 (0.08)	0.79	**0.007**	−0.48 (0.12)	0.62	**<0.001**
6-Hour Post	Reference			Reference			Reference		
12-Hour Post	−0.28 (0.10)	0.75	**0.006**	−0.19 (0.08)	0.83	**0.032**	−0.65 (0.12)	0.52	**<0.001**

Outcome	**Logarithm of 3-Hour Post DPhP Concentration**			**Logarithm of 3-Hour Post BDCPP Concentration**			**Logarithm of 3-Hour Post BCEtP Concentration**		
Covariate[B]	**Estimate (SE)**	**Factor**	**P-value**	**Estimate (SE)**	**Factor**	**P-value**	**Estimate (SE)**	**Factor**	**P-value**
Job Assignment									
Exterior	Reference			Reference			Reference		
Interior	0.05 (0.21)	1.05	0.812	−0.22 (0.20)	0.80	0.284	0.23 (0.18)	1.26	0.213
Overhaul	0.15 (0.21)	1.16	0.491	−0.14 (0.20)	0.87	0.480	0.29 (0.18)	1.34	0.119
Interior	Reference			Reference			Reference		
Overhaul	0.10 (0.21)	1.10	0.652	0.08 (0.20)	1.08	0.703	0.06 (0.17)	1.07	0.705

Outcome	**Logarithm of 6-Hour Post DPhP Concentration**			**Logarithm of 6-Hour Post BDCPP Concentration**			**Logarithm of 6-Hour Post BCEtP Concentration**		
Covariate[B]	**Estimate (SE)**	**Factor**	**P-value**	**Estimate (SE)**	**Factor**	**P-value**	**Estimate (SE)**	**Factor**	**P-value**
Job Assignment									
Exterior	Reference			Reference			Reference		
Interior	0.13 (0.21)	1.14	0.536	−0.03 (0.15)	0.97	0.820	0.13 (0.20)	1.13	0.524
Overhaul	0.25 (0.21)	1.28	0.237	0.27 (0.15)	1.31	0.077	0.35 (0.20)	1.41	0.095
Interior	Reference			Reference			Reference		
Overhaul	0.12 (0.21)	1.13	0.567	0.31 (0.15)	1.36	**0.048**	0.22 (0.19)	1.25	0.258

A. No univariable analysis was conducted for metabolites with less than 60% detection rates (BCPP, DBuP, DpCP, TBBA, DoCP, and DBzP).
B. Logarithm of pre-fire concentration was adjusted for in the model.

3.2. PBDE and brominated and chlorinated dioxin and furan serum results

The levels of the PBDEs which were detected most frequently (>60%) in serum samples are summarized in Table 6. Six compounds (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, and BDE-209) were detected in more than 60% of the samples. Several of these compounds were below the levels reported in the general population, and no analytes significantly increased from pre- to post-fire. Concentrations for these six compounds were also stratified by sex and compared to the general population in Supplemental Materials (Table S3) The remaining PBDEs are summarized in Supplemental Materials (Table S4).

Although the change from pre- to post-fire was not statistically significant, BDE-209 was detected more frequently and had statistically significantly greater GM concentrations (2.91 and 3.01 ng/g lipid for pre- and post-fire serum samples) than the general population (1.89 ng/g lipid; p-values < 0.001). Pre- and post-fire serum GM concentrations of BDE-209 in the overhaul group (3.82 and 3.53 ng/g lipid, respectively) were also statistically significantly greater than the general population (p-values < 0.001), while firefighters assigned to exterior and interior response had higher post-fire serum GM concentrations (2.69 and 2.86 ng/g lipid, correspondingly) compared to the general population (respective p-values <0.05). Pre-fire serum BDE-209 concentrations were also used as the dependent variable to see how previous job assignment or days since last assignment impacted exposures, but results were similar and not statistically significant (data not shown).

Firefighters also provided serum samples that were pooled by job assignment groupings and analyzed for brominated and chlorinated furans and chlorinated dioxins, summarized in Supplemental Materials (Table S5). Compared to the brominated furans, chlorinated dioxins and furans were detected more frequently in the serum. Firefighters were found to have statistically significantly higher pre-fire GM serum concentrations of 23478-PeCDF, and pre- and post-fire GM serum concentrations of 1,2,3,4,7,8-Hexachlorodibenzofuran (123478-HxCDF), 1,2,3,6,7,8-Hexachlorodibenzofuran (123678-HxCDF), and 2,3,4,6,7,8-Hexachlorodibenzofuran (234678-HxCDF) than the general population. Job assignment did not appear to have a strong effect on the serum concentrations. The few statistically significant findings by job assignment appeared to be related to the precision in the measurements (GSD) rather than the magnitude of the differences. Additionally, there were no statistically significant increases in serum concentrations from pre to post-fire.

4. Discussion

This study was designed to simulate a fire environment where firefighters responded to realistic scenarios and were assigned to common job assignments including interior, exterior and overhaul response. The fire environment included common home furnishings containing FRs. Specifically, this study characterized firefighters' exposure to FRs during common job assignments through urinary and serum samples.

We measured statistically significantly higher concentrations of BDCPP and DPhP in firefighters' urine post-fire compared to the general population. Interestingly, firefighters' pre-fire BDCPP concentrations were also statistically significantly higher than the general population, which was not true for DPhP or BCEtP. Additionally, we found DPhP concentrations in samples taken post-fire (3-h, 6-h, 12-h) were statistically significantly higher than pre-fire samples. The fact that BDCPP and DPhP are the most abundant OPFR urinary metabolites measured in this study is consistent with our previous environmental monitoring results (Fent et al., 2020b). Median air concentrations of TPhP (the parent compound of DPhP) were 3000-fold higher than any other OPFRs

Table 5
Univariable analysis using pre-fire urine metabolite concentrations[A] (μg/g creatinine) as the dependent variable.

Outcome	Logarithm of Pre DPhP Concentration			Logarithm of Pre BDCPP Concentration		
Covariate	Estimate (SE)	Factor	P-value	Estimate (SE)	Factor	P-value
Days Since Last Fire Scenario (Categorical)						
NA (N = 16)	Reference			Reference		
7 Days (N = 11)	−0.31 (0.27)	0.73	0.259	0.58 (0.28)	1.78	**0.045**
10 (N = 1) and 12 (N = 8)	−0.15 (0.29)	0.86	0.610	−0.20 (0.29)	0.82	0.508
7 Days	Reference			Reference		
10 and 12 Days	0.16 (0.31)	1.18	0.604	−0.77 (0.32)	0.46	**0.021**
Pre-Fire Group						
NA	Reference			Reference		
Exterior	−0.17 (0.34)	0.85	0.633	−0.31 (0.37)	0.73	0.409
Interior	0.04 (0.29)	1.04	0.899	0.62 (0.31)	1.86	0.055
Overhaul	−0.60 (0.30)	0.55	0.055	0.16 (0.33)	1.17	0.628
Exterior	Reference			Reference		
Interior	0.20 (0.38)	1.22	0.599	0.93 (0.41)	2.54	**0.030**
Overhaul	−0.44 (0.39)	0.65	0.273	0.47 (0.42)	1.60	0.275
Interior	Reference			Reference		
Overhaul	−0.64 (0.35)	0.53	0.074	−0.46 (0.37)	0.63	0.224

A. No univariable analysis was conducted for metabolites with less than 60% detection rates (BCPP, DBuP, DpCP, TBBA, DoCP, and DBzP).

analyzed in this study (408 μg/m^3) and TPhP was detected most frequently during overhaul as well. Surface wipe samples were also taken from turnout jackets worn by firefighters responding to these scenarios, and TDCPP (the parent compound of BDCPP) and TPhP were two of the most abundant compounds measured (Fent et al., 2020b). TPhP was also detected in bulk samples taken from headboard padding and chair cushions that were burned in the scenarios, while TDCPP was only detected in carpet padding (Table S6; Fent et al., 2020b). A previous publication found similar urinary results, reporting elevated concentrations of DPhP and BDCPP in firefighters' urine collected at the same training academy (Jayatilaka et al., 2017) where samples were collected for this study.

BCEtP pre-fire concentrations were lower than the general population, but the 6-h post-fire concentrations were statistically significantly increased from the pre-fire concentrations (though not statistically significantly higher than general population levels). Of note, we did not detect TCEP (the parent compound of BCEtP) in air or on turnout gear, although it was found in the bulk sample of carpet liner included in the scenarios (Table S6; Fent et al., 2020b). Nevertheless, the increase in urinary concentrations of BDCPP, DPhP, and BCEtP after firefighting suggest biological uptake of the parent compounds.

We stratified DPhP, BDCPP, and BCEtP urinary concentrations by sex and compared to the general population. Males in this study were more likely than their female counterparts to have concentrations above the male general population, but this is likely due in large part to the small sample size for females (n = 4). We also compared urinary concentrations by job assignment. Firefighters assigned to overhaul had statistically significantly higher 6-h BDCPP concentrations compared to interior firefighters. However, those who were previously assigned to interior response (a week or more prior) had statistically significantly higher pre-fire BDCPP urinary concentrations compared to those previously assigned to exterior or overhaul. Additionally, firefighters who last participated in a scenario 7 days prior had statistically significantly higher pre-fire urinary concentrations of BDCPP compared to those who were participating in their first scenario as part of this study. It is likely that the exposure from the previous scenario contributed to firefighters' elevated pre-fire BDCPP concentrations, particularly for those who were previously assigned to interior response. It is also possible the firefighters were exposed to FRs through their occupation. For example (Shaw et al., 2013), measured higher levels of BDCPP in California firefighters compared to the general population. Unfortunately, we did not survey firefighters in this study to determine whether they had responded to emergency fires in the period before specimen collections. A recent publication estimated BDCPP has an elimination half-life of 54 days (Wang et al., 2020) based on concentrations in human plasma and urine, much longer than previously thought (Carignan et al., 2013). Hence, we cannot rule out that work-related exposures from months ago or non-occupational exposures (e.g., diet or contaminated dust in the home) could contribute to the concentrations measured here.

DPhP urinary concentrations were more likely to increase post-fire (3-h, 6-h, 12-h) from pre-fire levels compared to all other analytes (including BDCPP) measured in this study. While TPhP appears to have slower permeation through the skin than many of the other OPFRs (absorption flux in ng cm^{-2} h^{-1}; TCEP = 10, TDCPP = 0.10, TPhP = 0.093) (Frederiksen et al., 2018), it was measured in air during the fires and after suppression at median concentrations that were several orders of magnitude higher than the other OPFRs (Fent et al., 2020b). DPhP post-fire concentrations were marginally higher for firefighters assigned to interior or overhaul compared to those assigned to exterior response. DPhP has a much shorter estimated half-life of 9.5 days (Wang et al., 2020) than BDCPP, which may explain why the firefighters' pre-fire urinary concentrations were near general population levels regardless of the previous job assignment or how long it had been since they participated in a fire scenario. Though differences are not statistically significant, DPhP concentrations were lower for those previously assigned to overhaul compared to those assigned to interior response. Previous studies have found interior response activities like fire suppression and search and rescue led to higher exposures than exterior response activities or overhaul (Fent et al., 2020a, 2020b). Other studies have also explored TPhP exposure in other industries. Estill et al. (2021) found nail salon technicians had DPhP urinary concentrations lower than the current study, but still higher than the general population, while an older study found aircraft technicians had DPhP concentrations similar to those reported here (Schindler et al., 2014).

BDE-209 was the only PBDE that appeared to be higher than general population levels. However, there was not a statistically significant change in serum concentrations of BDE-209 from pre- to post-fire for all firefighters or for firefighters stratified by job assignment. Thus, although BDE-209 was the most abundant PBDE measured in air (both during overhaul and the fire period) and deposited on turnout jackets and hoods used in this study, there is no evidence of significant uptake of BDE-209 over a 23-h period after firefighting as part of this study. Interestingly, firefighters assigned to overhaul had pre-fire serum concentrations that were higher than the general population, suggesting that they may have been exposed before starting the scenario.

However, when we evaluated the effect of previous job assignment and time since last fire scenario on pre-fire BDE-209 serum concentrations, no statistically significant effects were found. There may be a low-level source of chronic BDE-209 exposure among the firefighters in this study that contributed to the serum levels we measured. Alexander and Baxter (2016) found that BDE-209 was one of the most abundant PBDE contaminants on used gear, while Shen et al. (2015) found high levels of BDE-209 in dust samples taken from firehouses relative to samples taken from other occupational settings. Previous studies have also found BDE-209 serum levels for firefighters that were statistically significantly higher than the general population (Park et al., 2015; Shaw et al., 2013).

Table 6
Firefighter PBDE serum concentrations[A] (ng/g lipid) by job assignment compared to the general population (GP).

		Pre-fire Serum Concentration			Post-fire Serum Concentration			
Analyte	Job assignment	N (No. < LOD[B])	GM (ng/g lipid) (GSD)	P-value (vs GP)	N (No. < LOD[B])	GM (ng/g lipid) (GSD)	P-value (vs GP)	P-value (Pre vs Post)
BDE-28	**All firefighters**	36 (4)	0.53 (2.25)	**0.029[D]**	36 (2)	0.54 (2.15)	**0.027[D]**	0.922
	Exterior	12 (2)	0.43 (1.88)	**0.016[D]**	12 (0)	0.43 (1.81)	**0.011[D]**	0.498
	Interior	12 (2)	0.47 (2.06)	0.065	12 (2)	0.47 (1.87)	**0.039[D]**	0.226
	Overhaul	12 (0)	0.74 (2.69)	0.928	12 (0)	0.77 (2.59)	0.823	0.984
	General Population[C]	1637 (178)	0.72 (1.78)	Reference	**	**	Reference	
BDE-47	**All firefighters**	36 (0)	8.49 (2.59)	**0.008[D]**	36 (0)	8.37 (2.57)	**0.006[D]**	0.869
	Exterior	12 (0)	5.94 (1.88)	**0.001[D]**	12 (0)	5.73 (1.86)	**<0.001[D]**	0.172
	Interior	12 (0)	7.58 (2.28)	**0.038[D]**	12 (0)	7.60 (2.23)	**0.034[D]**	0.447
	Overhaul	12 (0)	13.59 (3.29)	0.955	12 (0)	13.47 (3.25)	0.974	0.921
	General Population[C]	1637 (0)	13.32 (1.89)	Reference	**	**	Reference	
BDE-99	**All firefighters**	36 (0)	1.58 (2.80)	**0.007[D]**	36 (0)	1.49 (2.76)	**0.003[D]**	0.816
	Exterior	12 (0)	1.08 (2.01)	**0.001[D]**	12 (0)	0.95 (2.01)	**<0.001[D]**	0.081
	Interior	12 (0)	1.32 (2.62)	**0.035[D]**	12 (0)	1.31 (2.46)	**0.024[D]**	0.135
	Overhaul	12 (0)	2.76 (3.30)	0.852	12 (0)	2.68 (3.23)	0.918	0.899
	General Population[C]	1637 (0)	2.59 (2.12)	Reference	**	**	Reference	
BDE-100	**All firefighters**	36 (1)	1.58 (2.52)	**<0.001[D]**	36 (0)	1.67 (2.28)	**<0.001[D]**	0.992
	Exterior	12 (0)	1.24 (1.56)	**<0.001[D]**	12 (0)	1.19 (1.52)	**<0.001[D]**	0.091
	Interior	12 (0)	1.60 (2.08)	**0.017[D]**	12 (0)	1.54 (2.10)	**0.014[D]**	0.204
	Overhaul	12 (1)	1.99 (3.91)	0.361	12 (0)	2.52 (2.87)	0.657	0.949
	General Population[C]	1637 (0)	2.90 (1.88)	Reference	**	**	Reference	
BDE-153	**All firefighters**	36 (0)	5.66 (2.42)	**<.001[D]**	36 (0)	5.53 (2.44)	**<0.001[D]**	0.907
	Exterior	12 (0)	4.61 (2.22)	**0.008[D]**	12 (0)	4.45 (2.23)	**0.006[D]**	0.347
	Interior	12 (0)	4.37 (2.05)	**0.003[D]**	12 (0)	4.33 (2.09)	**0.003[D]**	0.962
	Overhaul	12 (0)	9.00 (2.68)	0.769	12 (0)	8.80 (2.72)	0.715	0.790
	General Population[C]	1637 (0)	9.81 (1.93)	Reference	**	**	Reference	
BDE-209	**All firefighters**	36 (2)	2.91 (1.79)	**<0.001[E]**	36 (0)	3.01 (1.57)	**<0.001[E]**	0.687
	Exterior	12 (1)	2.35 (1.71)	0.191	12 (0)	2.69 (1.56)	**0.020[E]**	0.359
	Interior	12 (1)	2.75 (1.87)	0.062	12 (0)	2.86 (1.61)	**0.012[E]**	0.720
	Overhaul	12 (0)	3.82 (1.66)	**<0.001[E]**	12 (0)	3.53 (1.53)	**<0.001[E]**	0.257
	General Population[C]	1637 (27)	1.89 (1.64)	Reference	**	**	Reference	

A. PBDEs with less than 60% detection rate are summarized in Supplemental Materials (S4).
B. LOD: limit of detection. Observations below the LOD were substituted using LOD/square root of 2.
C. The data are from the National Health and Nutrition Examination Survey (NHANES) (2020). 2015–2016 data documentation, codebook, and frequencies. Brominated Flame Retardants (BFRs) - Pooled Samples (BFRPOL_I). Available at https://wwwn.cdc.gov/Nchs/Nhanes/2015-2016/BFRPOL_I.htm. Accessed 12 November 2020.
D. Results were significantly lower than the general population.
E. Results were significantly higher than the general population.
** GM and GSD of general population were listed in the pre serum columns.

Of note, BDE-209 has a half-life of 15 days, while tri- to hexaBDEs have half-lives in the range of one to four years (Sjödin et al., 2020; Thuresson et al., 2006). Hence, serum concentrations of BDE-209 represent relatively recent exposures (i.e., within the last month) while lower brominated congeners serum concentrations represent years of accumulated exposure possibly masking any exposures occurring in the last fire scenario.

While BDE-209 concentrations were above the general population, the other BDEs detected most frequently in this study were statistically significantly lower than the general population. To our knowledge, this is the first study reporting lower BDE levels for firefighters compared to the general population, indicating firefighters' exposure to this class of FRs may be decreasing following their usage restriction.

None of the serum concentrations of dioxins or furans increased from pre- to post-fire. In general, chlorinated furans were more likely to be above general population levels than chlorinated dioxins even before the fires (general population data were not available for brominated furans). Specifically, 23478-PeCDF pre-fire concentrations were statistically significantly above the general population. 23478-PeCDF is a Group 1 known human carcinogen, according to IARC (International Agency for Research on Cancer (IARC), 2010), and thus exposure to this compound should be reduced as much as possible. It should be noted that levels in wipe samples of the firefighters' gloves were below the LOD for 23478-PeCDF (Fent et al., 2020b). However, the analysis of chlorinated furans in wipe samples was qualitative in nature, so caution should be exercised when interpreting these findings.

The types and makeup of furnishings and additive FRs in those furnishings will vary greatly from one structure to another. Hence, while we attempted to create a representative residential fire that could be replicated across all three participant crews, these fires certainly do not represent potential exposures across all structure fires. The FRs that dominated in the environmental and biological samples collected in this study could be more or less prevalent in different structure fires. For example, PBDEs were phased out of production in the United States over the past decade, so furniture that has been manufactured more recently will be less likely to contain these chemicals. Therefore, caution should be exercised in generalizing these findings broadly across the U.S. fire service.

This study has some limitations. Most of the firefighters participating in this study were from the Midwest (i.e., Illinois, Wisconsin, Indiana) so a comparison with NHANES, a nationally representative sample, could overlook geographic differences. However, NHANES is the best comparison group available as regionally representative data for Midwest residents does not exist for these compounds. Although most of the urinary metabolites are specific for the parent compounds, it is important to note that some OPFRs have other metabolites (e.g., hydroxyl triphenyl phosphate for TPhP, 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate for TCPP) not included in this study. Additionally, DPhP is a metabolite for several other compounds including isopropylphenyl diphenyl phosphate, t-butylphenyl diphenyl phosphate, and 2-ethylhexyl diphenyl phosphate (Nishimaki-Mogami et al., 1988; Phillips et al., 2020; Shen et al., 2019). However, the metabolites

included in this study are those included in NHANES (Ospina et al., 2018), which allowed comparisons to concentrations found in the general population. We did not restrict firefighters from responding to fires as part of their occupation prior to the scenarios (or during the time period between scenarios) and it is possible participants recently responded to fires as part of their occupation (although this was not documented). Given the extended half-lives (i.e., several days) of several of these chemicals (e.g., DPhP, BDCPP, BDE-209), we cannot rule out the possibility that the firefighters' occupation or other non-occupationally related sources of exposure contributed to their metabolite levels even before the fire scenarios and specimen collections in this study. In fact, the data support that the previous fire-scenario assignment (at least 7 days prior) may have contributed to the pre-fire concentrations of BDCPP for some firefighters. Despite this potential confounder, we found post-fire urinary concentrations for several OPFR metabolites that were higher than pre-fire urinary concentrations. Additionally, the parent compounds (TPhP, TDCPP, BDE-209) of the most abundant metabolites (BDCPP, DPhP, BDE-209) were also the most abundant chemicals detected in air and deposited on turnout gear (as reported previously). BDE-209 concentrations were statistically significantly higher than the general population, suggesting firefighters may be chronically exposed to low levels of this chemical as part of their occupation.

This study provides further evidence that firefighters in full protective turnout gear can biologically absorb compounds that are produced or released during fires. While inhalation exposure is possible for firefighters on the exterior of the structure, interior firefighters wore SCBA throughout the response and overhaul firefighters donned SCBA before entering the structure post suppression. Hence, the dermal route likely played an important role in the absorption of the OPFRs. Participants in this study used commercial skin-cleansing wipes (Essendant baby wipes NICA630FW) and showered shortly after completing the scenarios, which likely removed some of the dermal contamination. While the impact of these measures should be further evaluated, higher biological levels may have been experienced if skin cleansing was delayed, which is often the case during emergency fire responses.

5. Conclusions

Firefighters can be exposed to certain PBDEs, OPFRs, and brominated and chlorinated furans and chlorinated dioxins when responding to structure fires containing modern home furnishings. Several FR biomarkers (BDE-209, DPhP, and BDCPP) were consistently detected in biological specimens at concentrations above the general population levels, and other compounds (23478-PeCDF) were above the general population levels during at least one collection period. Urinary concentrations of DPhP increased significantly from pre- to post-fire, suggesting absorption of the parent compound (TPhP) during the fire response. BCEtP concentrations were not above general population levels but did increase significantly pre- to post-fire. Job assignment appears to play an important role, as those who previously worked interior response had higher pre-fire BDCPP concentrations than those who had previously worked exterior operations. That the previous scenario occurred at least 7 days prior to the specimen collection suggests that BDCPP will remain in the body for several days following exposure. Future work should further investigate how job assignment and control interventions (e.g., routine laundering of turnout gear) impact the biological absorption of FRs during structural firefighting.

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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.2021.113782.

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Chemical prioritisation strategy in the European Human Biomonitoring Initiative (HBM4EU) – Development and results

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ABSTRACT

The European Human Biomonitoring Initiative (HBM4EU[1]) has established a European Union-wide human biomonitoring (HBM) programme to generate knowledge on human internal exposure to chemical pollutants and their potential health impacts in Europe, in order to support policy makers' efforts to ensure chemical safety and improve health in Europe.

A prioritisation strategy was necessary to determine and meet the most important needs of both policy makers and risk assessors, as well as common national needs of participating countries and a broad range of stakeholders. This strategy consisted of three mains steps: 1) mapping of knowledge gaps identified by policy makers, 2) prioritisation of substances using a scoring system, and 3) generation of a list of priority substances reflective of the scoring, as well as of public policy priorities and available resources.

For the first step, relevant ministries and agencies at EU and national levels, as well as members of the Stakeholder Forum each nominated up to 5 substances/substance groups of concern for policy-makers. These nominations were collated into a preliminary list of 48 substances/substance groups, which was subsequently shortened to a list of 23 after considering the total number of nominations each substance/substance group received and the nature of the nominating entities.

For the second step, a panel of 11 experts in epidemiology, toxicology, exposure sciences, and occupational and environmental health scored each of the substances/substance groups using prioritisation criteria including hazardous properties, exposure characteristics, and societal concern. The scores were used to rank the 23 substances/substance groups. In addition, substances were categorised according to the level of current knowledge about their hazards, extent of human exposure (through the availability of HBM data), regulatory status and availability of analytical methods for biomarker measurement.

Finally, in addition to the ranking and categorisation of the substances, the resources available for the project and the alignment with the policy priorities at European level were considered to produce a final priority list of 9 substances/substance groups for research activities and surveys within the framework of the HBM4EU project.

1. Introduction

Human biomonitoring (HBM) measures levels of chemicals directly in human biological samples (e.g. blood, urine, hair). This type of measurement aggregates exposure from all relevant routes and sources and, as such, is a powerful tool for tracing the uptake of chemicals in the human body (Angerer et al., 2007). Assessment of human exposure provides important information for health risk assessments, but it requires considerable coordination efforts, harmonised and comparable methods and financial investment. Therefore, the selection of substances

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for inclusion in HBM surveys needs to be well thought out. The identification of policy-relevant chemicals to be included in the Human Biomonitoring Initiative in Europe (HBM4EU) is a crucial step towards achieving these goals (Apel et al., 2020; Buekers et al., 2018; David et al., 2020; Louro et al., 2019).

HBM4EU is a joint effort of 30 countries, the European Environment Agency (EEA) and the European Commission (EC), which takes part in the project through the European Union (EU) Policy Board. The initiative aims to generate knowledge on human exposure to specific chemicals and chemical groups in Europe and on the human health impacts of this exposure. Running from 2017 to 2021, HBM4EU is co-funded by participating partners and the EC's Horizon 2020 research programme. It is organised to answer policy-relevant questions for priority chemicals, as identified by the partner countries' National Hubs (i.e. bodies set up at national level representing the national network of HBM activities), the EU Policy Board (representatives of EC services and EU agencies dealing with chemicals) and a Stakeholder Forum (comprised of non-governmental organisations (NGOs), industry and trade unions). The generated knowledge will support the efforts of policy makers to enhance chemical safety in Europe, as well as to set research priorities at the European level.

The selection of the first list of high-priority substances for action in HBM4EU was undertaken in 2016, at the stage of developing the proposal for this H2020 initiative. This step involved consortium partners and representatives from EU institutions (General Directorates and EU agencies in charge of chemical policy, monitoring and regulation), and took into account both national and EU level policy needs to better understand chemical exposure and health outcomes. This first prioritisation exercise resulted in 9 substances or substance groups: phthalates and Hexamoll® DINCH, bisphenols, per-/polyfluoroalkyl substances (PFAS), flame retardants, cadmium and chromium VI, polycyclic aromatic hydrocarbons (PAHs), anilines, chemical mixtures and emerging substances.

Because two additional rounds of chemical prioritisation were planned during the course of the project, a prioritisation strategy was developed to make the process more accountable, transparent, legitimate and useful for the next two rounds. This strategy consisted in a multi-step approach involving the consultation of the EU Policy Board, the National Hubs, and members of the HBM4EU Stakeholder Forum, which we present here. Its actual use for the first time and its results, namely the second list of HBM4EU priority substances, are also described. In addition, we offer recommendations for further improvement, emerging from the feedback of the various entities that participated in this second HBM4EU prioritisation round.

2. Chemical prioritisation strategy developed and applied in HBM4EU

In the first instance, a review of the literature was performed to collect criteria used for chemical prioritisation in HBM programmes worldwide. Prioritisation criteria used in the following HBM programmes were identified: the United States (US) National Health And Nutrition Examination Survey (NHANES), the Canadian Health Measures Survey (CHMS), the German Environmental Survey (GerES), the French Longitudinal Study of Children (ELFE), the French cross-sectional health survey (Esteban) and the Flemish Environment and Health Study (FLEHS) (Casteleyn et al., 2015; CDC 2002; 2003; 2006; Fillol et al., 2014; Fréry et al., 2012; Haines et al., 2017; Health Canada 2010; 2013; 2015; Kolossa-Gehring et al., 2012a; Schoeters et al., 2017). The prioritisation criteria were assessed in relation to their relevance to the objectives and specificities of the HBM4EU project (Ganzleben et al., 2017). The selected criteria related to hazardous properties, exposure characteristics, regulatory status, public concern and technical feasibility for HBM (HBM4EU 2017).

In brief, the strategy consisted of the implementation of three successive main steps, as described in Fig. 1. The first step was to *map knowledge needs* (nomination of substances along with their policy and knowledge needs; holding of a stakeholder workshop) and *initiate the prioritisation* (based on relatively simple frequency weightings to produce a short list of substances). The second step was to *rank nominated substances/substance groups* from the short list according to a priority score reflective of their level of concern and thus the relevancy to have them prioritised. In addition, a category (from A to D) was allocated to each substance to inform on the current level of knowledge on the substance, mainly from the perspective of HBM research. These substance categories aimed to support the prioritisation process by indicating the information gaps that research activities in HBM4EU could target. The third step consisted of consulting with the EU Policy Board and the HBM4EU Management Board to agree on a list of proposed priority substances, based on the ranked list of substances but also according to resources and policy considerations. Each of these steps is described in more detail below. The second priority list of substances, proposed according to this methodology, was finally approved by the HBM4EU Governing Board.

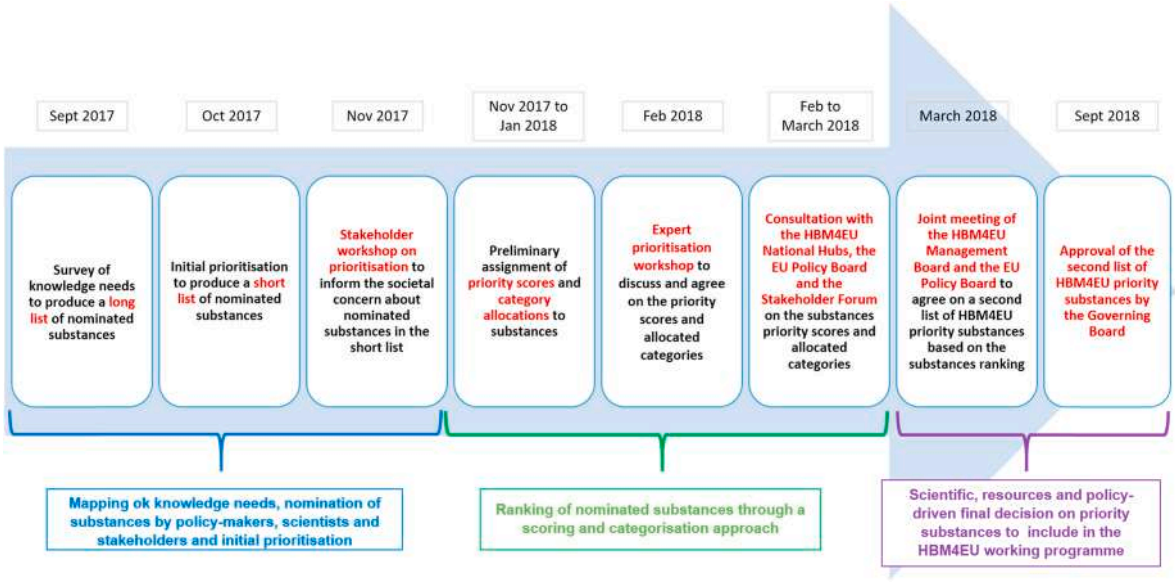

Fig. 1. Overview of the chemical prioritisation strategy under HBM4EU.

2.1. Mapping of knowledge needs for substances and initial prioritisation

The objective of the *mapping of knowledge needs* was to identify the activities and research needed on specific substances, as expressed by the EU Policy Board, the National Hubs and the HBM4EU Stakeholder Forum. These three groups of participants ensured an overarching knowledge input that would benefit the results generated under HBM4EU. It was therefore necessary to gain a comprehensive understanding of the specific needs of these groups of participants.

The key steps for determining the knowledge needs were to: 1) run an online survey for the nomination of substances; 2) produce a short list of nominated substances and 3) organise a stakeholder workshop to collect more input.

2.1.1. Online survey for the nomination of substances: polling European chemical research needs under HBM4EU

The online survey was structured around the selected set of prioritisation criteria consisting of the hazardous properties, exposure characteristics, regulatory status, public concern, and technical feasibility for HBM measurement. The complete online survey with questions is included in Supplementary Material - Annex 1. It ran from July to September 2017 and was opened to the following key entities: members of the EU Policy Board, the participating countries represented by their National Hubs and members of the HBM4EU Stakeholder Forum (Fig. 2).

Each entity could nominate up to 5 substances (or groups of substances) by completing the full online survey for each nominated substance/substance groups, as well as by defining the policy-related questions they thought HBM4EU research activities should address for each substance and how new knowledge generated under the project could benefit society. They were required to submit any available information and evidence on the nominated substance/substance groups involving questions related to the prioritisation criteria. Respondents could re-nominate substances that were already on the first list of HBM4EU prioritised substances, to emphasise their high priority and the fact that HBM-related research is still needed. These substances were communicated directly to the respective chemical group leaders (CGL) of the HBM4EU first priority list of substances (CGLs are tasked with developing scoping documents on current policy questions and proposals for relevant monitoring and research activities for the substances under their remit) and were not considered further in the process for obtaining the second HBM4EU priority list.

The survey participants nominating groups of substances were asked to provide a rationale for grouping these substances together. This included common analytical methods for measuring a panel of substances in a single biological sample, substances with similar use with possibility of substitution within the group or substances with similar toxicological profiles.

The survey results were collated to produce a long list of nominated substances and substance groups. This involved consolidating multiple nominations for individual substances and for groups of substances, where there was an overlap.

2.1.2. Producing a short list of substances and drafting background documents

Because the developed methodology for prioritising chemicals requires assessing information on the substances' hazards, exposures, regulations and HBM analytical feasibility, it was necessary to have a manageable number of substances to assess within the established timeframe. Therefore, the next step consisted in shortening the long list of all nominated substances down to a shorter list of approximately 25 substances/substance groups. The initial criterion for including a substance in the short list was to consider the number of nominations, on the basis of having been nominated by at least one member of the EU Policy Board and/or 9 National Hubs (representing just over one-third of the participating countries). In practice, there was less commonality across the nominations than expected, which meant the above criteria had to be adapted by including:

- all substances and groups prioritised by the EU Policy Board (with the objective of meeting EU knowledge needs for policy support), as well as
- substances nominated by two or more National Hubs, or by at least one National Hub and one member of the Stakeholder Forum.

For each substance/substance group on the short list, informative so-called draft background documents were produced with the information provided in the online survey, including details on toxicological information, effects on human health and exposure characteristics required in the later stages of the prioritisation process, as also knowledge gaps and proposed research efforts.

2.1.3. Stakeholder workshop on chemical prioritisation: including stakeholder needs in the process

A stakeholder workshop on prioritisation was held in November 2017 to apprehend stakeholders' perspectives on the societal relevance of new HBM4EU-generated knowledge on the substances from the short list, and to better understand stakeholders' substance priorities and the reasoning behind these priorities. Stakeholders were asked to vote for the three substances/substance groups from the short list that they considered as most important to include in the HBM4EU project activities. The number of votes obtained for each substance was converted into a score, which was later used for scoring the substances in light of the public concern criterion (one of the selected prioritisation criteria).

2.2. Ranking of nominated substances through a scoring and categorisation approach

The second main step of the strategy consisted of *ranking the substances* from the short list based on their priority score, which was calculated against a set of prioritisation criteria according to the methodology described in more detail below. The category allocated to the substances (reflective of the availability of HBM data in Europe, current analytical capacity to measure them in HBM studies, their current EU regulatory status and the level of knowledge about their hazards) was also used to propose an alternative ranking, i.e. according to the substance's priority score but this time among the substances in the different categories (A to D).

This step involved a panel of 11 experts, from a wide variety of fields such as epidemiology, toxicology, exposure sciences, and occupational and environmental health (details available in Supplementary Material - Annex 2). As the data and information provided in the online nomination survey (and gathered in draft background documents) formed the basis for the substance's scoring and categorisation, these experts were first tasked with reviewing and, if necessary, supplementing these documents with any missing important information. Each draft background document was reviewed by two experts, who were also asked to propose a priority score (according to a previously defined methodology) and a category to the substance(s) covered by the document they had to review. In order to reach consensus priority scores and categories among the 11 experts, these were then presented and discussed by the expert panel during a two-day scoring workshop held in February 2018, especially in case of divergent proposals from the two experts. Agreed priority score and category were included in the revised background document for each substance/substance groups.

2.2.1. Scoring the substances or substance groups included in the short list of nominations

The scoring of the substances involved a three-step process, which included: 1) setting a consensus weighting value to be applied to each prioritisation criterion; 2) scoring the substances against each chosen prioritisation criterion and 3) calculating the substance's overall score. The process is further described below.

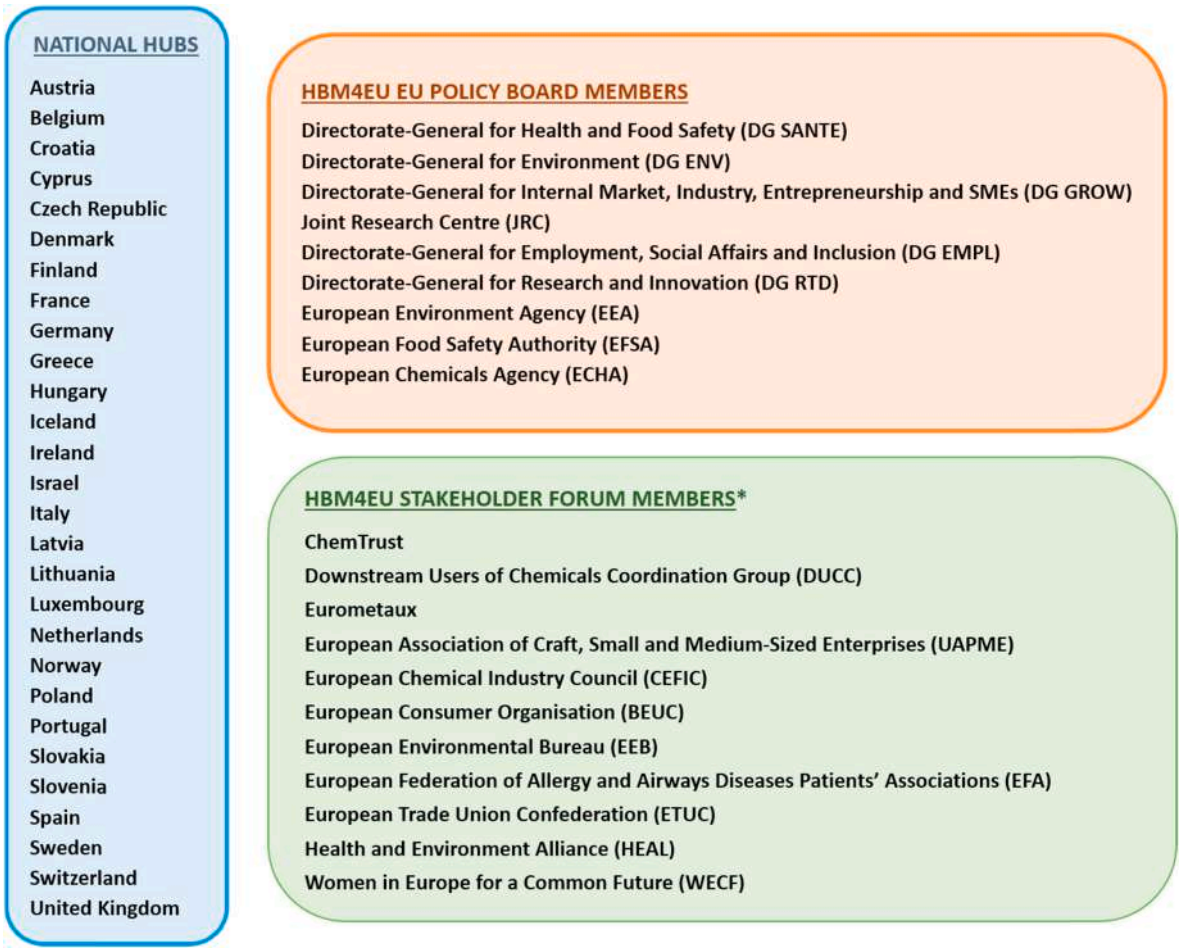

Fig. 2. Entities invited to nominate substances for prioritisation under HBM4EU.
* In 2019, two new stakeholders joined the Stakeholder Forum: Pesticide Action Network (PAN) and Plastics Europe. They did not take part in the 2017 round of chemical prioritisation.

2.2.1.1. Setting a consensus weighting value to be applied to each prioritisation criterion. An adapted Delphi method was adopted to weight the prioritisation criteria (hazards, exposure characteristics, regulatory status, public concern and technical feasibility for HBM measurement) according to their relative importance in the prioritisation process. The adapted Delphi method involved two rounds of consultation:

1) During the first round, a questionnaire was sent to the 11 experts, asking them to assign a percentage to each criterion to reflect its relative weight for the scoring step (i.e., their estimated importance for the priority setting). The sum of the weighting value assigned to each prioritisation criterion had to reach 100%. Experts had the possibility of justifying the given weighting values. Considering all expert responses, the median and boundary (minimum and maximum) values were calculated for each prioritisation criterion and were shared with all experts.
2) A conference call was organised to discuss the weighting values given during the first consultation round. Experts could explain the rationale for giving their values. Following these discussions, a second consultation round was organised during with each expert had the opportunity to modify the weighting values that he had given in the first round.

For each prioritisation criterion, the median of the weighing value given by the experts during the second consultation round was finally retained.

2.2.1.2. Scoring the substances against each selected prioritisation criterion. In order to score the substances against the criteria "hazardous properties" and "exposure characteristics", a systematic approach was implemented through the scoring rules that are summarised in Table 1 (scoring rules for different hazard endpoints) and Table 2 (scoring rules for different exposure characteristics). As informed by the information included in the substance's revised background documents, scores of 6, 3 or 1 were given, corresponding to, respectively, a High, Moderate or Low category of severity (towards hazard endpoints) or level (towards exposure characteristics). This approach is based on the GreenScreen® for Safer Chemicals method,[2] which has been designed to identify chemicals of high concern and safer alternatives using criteria to classify the human health and environmental hazard level for a chemical.

If no information was available to score a hazard endpoint/an exposure parameter or if the available data was considered inadequate, a Data Gap score of 2 was given. If available toxicological data did not suggest any related effect or mode of action toward an endpoint, a Low severity score of 1 was assigned for that endpoint instead of a Null score. This conservative approach is justified by the fact that toxicological tests may not currently be sensitive enough to detect effects.

Scores could deviate from the rules only if justified and accepted by the expert group involved in the scoring.

The scoring of the "public concern" criterion was informed in particular by the results of the vote held during the stakeholder

[2] https://www.greenscreenchemicals.org/learn/full-greenscreen-method.

Table 1
Scoring rules for endpoints of the *hazardous properties* criterion.

Hazardous properties						
Endpoint	Source of information	Severity level				Highest score by endpoint
		High (score of 6)	Moderate (score of 3)	Low (score of 1)	Data gap (score of 2)	
Carcinogenicity	EU – CLP regulation (H-Statements)	Carc. 1A or 1B (H350 or H350i)	Carc. 2 (H351)			
	EU – SVHC List	Candidate List				
	IARC classification	Group 1 or 2A	Group 2B	Group 4 (or Group 3 in specific cases)	Group 3	
	Peer-reviewed literature					
Mutagenicity	EU – CLP regulation (H-Statements)	Muta. 1A or 1B (H340)	Muta. 2 (H341)			
	EU – SVHC List	Candidate List				
	Peer-reviewed literature					
Reproductive toxicity	EU – CLP regulation (H-Statements)	Repr. 1A or 1B (H360F, H360FD, H360Fd)	Repr. 2 (H360Df, H361f, H361fd)			
	Peer-reviewed literature					
Developmental toxicity	EU – CLP regulation (H-Statements)	Repr. 1A or 1B (H360D, H360FD, H360Df, H362)	Repr. 2 (H360Fd, H361d, H361fd)			
	Peer-reviewed literature					
Endocrine activity	EU – SVHC List (article 57f)	Candidate List				
	EU - BKH List US - TEDX List		Cat. 1 or cat. 2 Inclusion in the list	Cat. 3		
	Peer-reviewed literature					
STOT RE (Systemic toxicity after repeated exposure)	EU – CLP regulation (H-Statements)	STOT -RE 1 (H373)	STOT-RE 2 (H373)			
	Adverse effects (e.g. on liver, kidneys, cardiovascular function) after chronic exposure to the substance, indicated from peer-reviewed literature	Yes	Suspected	Not identified		
Neurotoxicity[a]	Chemical Scorecard[b] Peer-reviewed literature	Strong evidence	Suspected	Not identified		
Immunotoxicity[a]	Chemical Scorecard [b] Peer-reviewed literature	Strong evidence	Suspected	Not identified		
Respiratory sensitiser[a]	EU – CLP regulation (H-Statements)	Resp. Sens. 1A or 1B (H334)				
	EU – SVHC List (article 57f) Peer-reviewed literature	Candidate List				
Skin sensitiser[a]	EU – CLP regulation (H-Statements)	Skin. Sens. 1A or 1B (H317)				
	Peer-reviewed literature					
Total score[c]						x
Adjusted total score for the hazardous properties criterion[d]						x/60 = X

CLP: Classification, Labelling and Packaging; EU: European Union; IARC: International Agency for Research on Cancer; STOT-RE: Specific target organ toxicity - repeated exposure; SVHC: Substances of Very High Concern; US: United States.

[a] Considered only if not yet addressed in another entry (to avoid double counting).

[b] Chemical Scorecard is an information service provided by the US Environmental Defense Fund that uses data from the US Environmental Protection Agency's Toxic Release Inventory plus other governmental and scientific agencies. Health effects are provided for more than 5000 chemicals.

[c] For each endpoint, the highest score was used to calculate the total score, which constitutes a conservative approach.

[d] The adjusted total score for the hazardous properties criterion was obtained by dividing the calculated total score by the highest possible score of 60 (10 endpoints of high severity category).

workshop on prioritisation. The number of stakeholder votes for each substance/substance group on the short list was translated into a corresponding score (e.g. a score of 4 for 4 votes). In addition, information on whether the substance is included in the SIN list[3] and/or in the Trade Union List for REACH authorisation[4] and whether NGO campaigns have been conducted regarding the substance were also considered (Table 3).

2.2.1.3. Calculating the overall score for each substance. The adjusted total score for each selected criterion (hazardous properties, exposure characteristics and the public concern) was multiplied by its respective median weighting value. Finally, the sum of the products resulted in the overall priority score for each substance (Table 4).

2.2.2. Categorising the substances or substance groups included in the short list of nominations

The categorisation step aimed to assign a category to each substance to reflect the level of knowledge on the availability of HBM data in

[3] http://sinlist.chemsec.org/.
[4] https://www.etuc.org/IMG/pdf/TUListREACH.pdf.

Table 2
Scoring rules for the parameters of the *substance exposure characteristics* criterion.

Exposure characteristics							
Parameter		Source or information	Severity level High (score of 6)	Moderate (score of 3)	Low (score of 1)	Data gap (score of 2)	Highest score per parameter
Persistency and/or bioaccumulation potential		EU – SVHC List (articles 57d and 57e for PBT and vPvB)	Candidate List				
		Peer-reviewed literature/ Institutional report	Persistent and evidence of bioaccumulation or significant biological half-life in mammals	Persistent (without evidence of bioaccumulation) or significant biological half-life in mammals			
Tonnages		ECHA	>1000 tpa	10-1000 tpa	<10 tpa		
Extent of exposure		ECHA	EU wide	Country/regional	Hotspot		
Routes of exposure		Peer-reviewed literature/ Institutional report	Multipathway exposure (oral, inhalation, dermal)	Multipathway (two routes of exposure only)	One route of exposure		
Passage of placental barrier		Peer-reviewed literature/ Institutional report	Strong evidence	Limited evidence	No		
Exposed populations	Workers	ECHA/Peer-reviewed literature/ Institutional report	Widespread (professional use and use in different industrial sectors	Some professional/industrial use	Intermediate use only		
	General population		Evidence of wide exposure (multiple media) - dispersive use	Limited evidence of exposure through external media	No significant exposure		
	Vulnerable groups exposed		Neonates, children, pregnant women				
Level of concern of the exposure	HBM data	ECHA/EFSA/Peer-reviewed literature/ Institutional report	Recent HBM data above or close to an established health-based HBM guidance value		Recent HBM data well below an established health-based HBM guidance value		
	External exposure data		Recent external exposure data above or close to a regulatory reference value		Recent external exposure data well below a regulatory reference value		
Total score[a]							y
Adjusted total score for the exposure characteristics criterion[b]							y/60 = Y

ECHA: European Chemicals Agency; EFSA: European Food Safety Authority; EU: European Union; PBT: Persistent, Bioaccumulative and Toxic; SVHC: Substances of Very High Concern; tpa: tonnes per annum; vPvB: very Persistent and very Bioaccumulative.

[a] For all parameters, the highest score was used to calculate the total score, which constitutes a conservative approach.

[b] The adjusted total score for the exposure characteristics criterion was obtained by dividing the calculated total score by the highest possible score of 60 (10 parameters of high severity category).

Table 3
Scoring rules for the *public concern* criterion.

Level of public concern	Related score
Stakeholder votes reflecting their interest in certain substances (or groups) from the short list of nominated substances during the stakeholder workshop on prioritisation	Number of votes translated into scores (maximum score = 9)
Inclusion in the SIN List and/or the Trade Union Priority List for REACH Authorisation	No = 0; Yes = 1
Recent NGO campaigns/media coverage	No = 0; Yes = 1
Total score	z
Adjusted total score for the public concern criterion[a]	z/11 = Z

[a] The adjusted total score was obtained by dividing the total score by the highest possible score.

Table 4
Calculation method of the overall priority score for substances included in the short list of nominations.

Prioritisation criterion	Criterion adjusted total score	Weighting value (W_i)	Product of the criterion adjusted total score by W_i
Hazardous properties	X	W_1	$X*W_1$
Exposure characteristics	Y	W_2	$Y*W_2$
Public concern	Z	W_3	$Z*W_3$
Overall priority score of the substance			$\Sigma[X*W_1 + Y*W_2 + Z*W_3]$

particular and, to a lesser extent, on the hazards, regulatory status or analytical capabilities for implementing HBM. Five substance categories (A to E) were defined in the HBM4EU project, as informed in Table 5.

Because category E substances are not yet constitutively identified substances, none of the substances nominated during the prioritisation process would belong to this category. Hence, substances were classified into the categories A to D, based on the information contained in the revised background document. For substance groups, categorisation was performed either for several substances included in the group or at least for an identified representative substance of the group.

The aim of allocating the substances to these categories was to ensure a balanced workload across the different areas of activity of the HBM4EU project by including substances from categories A to D in the

Table 5
Definition of category A to E substances under HBM4EU.

Substance's category within HBM4EU	Definition
Category A	Substances for which HBM data are sufficient to provide an overall picture of exposure levels across Europe, and interpretation of biomonitoring results in terms of health risks is possible. Risk management measures have been implemented at national or European level. Improvement of knowledge for these substances will therefore focus on policy-related research questions and evaluation of the effectiveness of existing regulatory measures.
Category B	Substances for which HBM data exists, but not sufficiently to have a clear picture across Europe. Also, knowledge on the extend of exposure, levels and impact on the human health should be improved, in order to give policy makers relevant and strategic data to establish appropriate regulations and improve chemical risk management. Analytical method and capacities to monitor the substances across Europe might have to be improved.
Category C	Substances for which HBM data scarcely or does not exists. Efforts to develop an analytical method to obtain relevant HBM results need to be done. Hazardous properties of the substances are identified, yet greater knowledge on toxicological characteristics and effects on the human health is needed. Interpretation of HBM data is not possible, due to the lack of HBM guidance values.
Category D	Substances for which a toxicological concern exists but HBM data are not available. HBM4EU research may be focused on the development of suspect screening approaches permitting to generate a first level of data enabling to document the reality of human exposure and better justify further investment in a full quantitative and validated method development.
Category E	Substances not yet identified as of toxicological concern and for which no HBM data are available. A bottom-up strategy will be applied, consisting to non-targeted screening approaches coupled to identification of unknowns capabilities for revealing, and further identifying, new (i.e. not yet known) markers of exposure related to chemicals of concern for HBM (parent compound or metabolite).

final list of priority substances. Indeed, according to the definitions of the substance categories, substances classified as A or B would require more public policy-oriented work, whereas C or D substances would require more research efforts such as developing HBM analytical methods or identifying biomarkers of effect.

2.3. Scientific, resources and policy driven final decision on the second HBM4EU priority list

The ranked list of substances was sent to the HBM4EU Management Board and the EU Policy Board, allowing them to weigh the scientific and policy merit of conducting research on each substance. Both boards met separately to discuss their priorities for action. Finally, a joint discussion with members of each of the two boards took place to agree on the final list of HBM4EU priority substances, considering also the resources available to conduct research activities during the 2019–2021 period.

2.4. After chemical prioritisation: feedback survey and suggestions for improvement

A survey was sent out in June 2019 to obtain feedback from the different participants who contributed to the second round of HBM4EU chemical prioritisation. This survey was designed to garner suggestions on how to further refine and streamline the overall strategy, including the scientific aspects of the elaborated method. The feedback received was taken into account to improve the overall process ahead of the third round of HBM4EU chemical prioritisation, which will take place in 2020–21.

3. Results

3.1. Mapping of knowledge needs for substances and initial prioritisation

3.1.1. Results of the online survey for the nomination of substances for research under HBM4EU

One hundred and thirty-two substances with policy needs were nominated in the online survey (this initial list is available here). Respondents were from 24 National Hubs, 4 members of the Stakeholder Forum and 6 members of the EU Policy Board.

As mentioned in section 2.1.1, re-nominations of substances that were part of the first list of HBM4EU priority substances were communicated to the respective CGLs and consequently removed to produce a 92-nomination list (cf. Annex 3 - Supplementary Material). This list was further refined by looking for overlaps across nominations:

- Single substances were grouped when they were associated to each other. For example, "mercury" was combined with "mercury and its compounds".
- Substance groups were used when consolidating some of the nominated single substances or smaller groups into larger groups of substances.

Finally, 23 single substances and 25 substance groups were obtained in the so-called "long list" of nominated substances, which is available here.

3.1.2. Results from the initial prioritisation to produce a short list of substances

Following application of the established criteria to reduce the long list of nominations (see section 2.1.2), a "short list" of 23 substances/substance groups was produced. This was a manageable number of substances in order to apply the further steps of the prioritisation strategy (i.e. the scoring and categorisation) in the allotted time. This short list is available in Annex 4 of the Supplementary Material.

Fig. 3 summarizes the overall process starting from all the nominations received up to the short list of nominated substances.

3.2. Ranking of nominated substances included in the short list through the scoring and categorisation approach

3.2.1. Consensual weighting values for the prioritisation criteria

When implementing the adapted Delphi approach, the 11 experts agreed that two of the five prioritisation criteria (i.e. regulatory status, and technical feasibility for HBM measurement) were not of highest relevance for the scoring step. Indeed, scoring the substances according to their current regulatory status was not considered appropriate, because this would produce a bias towards already regulated substances for which more follow-up activities are needed instead of gaining new knowledge. Likewise, as the HBM4EU project entails activities aiming to develop analytical methods for measuring biomarkers, it was not considered informative to de-prioritise lesser-known substances for which no analytical biomonitoring methods currently exist (C or D category substances). While finally not used for the scoring step, these two prioritisation criteria were nonetheless useful for the categorisation step, as this defines the type of work to be perform in HBM4EU.

Table 6 indicates the weighting values assigned to each of the three selected prioritisation criteria (hazardous properties, exposure characteristics and the public concern) during the second round of consultation with the 11 involved experts. The median values were considered thereafter as the consensus weighting value to be applied to the adjusted score of each prioritisation criterion.

Fig. 3. Overview of the process to obtain the short list of substances/substance groups in the second HBM4EU prioritisation round.

Table 6
Weighting values assigned to the prioritisation criteria by experts during a second round of consultation as part of an adapted Delphi method and resulting consensus weighting values.

Prioritisation criterion	Weighting values (%) assigned by the experts											Resulting consensus weighting value (median)
	Expert number											
	1	2	3	4	5	6	7	8	9	10	11	
Hazardous properties	30	35	50	40	40	40	30	40	40	50	25	40%
Exposure characteristics	50	45	40	40	40	40	50	40	40	30	50	40%
Public concern	20	20	10	20	20	20	20	20	20	20	25	20%

3.2.2. *Ranking the substances/substance groups according to their priority score (and category)*

The substance's/substance group's scores against the hazard, exposure and public concern criteria that were discussed and approved by the expert panel involved in the scoring and categorisation steps, have been used to calculate the substance's/substance group's overall priority score following the approach described in section 2.2.1. These scores (individual score against each prioritisation criterion and overall priority score) and the category assigned by the experts to each substance/substance groups are available in the substance's revised background documents under https://www.hbm4eu.eu/prioritisation-step-5/. As an example, the scoring and categorisation of acrylamide is also provided in Supplementary Material - Annex 5.

The substances (or selected "lead" substances representing a nominated substance group) from the short list were then ranked according to their overall priority score (Table 7). As several single "lead" substances belonging to wide groups of substances have been scored (e.g. nano titanium dioxide, nano silver and carbon nanotubes were scored within the nominated nanomaterials group), 29 substances were ranked although only 23 substances/substance groups were included in the short list of substances' nominations. The substance's category, as indicator of the type of activity to be undertaken in the project for each substance (either policy-oriented activities as for example derivation of HBM guidance values or rather research activities as development of analytical methods) was specified next to the priority score. An alternative way of ranking the substances consisted in considering the priority score but this time among the substances from a certain category (ranking not shown).

3.3. *Finalisation and approval of the second HBM4EU prioritisation list*

The EU Policy board and the HBM4EU Management Board agreed on a final substances priority list after having engaged discussions on the substance's ranking and on the priorities for action from a policy perspective. The resources available for the project were considered in order to calibrate the number of substances to be included in the final list. This consideration also explains why few substances that had been ranked highly were not included in the final list. As an example, nanomaterials were not included due to the complexity to characterize them and the considerable resources that would be required for the development of analytical methods to measure them in biological matrices.

The second HBM4EU substance priority list, later approved by the HBM4EU Governing Board, is presented in Table 8.

3.4. *Results of the feedback survey and recommendations for improvement of the chemical prioritisation strategy*

In June 2019, a survey for the refinement of the prioritisation strategy was sent in anticipation of the third round of prioritisation under HBM4EU. Detailed information on this survey is available in the Annex 6 of the Supplementary Material. Main suggestions and improvements made are described below and will be taken into account for future rounds of prioritisation.

First, considering the nomination of groups of substances, the question was raised if it had to remain an option, considering the methodological difficulties that have been encountered to score such groups. The majority of respondents were still in favour of keeping this possibility. However, the nomination of a group should be manageable with regard to the resources of the programme. Substances of a given group

Table 7
Ranking of nominated substances or representative substance(s) for a substance group based on their overall priority score, along with the allocated knowledge-level representing category (A to D).

Rank	Substance (substance group)	Hazard score	Exposure score	Public concern score	Overall score[a]	Substance category (A to D)[b]
1	Arsenic (and its inorganic compounds)	27.2	38.0	9.0	74.2	B
2	Lead	25.3	36.0	9.0	70.3	A
3	Acrylamide	27.2	36.8	5.4	69.4	B
4	Aflatoxin B1 (*Mycotoxins*)	30.8	27.2	5.4	63.4	B
5	Chlorpyrifos (*Pesticides*)	13.3	29.2	20.0	62.5	B
6	Dimethoate (*Pesticides*)	12.4	31.2	18.0	61.6	C
7	Pyrethroids (*Pesticides*)	16.0	27.2	18.0	61.2	B
8	Permethrin (*Pesticides*)	14.0	28.0	18.0	60.0	B
9	Mercury (*Mercury & its organic compounds*)	17.2	28.0	10.8	56.0	A
10	DDAC (*Quaternary ammonium salts*)	9.2	32.8	12.8	54.8	C
11	Methylmercury (*Mercury & its organic compounds*)	22.0	23.2	9.0	54.2	B
12	Nano titanium dioxide (*Nanomaterials*)	16.0	26.8	10.8	53.6	D
13	4,4-MDI, 2,4-TDI & 2,6-TDI (*Diisocyanates*)	18.0	28.0	7.2	53.2	C
14	Glyphosate (*Pesticide*)	7.2	32.0	12.8	52.0	B
15	Deoxynivalenol (DON) (*Mycotoxins*)	18.0	28.0	5.4	51.4	C
16	BP-3 (*UV filters-Benzophenones*)	12.8	29.2	9.0	51.0	B
17	D4 (*Cyclic Siloxanes*)	5.6	33.2	11.0	49.8	C
18	N,N-dimethylformamide (DMF) (*Reprotoxic aprotic solvents*)	16.0	30.0	3.6	49.6	B
19	Nano silver (*Nanomaterials*)	14.0	26.0	9.0	49.0	D
20	BHT (2,6-di-tert-butyl-p-cresol)	14.0	32.8	1.8	48.6	C
21	Fumonisin B1 (*Mycotoxins*)	18.0	24.0	5.4	47.4	C
22	Fipronil (*Pesticide*)	16.8	25.2	3.6	45.6	C
23	Perchlorate	13.2	30.0	1.8	45.0	C
24	1-methyl-2-pyrrolidone (NMP) (*Reprotoxic aprotic solvents*)	12.0	27.2	3.6	42.8	B
25	UV-328 (*Phenolic benzotriazoles*)	12.0	27.2	3.6	41.0	C
26	Carbon nanotube (*Nanomaterials*)	12.8	18.8	9.0	40.6	D
27	BENPAT (*Substituted phenylenediamines*)	15.2	24.8	0	40.0	D
28	POE-tallow amine	12.0	20.0	3.6	35.6	C
29	N,N-diethyl-m-toluamide (*Pesticides*)	7.2	25.2	0.0	32.4	C

[a] The overall priority score was obtained by adding the weighted prioritisation criteria scores.

[b] An A to D category was allocated to the substance considering its knowledge level as informed in the revised background document and according to the definitions of the categories.

Table 8
Approved second HBM4EU list of priority substances.

No	Single substance or group of substances	Substance(s) considered for the scoring	Overall priority score	Substance Category
1	Lead (and its compounds)	Lead	70.3	A
2	Mercury (and its organic compounds)	Mercury	56.0	A
		Methylmercury	54.2	B
3	Arsenic inorganic compounds	Inorganic arsenic compounds, including diarsenic trioxide	74.2	B
4	Acrylamide	Acrylamide	69.4	B
5	Mycotoxins	Aflatoxin B1	63.4	B
		Deoxynivalenol	51.4	C
		Fumonisin B1	47.4	C
6	Pesticides	Chlorpyrifos	62.5	B
		Dimethoate	61.6	C
		Pyrethroids	61.2	B
		Permethrin	60.0	B
		Glyphosate	52.0	B
		Fipronil	45.6	C
7	UV filters - Benzophenones	Benzophenone-3	51.0	B
8	Aprotic solvents	N,N-dimethylformamide	49.6	B
		1-methyl-2-pyrrolidone	42.8	B
9	Diisocyanates	4,4-MDI, 2,4-TDI & 2,6-TDI	53.2	C

may have structural similarities or the same analytical methods, offering the possibility for work packages to include a set of substances (i.e. a group) in their programmes in a rational way. The online survey for nominating substances in the second prioritisation round suggested that a possible rationale for including several substances in a single group may be based on a similar toxicological profile or similar uses. However, a rationale for grouping substances based on similar uses can possibly lead to the nomination of a wide group of substances of very different types, making it difficult to work on all of them within the project. Therefore, more relevant rationales for grouping substances, e.g. common analytical methods is recommended (e.g. all species of inorganic arsenic such as arsine, arsenate, dimethylarsinic acid (DMA), methylarsonic acid (MMA) may all be measured by a single speciation method).

Secondly, regarding the scoring method for groups of substances, a legitimate discussion was raised on the scoring of a "representative" lead substance of the group. An alternative approach consists in considering a "worst-case scoring", performed on the most harmful characteristics identified for substances within the group. Because there may be significant differences across substances within a group, due for example to the heterogeneity in data availability, the most cautious approach should always prevail when considering risk assessment. This enhances the need of a robust and well-justified rationale behind the nominations of groups, as mentioned above.

Thirdly, the likelihood that a specific substance is part of a mixture of daily life exposure could additionally be taken into account as a parameter to score the exposure criterion.

Finally, regarding the overall nomination process, interesting suggestions have been made to improve its efficiency. Indeed, each entity nominating substances had to complete the entire online survey for each substance/substance groups (with an upper limit of 5 nominations). The survey is a time-consuming procedure that had to be completed even if the nominated substance may not be selected in the final prioritisation process. It was therefore suggested that each nominating entity first

provides initial expression of interest. Then, a short list of substances would be set on the basis of the interest of the nominating entities and shared with them, to allow coordinating efforts on providing the relevant documentation for a substance or group of this list.

4. Discussion

It is the first time that a pan-European HBM project has been implemented, with currently 30 countries being part of the initiative already (North Macedonia and Estonia joined the initiative in 2020). The prioritisation process had to propose a method allowing the selection of substances of common priority across participating countries, the EC and a range of stakeholders, but also to determine the research efforts needed at national levels.

4.1. Comparison with other chemical prioritisation process

The HBM4EU prioritisation process can be compared to other substance prioritisation activities at the EU level, for example the EU Directive 2000/60/EC, commonly known as the EU Water Framework Directive (WFD) (Daginnus et al., 2011). This was also a policy-oriented prioritisation process for monitoring chemicals in water bodies. The process for the WFD was finalised with Decision 2455/2001/EC on the first list of priority substances and is revised and updated every 6 years. Under the WFD, priority substances are identified based on a two-year consultation process for which a combined monitoring-based and modelling-based priority-setting procedure has been developed. Background documents are produced for the priority substances as well as a procedure for the identification of priority hazardous substances. An expert advisory group, consisting of experts from Member States, European Free Trade Association (EFTA) countries, the Scientific Committee on Toxicity, Ecotoxicity and the Environment (SCTEE), the European Chemicals Bureau and stakeholders from industry, water suppliers and environmental groups, are consulted. In the WFD, substances are prioritised taking into account (Bodar et al., 2003): 1) risk assessments carried out under existing chemically relevant EU Directives and Regulations, e.g. ECHA and the European Food Safety Authority (EFSA) (Bodar et al., 2003; ECHA 2016; EFSA 2009; EU Regulation No 528/2012); 2) targeted risk-based assessments focusing on aquatic ecotoxicity and human toxicity *via* the aquatic environment; 3) simplified risk-based assessments based on intrinsic hazards, widespread environmental contamination, production volumes and use patterns. The priority list of substances under the WFD focuses only on single substances (Daginnus et al., 2011; Faust et al., 2019; INERIS 2009).

Similar to the WFD, the time for implementation of the proposed HBM4EU prioritisation process is quite long (approximately 1 year). The HBM4EU prioritisation process also includes consulting an expert group, as well as with partners participating in the project, i.e. policy makers, scientists and stakeholders who nominated substances to be included for monitoring and research activities within the project. This approach aims to ensure the legitimacy, credibility and societal relevance of the process. Within HBM4EU, a stakeholder workshop was also conducted to address public concerns, a step that was not included in the WFD priority-setting process.

In contrast to the WFD, the HBM4EU priority-setting process did not include any type of risk assessment studies, nor did it focus only on single substances, cf. group of priority substances on mixtures, emerging chemicals, flame retardants, PAHs and pesticides.

In Europe, other national biomonitoring programmes are also known to have a priority strategy for substances that share some similarities with that of HBM4EU.

In France, the National Nutrition and Health Survey (ENNS) was conducted in 2006–2007 to meet the objectives on biomonitoring, chronic disease surveillance and nutritional surveillance. More recently, the Grenelle I Act (No, 2009–967 of August 3, 2009) led to the development of a French National Biomonitoring programme, in which two distinct studies were designed: 1) the Health Study on Environment, Biomonitoring, Physical Activity and Nutrition (called "Esteban"), which is a nationwide cross-sectional survey of the mainland population aged 6–74 years and 2) the ELFE cohort (Longitudinal Study from Childhood) constituting the perinatal component of the French National Biomonitoring programme (Balicco et al., 2017; Dereumeaux et al., 2016; Fillol et al., 2014; Fréry et al., 2012). The prioritisation process relies on members of government agencies to validate an initial list of pollutants and on a group of French-speaking and international HBM experts to establish the selection criteria, to rate the chemicals using a graded score and to review, validate and establish a provisional final list. The final list is reviewed, revised and recommended by an "emerging risk" group of the National Environmental Health Plan (PNSE).

In Belgium, Flanders has established the Flemish Environment and Health Study (FLEHS) (Schoeters et al. 2012, 2017). The prioritisation of chemicals was based on international lists and expert advice using weighted scoring, followed by a step-by-step procedure implemented to first categorise criteria and then select and score the chemicals. Scientific experts and the strategic advisory board for the Ministry of Environment, Health and Energy and the Socio-economic Board (representatives of employers and employees) were asked to make recommendations.

Germany has one of the longest running HBM programmes in Europe. The German Environmental Survey (GerES) has been running since 1985, and its prioritisation strategy is based on existing international lists and further information on hazardous chemicals and the degree of exposure of the general public in Germany and expert judgments (government authorities, industry and science sectors) (Kolossa-Gehring et al. 2012b, 2017; Schulz et al. 2007, 2017). The selection of chemicals for GerES also focuses on a cooperation project between the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) and the German Chemical Industry Association (VCI) to select new substances and to develop new analytical HBM methods.

In Canada, the Canadian Health Measures Survey (CHMS) prioritisation approach is based on expert advice (workshop of experts), stakeholder consultations *via* questionnaires, and the Health Canada regulatory programme needs mandated by the Chemicals Management Plan. The process was adapted and adjusted during each of the first three cycles of the CHMS (Haines et al., 2017).

In the US, the main HBM programme is NHANES, running since the 1970s (CDC 2009, 2019). It comprises a participatory approach led by the Center for Disease Control (CDC) *via* notices in the Federal Register to establish criteria for inclusion or removal of chemicals and for the nomination of chemicals to measure in the biomonitoring program as part of NHANES. An expert panel of outside reviewers and CDC scientists scores nominated individual chemicals or categories of chemicals using weighted criteria to categorise these into five priority groups. Another recent initiative is the Environmental Influences on Child Health Outcomes (ECHO) by the US National Institutes of Health. With multiple cohorts of participating children, it will take into account longitudinal studies to investigate environmental exposures (including chemicals) on child health and development. It will investigate the exposure to about 200 chemicals already present in NHANES as well as new chemicals. ECHO's prioritisation strategy is based on database and literature research for chemicals in environmental media and in consumer products that are potentially toxic, and that have not been measured in NHANES (Pellizzari et al., 2019).

4.2. Specificity and limitation of the HBM4EU chemical prioritisation process

With different types of approaches for each country and HBM programme, there are some commonalities that were taken into account and adapted to the objectives of HBM4EU. The aim of the HBM4EU initiative is not only to measure the internal exposure to well-known

substances in the European population, but also to focus on human exposure to lesser known or emerging substances for which analytical methods are not yet available, and toxicological and exposure data is currently insufficient.

As an H2020 project, HBM4EU addresses societal challenges to health and well-being for European citizens. It is a main objective of the project to bridge the divide between science and policy at the European level and to generate results that meet the knowledge needs of EU policy makers. Priority was therefore given to the nomination of substances by members of the EU Policy Board, with the aim of delivering on this key objective. Input from the National Hubs was also highly valued and helped to ensure that the project also serves the knowledge needs of national policy makers and to determine whether national and European level priorities are aligned. Selected substances are the subject of research at European level. It is therefore important that HBM4EU addresses knowledge gaps on chemical exposure and resulting health impacts that have relevance at the European level and that can generate results that benefit the pan-European population. Substances that are exclusively of local or national concern were therefore not considered. Input from the Stakeholder Forum made it possible to assess the social relevance of research activities on nominated substances and drew in additional evidence and knowledge. As such, the strategy for the prioritisation of substances was not based entirely on scientific evidence. It was also guided by an imperative to produce knowledge in support of policy making at European level.

A literature review was carried out to discern and adapt the prioritisation strategy from international experiences. The prioritisation criteria that were selected and used for the scoring and the whole prioritisation process needed to be streamlined, also including time restrictions. During the prioritisation process, substances were allocated to categories reflecting the availability of existing data on hazard and exposure. The substance's regulatory status and availability of analytical methods to perform HBM were also taken into account. The aim was to ensure that HBM4EU focused not just on well-known and -studied substances, but also covered ones with limited data. Indeed, the particularity of this project in comparison to other biomonitoring programmes is that it includes also research activities dedicated, for example, to the identification of biomarkers of effect or the development of analytical methods. Therefore also, when data gaps have been identified for certain hazards or exposure parameters during the scoring step, a Low score was attributed instead of a null score to avoid "disadvantaging" lesser-known substances during the ranking of the substances. This special attention to have lesser-known substances included in the second priority list has the merit to constitute an anticipatory approach by generating knowledge on data poor substances.

Despite efforts made to implement an objective and systematic scoring method, expert judgment was inevitable to score some parameters, in particular for data-poor substances. For example, the CLP classification was considered for scoring some endpoints (i.e. an objective score relying on solid evidence), but where no official classification has been proposed, then evidence identified in the peer-reviewed literature were considered. In this case, the expert's judgement has a more prominent influence on the given score. Nevertheless, each given score was discussed with a panel of experts from a variety of fields of expertise, in order to reduce the subjectivity in the scoring. The evaluation of societal concern can also be liable to some subjectivity. Scoring this criterion relied mainly on the stakeholder's reflections and information about the substances captured during a stakeholder workshop (including whether NGO campaigns related to the substances were recently conducted), but also on lists established by diverse stakeholders. In this consideration however, the weight given under the prioritisation process to the scoring of societal concern was lower (20%) than the ones given to the scoring of hazards and exposure characteristics (40% each). However, to help define this societal concern, it may worth better considering differences in terms of occupational health aspects versus the general population or environmental aspects. Specific questions on such aspects can be asked before prioritisation in the survey used by the entities to nominate substances, to provide further arguments in support of the nomination of substances or groups of substances. Finally, every step of the process, justifications of the choices made and results are fully documented on the HBM4EU webpages, in order to account for the choices made in a completely transparent way.

4.3. Reflections for a further refined chemical prioritisation process

Steps for a further refined prioritisation strategy are presented below:

1) Pre-nomination step: the EU Policy Board, the Stakeholder Forum as well as the National Hubs provide an initial expression of interest for up to 5 substances or groups of substances together with a rationale for nominating them.
2) Long list of nominated substances/groups: this list will be publicly available and will collate all the nominated substances/groups of substances and the nominating parties who nominated them.
3) Short list of nominated substances: based on the long list of nominated substances/groups of substances, partners involved in the prioritisation process will produce a short list of approximately 25 substances/groups, ranked according to the number of times they were nominated and to who nominated them (i.e. following the same criteria used during the second round of prioritisation, as mentioned above).
4) Compiling data: after producing and sharing the short list, nominating parties will have 2 months to complete the online survey requesting information related to the prioritisation criteria. Based on the information provided in the survey on each nominated substances/groups of substances, background documents will be produced within 3 months. In the meantime, a Stakeholder Forum workshop will be organised to evaluate the societal concern of the substances/groups of substances on the short list.
5) Scoring and ranking process: the substances/groups of substances of the short list will be scored and categorised by experts, on the basis of the information gathered in the background documents, allowing for the elaboration of a ranked list of prioritised substances.

Before the end of the HBM4EU project, a third round of prioritisation will be run based on the feedback and suggestions for improvement presented in this paper. This work will then benefit any follow-up HBM initiative that takes place. Currently, discussions are ongoing for the set-up of a partnership under Horizon Europe, the European Partnership for the Assessment of Risks from Chemicals (PARC).

5. Conclusion

To prioritise chemicals for inclusion in a European-wide HBM initiative, a structured and transparent process was developed using a participatory approach. Prioritisation must reflect the selection of chemicals of interest considering the diversity of needs for such initiative, as also the policy needs from the participating countries and agencies and the current concerns of European citizens. The prioritisation strategy for substances and substance groups developed in HBM4EU served to guide biomarker selection for the biomonitoring studies initiated in HBM4EU and the associated research to improve interpretation of biomarker data in terms of health risks and exposure sources. The process was considered to be transparent and science-based, as shown by the feedback that was obtained.

The strategy for the second round of prioritisation of HBM4EU priority substances was implemented over a one-year period (from July 2017 to June 2018). The list of prioritised substances and their respective selected CGLs was approved by the HBM4EU Governing Board composed of the programme sponsors in the participating countries, the European Chemicals Agency (ECHA), the EEA and EFSA in July 2018,

after which research activities could start. The second list included acrylamide, aprotic solvents, arsenic, diisocyanates, lead, mercury, mycotoxins, pesticides (including chlorpyrifos, dimethoate, pyrethroids, glyphosate and polyethoxylated (POE)-tallow amine, and fipronil), and a type of UV filters (benzophenones).

To further maintain harmonised and comparable results of prioritisation processes, we recommend that any future HBM project take this prioritisation process into account. The prioritisation strategy developed under HBM4EU, as well as the third list of priority substances, can be used in any follow-up HBM initiative.

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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.2021.113778.

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Combined effects of chronic $PM_{2.5}$ exposure and habitual exercise on renal function and chronic kidney disease: A longitudinal cohort study

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ABSTRACT

Background: We investigated the combined effects of chronic $PM_{2.5}$ exposure and habitual exercise on the decline of renal function and the incidence of chronic kidney disease (CKD) in a large cohort in Taiwan.

Methods: The present data analysis included a total of 108,615 participants aged 18 years or above who were recruited between 2001 and 2016. All participants underwent at least two medical examinations. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation. The incident of eGFR decline ≥30% was defined as a decline in eGFR of ≥30% during the study period, while the incident CKD was defined as an eGFR <60 mL/min/1.73 m^2 or a newly self-reported physician-diagnosed CKD in the subsequent visits. The satellite-based spatiotemporal model was used to estimate $PM_{2.5}$ exposure at each participant's address. Information on habitual exercise was collected using a standard self-administered questionnaire. The Cox regression model with time-dependent covariates was used for data analyses.

Results: Higher habitual exercise was associated with lower risks of renal function decline and CKD development, whereas higher $PM_{2.5}$ exposure was associated with higher risks of renal function decline and CKD development. We found no significant interaction effect between $PM_{2.5}$ and habitual exercise, with an HR (95% CI) of 1.02 (0.97, 1.07) for incident eGFR decline ≥30% and 1.00 (0.95, 1.05) for CKD development. Compared to participants with inactive-exercise and high-$PM_{2.5}$, participants with high-exercise and low-$PM_{2.5}$ had 74% and 61% lower risks of renal function decline and CKD development, respectively.

Conclusion: Increased habitual exercise and reduced $PM_{2.5}$ exposures are associated with lower risks of renal function decline and CKD development. Habitual exercise reduces risks of renal function decline and CKD development regardless of the levels of chronic $PM_{2.5}$ exposure. Our study suggests that habitual exercise is a safe approach for kidney health improvement even for people residing in relatively polluted areas and should be promoted.

1. Introduction

Chronic kidney disease (CKD) is a global public health challenge. In 2017, there were 697 million CKD patients worldwide, increasing by 27% over past 10 years (James et al., 2018). CKD contributed to 1.2 million deaths and was ranked as the 12th leading cause of global death in 2017 (Roth et al., 2018). The most severe stage of CKD, end-stage renal disease, requires costly dialysis or transplant, seriously affects patients' quality of life, and results in an enormous economic burden.

Regular exercise may improve kidney function and reduce the risk of

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CKD progression (Castaneda et al., 2001; Guo et al., 2020b; Jafar et al., 2015; Robinson-Cohen et al., 2009, 2014). The World Health Organization (WHO) recommends an adult to undertake at least 150 min of moderate-intensity physical activity per week to prevent non-communicable diseases (WHO, 2010). However, exercise may increase the inhalation of air pollutants due to higher ventilation. Air pollution has been shown as a novel risk of reduced renal function and CKD development by increasing evidences (Bowe et al., 2017, 2018; Chan et al., 2018; Mehta et al., 2016). Thus, there is an emerging public concern whether the increased intake of air pollutants due to exercise may exacerbate the adverse health effects on kidney health.

There are more than 91% of world population lives in a place where air quality does not meet the WHO guideline (WHO, 2016). The risk-benefit relationship between air pollution and exercise needs to be addressed urgently to inform people whether it is safe to perform habitual exercise in polluted regions. Few studies have investigated the combined effects of habitual exercise and air pollution exposure on hypertension, respiratory diseases, and mortality (Andersen et al., 2015; Fisher et al., 2016; Guo et al., 2020a, 2020c; Kubesch et al., 2018; McConnell et al., 2002; Sun et al., 2020). There is little information on the combined effects on kidney health so far. We previously investigated the association between chronic exposure to $PM_{2.5}$ and incident CKD using a large longitudinal cohort. We observed that every 10 $\mu g/m^3$ increase in $PM_{2.5}$ was associated a higher risk of 6% in CKD development in that study [Hazard Ratio (HR): 1.06; 95% confident interval(CI): 1.02, 1.10] (Chan et al., 2018). We have also investigated the association between habitual exercise and incident CKD using the same cohort and our results show habitual exercise was associated with a lower risk of CKD and impaired renal function (Guo et al., 2020b). We therefore extended our previous research to investigate the combined effects of habitual exercise and chronic $PM_{2.5}$ exposure on reduced renal function and development of CKD based on the same longitudinal cohort in Taiwan, where the annual $PM_{2.5}$ concentration was 2.6 times of the limit recommended by the WHO (WHO, 2006).

2. Methods

2.1. Study design and participants

The present study was based on an ongoing longitudinal cohort in Taiwan. Details of the cohort have been described in our previous publications (Guo et al., 2020a; Lao et al., 2019b). In brief, a private firm, the MJ Health Management Institution, has provided a standard medical screening program for Taiwan residents since 1994 (Chang et al., 2016). Residents who joined the program were encouraged to visit clinics on a yearly basis and to receive a series of standard medical examinations and to complete an extensive self-administrated questionnaire. Between 1996 and 2016, around 0.60 million Taiwan residents were recruited in the program and 44% of them had at least two medical visits. Each participant was required to sign an informed consent form prior to each medical examination authorizing the institution to release the data for research purpose. Ethical approval for this study has been obtained from the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee. Details of participant selection were described in supplementary file (Online Resource 1 & 2). Finally, a total of 108,615 participants and 104,092 participants with a follow-up period of ≥3 years were included in analysis to investigate the combined effect on incidence of eGFR decline ≥30% and incidence of CKD, respectively.

2.2. Air pollution exposure assessment

The details of estimating $PM_{2.5}$ exposure have been published elsewhere (Lao et al., 2019b; Li et al., 2005; Lin et al., 2015; Zhang et al., 2017). Briefly, We developed a satellite-based spatiotemporal model based on the aerosol optical depth (AOD) data at a resolution of 1 km^2 (Li et al., 2005; Lin et al., 2015). The AOD data was derived from the Moderate Resolution Imaging Spectroradiometer (MODIS) carried on Terra and Aqua satellites of the National Aeronautics and Space Administrations (NASA) (Li et al., 2005; Lin et al., 2015). The model was validated by comparing the estimated $PM_{2.5}$ exposure with data from more than 70 monitoring stations across Taiwan. The correlation coefficients for yearly average concentration ranged from 0.72 to 0.83 (Zhang et al., 2017).

The address of each participant was geocoded into latitude and longitude data and the estimated $PM_{2.5}$ exposure was matched with the individual address. The 2-year average of $PM_{2.5}$ concentrations was used as a proxy of chronic exposure, which corresponds to the average of yearly $PM_{2.5}$ concentrations of the calendar year of medical examination and the previous year. Both the continuous (per 10 $\mu g/m^3$) and category (participants were grouped into three categories based on the tertile cut-off points of $PM_{2.5}$, i.e. low: ≤22.40, moderate: 22.40–26.0, and high: >26.01 $\mu g/m^3$) of $PM_{2.5}$ were used for data analysis.

2.3. Assessment of habitual exercise

Details of assessing habitual exercise have been described elsewhere (Guo et al., 2020a, 2020b; Lao et al., 2019a; Wen et al., 2011; Zhang et al., 2018b). First, a standard self-administrated questionnaire was used to collect information on weekly exercise that participants generally engaged during the month before medical examination. Weekly exercise was classified into four intensity categories with examples provided under each category: light (e.g. walking), moderate (e.g. brisk walking), medium-vigorous (e.g. jogging), and high-vigorous (e.g. rope skipping). A standard metabolic equivalent of task (MET; 1 MET = 1 kcal/kg/hour) value was assigned to four intensity categories: 2.5 (light), 4.5 (moderate), 6.5 (medium-vigorous), and 8.5 (high-vigorous), respectively(Ainsworth et al., 2000; Wen et al., 2011). If participants reported activities in more than one category, a weighted MET was calculated based on time spent in each category. Afterwards, the weekly exercise volume (MET-h) of each participant was calculated as the product of intensity (MET) and duration (hours) of exercise. Participants were then classified into three exercise groups based on the tertile cut-off point of exercise-volume (MET-h): inactive (0 MET-hour), moderate (0–8.75 MET-hour), and high (>8.75 MET-hour) for data analysis. We did not use the continuous MET-h for data analysis because 0 MET-h was assigned to all participants in inactive group.

2.4. Outcome ascertainment

Glomerular filtration rate (GFR) is regarded as the best overall index of kidney function (KDIGO, 2013), while estimated glomerular filtration rate (eGFR) is widely used as an alternative for GFR in research (KDIGO, 2013). We used the following two health outcomes based on eGFR in this study.

- Incidence of eGFR decline ≥30%: in the cohort of 108,615 participants with 468,154 medical examination records, a participant was defined as an incident of eGFR decline ≥30% if s/he has a decline of eGFR ≥30% during the study period. The decline of eGFR was calculated using the formula: $\frac{(baseline\ eGFR - follow\text{-}up\ eGFR)}{baseline\ eGFR} \times 100\%$. We used the cutoff point of ≥30% for decline in eGFR because it was reported that an eGFR decline ≥30% was more strongly associated with the risk of end-stage renal disease (ESRD), an end point of CKD progression (Coresh et al., 2014). It is also widely used as a parameter of renal function outcomes in previous studies (Bowe et al., 2017, 2018).
- Incidence of CKD: in the cohort of 104,092 participants with 446,119 medical examination records, a participant was defined as an incident CKD if s/he had an eGFR <60 mL/min/1.73 m^2 or self-reported physician-diagnosed CKD in the subsequent visits. eGFR <60 mL/

min/1.73 m^2 was widely used as the diagnosing criteria of CKD in many studies and guidelines (Bowe et al., 2017; 2018; Chan et al., 2018; KDIGO, 2013).

The end point of the health outcome of CKD was defined as the first occurrence of CKD or the final visit if CKD did not occur over the study period. Similarly, the endpoint of eGFR decline ≥30% was defined as the first occurrence of eGFR decline ≥30% or the final visit if eGFR decline ≥30% did not occur constantly over the study period.

To calculate eGFR, overnight fasting venous blood samples were drawn in the morning and serum creatinine was analyzed through uncompensated Jaffe method containing an alkaline picrate kinetic test on a HITACHI 7150 (before 2005) or a TOSHIBA C8000 (after 2005) analyzer. Based on serum creatinine level, age, and gender, eGFR was calculated using the following Modification of Diet in Renal Disease (MDRD) equation:

$$186.3 \times (serum\ creatinine)^{-1.154} \times age^{-0.203} \times (0.742\ for\ women)$$

where serum creatinine is in mg/dL.

2.5. Covariates

Details about the procedure of medical examination and quality-control measures have been described in previous studies (Chan et al., 2018; Chang et al., 2016; Guo et al., 2020b; Zhang et al., 2018a). Weight and height were measured by an auto-anthropometer (KN-5000A, Nakamura) with participants wearing light clothes. Body mass index (BMI) was calculated as the weight (kg) divided by the square of the height(m). Seated blood pressure including systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by a computerized auto-mercury sphygmomanometer (CH-5000, Citizen). Overnight fasting venous blood samples were drawn to measure glucose and lipids (total cholesterol (TC), triglycerides (TG), high lipoprotein cholesterol (HDL-C), and low lipoprotein cholesterol (LDL-C)) on an automated biochemical analyzer (HITACHI 7150 before 2005 or TOSHIBA C8000 since 2005). Urinary total protein was analyzed using a ROCHE Miditron or ROCHE Cobas U411semi-automated computer-assisted urinalysis system.

Besides, a standard self-administered questionnaire was used to collect information on the participants' demographic characteristics, behavioral and lifestyle factors, and medical history.

Based on previous literature, we included the following covariates in the present study: age (years), gender (male or female), education levels (lower than high school, high school, college or university, or post-graduate), smoking status (never, ever[smoked at least once but quit later], or current[more than once a week]), alcohol drinking (seldom [<once/week], occasional[1–3 times/week], or regular[>3 times/week]), occupational exposure to dust or solvent in the workplace (yes or no), physical labor at work (sedentary jobs [e.g. clerk], jobs that require approximately half sedentary and half standing/walking [e.g. nurse], jobs that mostly require walking and standing [e.g. retail salesperson], or jobs that require vigorous physical activity[e.g. porter]), BMI (kg/m2), diabetes (defined as fasting blood glucose ≥126 mg/dL or self-reported physician-diagnosed diabetes), hypertension (defined as an SBP ≥140 mm Hg or a DBP ≥90 mm Hg, or self-reported physician-diagnosed hypertension), dyslipidemia (defined as TC ≥ 240 mg/L, TG ≥ 200 mg/dL, HDL- C < 40 mg/dL, or LDL-C ≥160 mg/dL), self-reported CVD or stroke (yes or no) and any self-reported form of cancer (yes or no), baseline eGFR, urinary total protein (negative[<0.1 g/L], trace [0.1~0.2 g/L], 1 plus [0.2~1.0 g/L], 2 plus [1.0~2.0 g/L], 3 plus [2.0~4.0 g/L], and 4 plus [>4.0 g/L]), season (spring, summer, fall, or winter) and calendar year of baseline visit.

2.6. Statistical analysis

Cox regression models with time-dependent covariates was used to investigate the effects of $PM_{2.5}$ or exercise on incidences of eGFR decline ≥30% and CKD, respectively, with a city-level random intercept added to account for within-city clustering. The time-scale used in the Cox regression model was time-in-study (i.e. follow-up time). The $PM_{2.5}$ concentration, habitual exercise and all covariates were treated as time-dependent variables in the models except for gender, baseline eGFR, and baseline calendar year. Three statistical models were developed with gradual addition of aforementioned covariates: Crude Model: without adjustment; Model 1: adjusted for age, gender, education, season, baseline calendar year, smoking status, alcohol drinking, occupational exposure, and physical labor at work; Model 2 further adjusted for BMI, diabetes, hypertension, dyslipidemia, self-reported cardiovascular disease, self-reported cancer, baseline eGFR, and urinary total protein. A trend test was performed across exercise and $PM_{2.5}$ categories. Hazard ratios (HRs) with 95% confident interval (CI) were presented with the inactive-exercise group or the low-$PM_{2.5}$ as the reference. Mutual adjustments for exercise and $PM_{2.5}$ were performed for comparison (i.e. we further included exercise in the model for assessing main effect of $PM_{2.5}$ or $PM_{2.5}$ in the model for assessing the main effect of exercise). We subsequently evaluated the potential interaction effect of $PM_{2.5}$ and exercise by adding a product term of "continuous $PM_{2.5}$(every 10 $\mu g/m^3$) × category-exercise" into the fully adjusted model.

We then performed subgroup analyses stratified by $PM_{2.5}$ or exercise categories, separately, to assess the effects of habitual exercise or $PM_{2.5}$ in each stratum. To examine the combined effects of $PM_{2.5}$ and exercise, participants were classified into nine groups based on their $PM_{2.5}$ and exercise categories, and those with inactive-exercise and high-$PM_{2.5}$ were served as the reference group.

A series of sensitivity analyses were performed. 1) We excluded participants with baseline diabetes, cardiovascular diseases, or cancer to eliminate the potential comorbidity effects (for the outcome eGFR decline ≥30%, participants with baseline CKD or those reported physician-diagnosed kidney disease were also excluded); 2) We used the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (KDIGO, 2013) to calculate eGFR for comparison; 3) We used annual average $PM_{2.5}$ concentration at the previous year of medical examination to examine the stability of $PM_{2.5}$ effects; 4) We restricted the analysis within the elderly aged ≥65 years to examine whether the combined effects of $PM_{2.5}$ and exercise on renal function or CKD were different in the elderly; 5) We used the inverse probability weight method to control for potential bias caused by missing values.

Statistical analyses were performed using R 3.2.5 (R Core Team, Vienna, Austria). A two-tailed P value < 0.05 defined the statistical significance.

3. Results

A total of 108,615 participants with 468,154 observations were included in the study to investigate the combined effects of habitual PA and $PM_{2.5}$ on the incidence of eGFR decline≥30%. Around 76.2% of the participants underwent more than two medical examinations with a median number of 4 (ranged from 2 to 26). The median interval of examinations was 17 months (IQR, 12–29 months). We identified 4,825 participants whose eGFR declined more than 30% during the study period. The mean follow-up duration was 6.7 years (standard deviation (SD: 3.2)).

A total of 104,092 participants without CKD at baseline were included in the analysis to investigate the combined effects of habitual exercise and $PM_{2.5}$ on the incidence of CKD. Similarly, around 76.2% of the participants underwent more than two medical examinations with a median number of 4 (ranged from 2 to 26). The median interval of examinations was 17 months (IQR, 12–29 months). We identified 4,850 incident cases of CKD. The mean follow-up duration was 6.7 years (SD:

3.2).

The general characteristics of participants at baseline and all observations are summarized in Table 1. Slightly more than half of participants were males. The majority had higher education level, never smoked, and seldom drank. More than 60% participants had a mostly sedentary job. Fig. 1 shows the temporal and spatial distribution of $PM_{2.5}$ concentrations by year. $PM_{2.5}$ concentration reached the peak in 2004 and declined gradually in the following years. The majority of participants lived in the western parts of Taiwan.

Table 2 shows the main effects of habitual exercise and $PM_{2.5}$ on the incidences of eGFR decline ≥30% and CKD respectively. Participants who undertook a moderate/high volume of exercise were associated with a lower risk of incidences of eGFR decline ≥30% and CKD. However, exposure to a moderate/high level of $PM_{2.5}$ was associated with a higher risk of incident eGFR decline ≥30% and CKD. All estimates remained stable to additional adjustment for $PM_{2.5}$ or exercise. Significant trends for associations were shown across exercise or $PM_{2.5}$ levels. Besides, Overall interactions between habitual exercise and ambient $PM_{2.5}$ were not statistically significant with an HR of 1.02 (0.97, 1.07) for the incidence of eGFR decline ≥30% and 1.00 (95% CI: 0.95, 1.05) for the incidence of CKD.

Beneficial effects of exercise were observed in subgroup analysis stratified by $PM_{2.5}$ categories. In contrast, harmful effects of $PM_{2.5}$ exposure were observed in each exercise stratum (Table 3). The

Table 1
Characteristics of the participants.

Characteristic	Incidence of eGFR decline ≥30%				Incidence of CKD			
	Baseline [a] (n = 108,615)		All visits [b] (n = 468,154)		Baseline [c] (n = 104,092)		All visits [d] (n = 446,119)	
Age (year)	39.1	(11.8)	43.4	(12.0)	38.4	(11.3)	42.5	(11.4)
Male (n, %)	56,600	(52.1)	255,743	(54.6)	54,017	(51.9)	241,194	(54.1)
Education (n, %)								
Lower than high school	14,672	(13.5)	59,105	(12.6)	12,910	(12.4)	50,535	(11.3)
High school	22,046	(20.3)	88,588	(18.9)	21,218	(20.4)	84,987	(19.1)
College or university	59,335	(54.6)	255,569	(54.6)	57,732	(55.5)	247,730	(55.5)
Postgraduate	12,562	(11.6)	64,892	(13.9)	12,232	(11.8)	62,867	(14.1)
Smoking status (n, %)								
Never	81,389	(74.9)	354,265	(75.7)	78,127	(75.1)	338,050	(75.8)
Former	5,995	(5.5)	30,264	(6.5)	5,578	(5.4)	27,927	(6.3)
Current	21,231	(19.6)	83,625	(17.9)	20,387	(19.6)	80,142	(18.0)
Alcohol consumption (n, %)								
Seldom	93,428	(86.0)	397,059	(84.8)	89,661	(86.1)	378,774	(84.9)
Occasional	10,436	(9.6)	48,457	(10.4)	9,944	(9.6)	46,019	(10.3)
Regular	4,751	(4.4)	22,638	(4.8)	4,487	(4.3)	21,326	(4.8)
Physical labor at work (n, %)								
Sedentary jobs	69,454	(64.0)	316,238	(67.6)	66,522	(63.9)	300,656	(67.4)
Jobs that require approximately half sedentary and half standing/walking	28,667	(26.4)	113,040	(24.2)	27,513	(26.4)	108,301	(24.3)
Jobs that mostly require walking and standing	8,562	(7.9)	32,240	(6.9)	8,195	(7.9)	30,782	(6.9)
Jobs that require vigorous physical activity	1,932	(1.8)	6,636	(1.4)	1,862	(1.8)	6,380	(1.4)
Habitual exercise (n, %)								
Inactive (0 MET-h)	49,486	(45.6)	160,613	(34.3)	47,951	(46.1)	155,718	(34.9)
Moderate (≤8.75 MET-h)	32,600	(30.0)	152,539	(32.6)	31,307	(30.1)	146,468	(32.8)
High (>8.75 MET-h)	26,529	(24.4)	155,002	(33.1)	24,834	(23.9)	143,933	(32.3)
Occupational exposure (n, %) [e]	9,127	(8.4)	36,581	(7.8)	8,806	(8.5)	35,342	(7.9)
BMI (kg/m^2)	23.0	(3.5)	23.3	(3.5)	22.9	(3.5)	23.3	(3.5)
Urinary total protein (n, %)								
negative or normal (<0.1 g/L)	103,422	(95.2)	451,082	(96.4)	99,484	(95.6)	431,548	(96.7)
trace (0.1–0.2 g/L)	4,423	(4.1)	13,241	(2.8)	4,058	(3.9)	11,905	(2.7)
1 plus (0.2–1.0 g/L)	583	(0.5)	2,729	(0.6)	429	(0.4)	1,945	(0.4)
2 plus (1.0–2.0 g/L)	187	(0.2)	886	(0.2)	121	(0.1)	592	(0.1)
3 plus (>2.0 g/L)			216	(0.05)			129	(0.03)
Diabetes(n, %)	3,689	(3.4)	22,622	(4.8)	3,288	(3.2)	20,066	(4.5)
Hypertension (n, %)	14,483	(13.3)	75,679	(16.2)	12,760	(12.3)	65,617	(14.7)
Dyslipidemia (n, %)	42,559	(39.2)	198,118	(42.3)	40,033	(38.5)	185,872	(41.7)
Cardiovascular disease (n, %)	2,524	(2.3)	14,874	(3.2)	2,153	(2.1)	12,343	(2.8)
Cancer (n, %)	1,127	(1.0)	7519	(1.6)	1,002	(1.0)	6,476	(1.5)
$PM_{2.5}$ (ug/m^3) [f]	26.8	(7.9)	26.3	(7.4)	26.8	(7.8)	26.3	(7.4)
$PM_{2.5}$ by exercise categories (ug/m3)								
Inactive (0 MET-h)	26.8	(7.8)	26.6	(7.5)	26.8	(7.8)	26.6	(7.5)
Moderate (≤8.75 MET-h)	26.9	(7.8)	26.3	(7.4)	26.9	(7.8)	26.3	(7.4)
High (>8.75 MET-h)	26.9	(7.9)	26.0	(7.4)	26.8	(7.9)	26.0	(7.4)
Season (n, %)								
Spring	26,532	(24.4)	114,240	(24.4)	25,561	(24.6)	109,028	(24.4)
Summer	30,837	(28.4)	135,582	(29.0)	29,233	(28.1)	128,502	(28.8)
Fall	28,837	(26.6)	127,662	(27.3)	27,673	(26.6)	121,844	(27.3)
Winter	22,409	(20.6)	90,670	(19.4)	21,625	(20.8)	86,745	(19.4)

Abbreviations: CKD, chronic kidney disease; BMI, body mass index.
Values are presented as mean (standard deviation) for continuous variables and count (%) for categorical variables.
[a] Baseline characteristics of the 108,615 participants for analysis of the combined effects of $PM_{2.5}$ and habitual exercise on eGFR decline ≥30%.
[b] Characteristics of the 468,154 observations from the 108,615 participants.
[c] Baseline characteristics of the 104,092 participants for analysis of the combined effects of $PM_{2.5}$ and habitual exercise on CKD development.
[d] Characteristics of the 446,119 observations from the 104,092 participants.
[e] Classified as exposure to dust or organic solvents in the workplace, established by asking, 'Are there any occupational hazards in your workplace?'
[f] Refers to the average $PM_{2.5}$ levels of the year of the visit and the year before the visit.

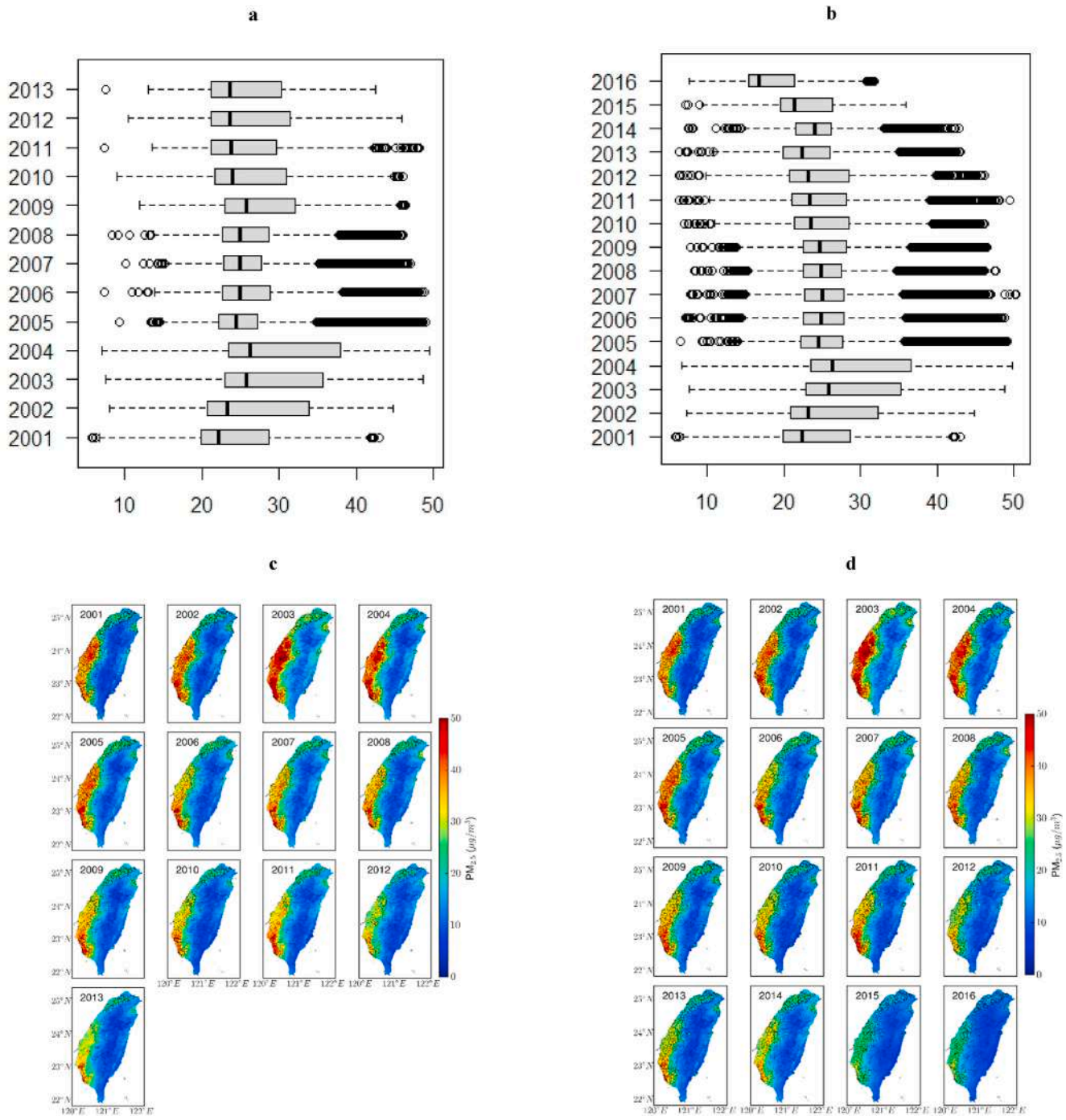

Fig. 1. The spatial and temporal distribution of the two-year average of $PM_{2.5}$ concentrations by year in Taiwan.
Panels a and b represent the temporal distributions of the 2-year average $PM_{2.5}$ concentrations by year. Boxes cover the 25–75th percentiles (IQR) with centre lines indicating the median concentration. Whiskers extend to the highest observations within three IQRs of the box, with more extreme observations shown as circles. Panels a illustrates the distribution of baseline $PM_{2.5}$ concentrations from 108,615 participants. Panels b shows the distribution of $PM_{2.5}$ exposure of 468,154 medical visits from the 108,615 participants. Panel c and d represent the spatial distributions of the participants. Circles indicate the locations of the participants. Panel c depicts the address locations (circles) of the 108,615 participants at baseline by year; Panel d depicts the address locations (circles) of the 108,615 observations from the 468,154 participants by year.

combined effects of habitual exercise and $PM_{2.5}$ are shown in Fig. 2 and Fig. 3 for the incidences of eGFR decline ≥30% and CKD, respectively. Overall, participants with low level of $PM_{2.5}$ exposure combined with high-volume of exercise had the lowest risk of renal function decline and CKD development. There was a prominently downward trend in the risk of developing eGFR decline ≥30% and CKD, with exposure to lower level of $PM_{2.5}$ in each stratum of exercise. The decreasing trend patterns of the effects of habitual exercise on these two outcomes were relatively flat across $PM_{2.5}$ stratums.

Sensitivity analyses generally yielded similar results (Online Resource 3–7).

4. Discussion

In this large Taiwanese cohort study, we found that habitual exercise significantly reduced the risk of renal function decline and CKD

Table 2
Associations of eGFR decline and CKD development with habitual exercise or $PM_{2.5}$ exposure in Taiwanese adults.

Models	Association with eGFR decline ≥30%															
	Main effects of exercise								Main effects of $PM_{2.5}$ exposure							
	HR (95% CI)		P		HR (95% CI) [a]		P		HR (95% CI)		P		HR (95% CI) [a]		P	
Crude model																
Moderate-exercise/$PM_{2.5}$	0.75	(0.70, 0.80)	<	.001	0.76	(0.70, 0.81)	<	.001	1.36	(1.26, 1.46)	<	.001	1.34	(1.24, 1.43)	<	.001
High-exercise/$PM_{2.5}$	0.63	(0.59, 0.67)	<	.001	0.65	(0.60, 0.69)	<	.001	2.31	(2.09, 2.56)	<	.001	2.24	(2.02, 2.48)	<	.001
Test for trend	0.79	(0.76, 0.82)	<	.001	0.80	(0.77, 0.83)	<	.001	1.48	(1.41, 1.55)	<	.001	1.45	(1.38, 1.53)	<	.001
Per 10 μg/m^3									2.05	(1.88, 2.24)	<	.001	1.98	(1.81, 2.16)	<	.001
Model 1																
Moderate-exercise/$PM_{2.5}$	0.78	(0.72, 0.83)	<	.001	0.79	(0.73, 0.84)	<	.001	1.51	(1.41, 1.63)	<	.001	1.50	(1.39, 1.61)	<	.001
High-exercise/$PM_{2.5}$	0.64	(0.60, 0.69)	<	.001	0.66	(0.61, 0.71)	<	.001	2.80	(2.52, 3.11)	<	.001	2.75	(2.48, 3.06)	<	.001
Test for trend	0.80	(0.77, 0.83)	<	.001	0.81	(0.78, 0.84)	<	.001	1.63	(1.55, 1.72)	<	.001	1.62	(1.54, 1.70)	<	.001
Per 10 μg/m^3									2.90	(2.63, 3.19)	<	.001	2.85	(2.59, 3.14)	<	.001
Model 2																
Moderate-exercise/$PM_{2.5}$	0.87	(0.81, 0.93)	<	.001	0.87	(0.81, 0.94)	<	.001	1.54	(1.44, 1.66)	<	.001	1.54	(1.43, 1.66)	<	.001
High-exercise/$PM_{2.5}$	0.70	(0.65, 0.75)	<	.001	0.71	(0.66, 0.76)	<	.001	2.86	(2.58, 3.18)	<	.001	2.84	(2.56, 3.15)	<	.001
Test for trend	0.84	(0.81, 0.87)	<	.001	0.84	(0.81, 0.87)	<	.001	1.65	(1.57, 1.74)	<	.001	1.65	(1.57, 1.73)	<	.001
Per 10 μg/m^3									3.18	(2.88, 3.50)	<	.001	3.15	(2.86, 3.47)	<	.001
	Association with CKD development															
	Main effects of exercise								Main effects of $PM_{2.5}$ exposure							
	HR (95% CI)		P		HR (95% CI) [a]		P		HR (95% CI)		P		HR (95% CI) [a]		P	
Crude model																
Moderate-exercise/$PM_{2.5}$	1.06	(0.98, 1.15)		.13	1.07	(0.99, 1.16)		.07	1.29	(1.20, 1.39)	<	.001	1.30	(1.21, 1.40)	<	.001
High-exercise/$PM_{2.5}$	1.30	(1.21, 1.40)	<	.001	1.33	(1.24, 1.43)	<	.001	1.77	(1.59, 1.96)	<	.001	1.81	(1.63, 2.01)	<	.001
Test for trend	1.15	(1.11, 1.19)	<	.001	1.16	(1.12, 1.21)	<	.001	1.32	(1.26, 1.38)	<	.001	1.34	(1.27, 1.40)	<	.001
Per 10 μg/m^3									1.90	(1.75, 2.06)	<	.001	1.95	(1.80, 2.12)	<	.001
Model 1																
Moderate-exercise/$PM_{2.5}$	0.96	(0.88, 1.03)		.25	0.96	(0.89, 1.04)		.36	1.42	(1.32, 1.53)	<	.001	1.41	(1.31, 1.52)	<	.001
High-exercise/$PM_{2.5}$	0.81	(0.75, 0.87)	<	.001	0.82	(0.76, 0.89)	<	.001	2.17	(1.95, 2.41)	<	.001	2.15	(1.93, 2.39)	<	.001
Test for trend	0.89	(0.86, 0.93)	<	.001	0.90	(0.87, 0.93)	<	.001	1.46	(1.39, 1.53)	<	.001	1.45	(1.38, 1.53)	<	.001
Per 10 μg/m^3									2.70	(2.46, 2.96)	<	.001	2.68	(2.45, 2.93)	<	.001
Model 2																
Moderate-exercise/$PM_{2.5}$	0.93	(0.86, 1.01)		.07	0.94	(0.87, 1.01)		.11	1.40	(1.30, 1.50)	<	.001	1.39	(1.29, 1.50)	<	.001
High-exercise/$PM_{2.5}$	0.79	(0.73, 0.85)	<	.001	0.80	(0.75, 0.87)	<	.001	2.16	(1.94, 2.40)	<	.001	2.14	(1.92, 2.37)	<	.001
Test for trend	0.89	(0.85, 0.92)	<	.001	0.89	(0.86, 0.93)	<	.001	1.45	(1.38, 1.53)	<	.001	1.44	(1.37, 1.52)	<	.001
Per 10 μg/m^3									2.66	(2.43, 2.90)	<	.001	2.63	(2.41, 2.88)	<	.001

Abbreviations: CKD, chronic kidney disease; HR, hazard ratio.
The low, moderate, and high level of $PM_{2.5}$ was ≤22.40, 22.40–26.01, and >26.01 μg/m^3, respectively.
The inactive, moderate, and high volume of exercise was 0, 0–8.75, and >8.75 MET-h, respectively.
Crude Model: without adjustment; Model 1: adjusted for age, gender, education, season, baseline calendar year, smoking status, alcohol drinking, occupational exposure, and physical labor at work; Model 2 further adjusted for BMI, diabetes, hypertension, dyslipidemia, self-reported cardiovascular disease, self-reported cancer, baseline eGFR, and urinary total protein.
[a] Further adjusted for $PM_{2.5}$ (for the association between exercise and eGFR decline ≥30%/CKD development) or exercise (for the association between $PM_{2.5}$ and eGFR decline ≥30%/CKD development).

development even at high level of $PM_{2.5}$, whereas ambient $PM_{2.5}$ was associated with higher risk of decreased renal function and CKD development at all levels of habitual exercise. We present a novel finding of no significant interaction between ambient $PM_{2.5}$ and habitual exercise, which suggests that the level of ambient air pollution did not significantly modify beneficial effects of exercise on renal function exacerbation and CKD development.

We found that regular exercise was associated with lower risk of renal function decline and CKD development, which corroborates existing evidence (Guo et al., 2020b; Robinson-Cohen et al., 2009, 2014). Exercise brings benefits on cardiovascular health via improving cardiovascular endothelial function, insulin sensitivity, lipidic dysmetabolism, and anti-inflammation, while reducing plasma viscosity and insulin resistance (Evensen et al., 2018; Linke et al., 2008; Schauer et al., 2020; Wang et al., 2017). Because there is a close relationship between CKD and cardiovascular diseases, where the disease of one organ leads to the abnormality of the other (Di Lullo et al., 2015; Subbiah et al., 2016), similar biological mechanisms are conceivable to be extended to renal vasculature.

Our findings of adverse effects of long-term exposure to ambient $PM_{2.5}$ on renal function decline and incident CKD are in agreement with previous cohort studies (Bowe et al., 2017, 2018; Chan et al., 2018; Mehta et al., 2016). Inflammation and oxidative stress are hypothesized as principal biological mechanisms to explain the positive association between air pollution and kidney diseases (Webster et al., 2017), which have been demonstrated in prior studies (Sørensen et al., 2003; Zhang et al., 2017). However, the HR for the association of ambient $PM_{2.5}$ with incident CKD in this study was greater than the one reported in our previous study (2.66 vs. 1.06) (Chan et al., 2018). It is possible that previous study did not consider the city-level random effects (Beelen et al., 2014) and did not use time-dependent covariates in the cox models (Bellera et al., 2010). The $PM_{2.5}$ concentration increased before 2005 and declined since then (Fig. 1). Another previous cohort study also showed that risk of $PM_{2.5}$ on incidence of CKD increased when using time-varying exposure in analysis compared with using baseline exposure (Bowe et al., 2018).

To our knowledge, current evidence exploring combined effects of air pollution and habitual exercise on kidney diseases are limited. Our study, showing that healthy benefits of a higher level of habitual exercise with respect to renal function and CKD in Asian adults were not statistically significantly moderated by higher levels of chronic $PM_{2.5}$ exposure, are novel. Some previous studies have investigated the effect modification of the association between exercise and other diseases by different levels of air pollution. Studies from Danish Diet, Cancer, and

Table 3
Subgroup analyses stratified by habitual exercise or $PM_{2.5}$ categories in Taiwanese adults.

eGFR decline ≥30%									
Stratified by $PM_{2.5}$	Low-$PM_{2.5}$			Moderate-$PM_{2.5}$			High-$PM_{2.5}$		
	HR (95% CI)		*p*	HR (95% CI)		*p*	HR (95% CI)		*p*
Habitual Exercise									
Moderate	0.87	(0.77, 0.99)	.03	0.89	(0.79, 1.00)	.05	0.89	(0.79, 1.01)	.08
High	0.75	(0.66, 0.85)	<.001	0.71	(0.62, 0.80)	<.001	0.65	(0.57, 0.74)	<.001
Trend test	0.87	(0.82, 0.92)	<.001	0.84	(0.79, 0.90)	<.001	0.81	(0.76, 0.87)	<.001
Stratified by exercise	Inactive-exercise			Moderate-exercise			High-exercise		
	HR (95% CI)		*p*	HR (95% CI)		*p*	HR (95% CI)		*p*
$PM_{2.5}$									
Moderate	1.49	(1.32, 1.69)	<.001	1.51	(1.33, 1.72)	<.001	1.55	(1.36, 1.76)	<.001
High	2.57	(2.18, 3.03)	<.001	2.81	(2.34, 3.37)	<.001	2.58	(2.13, 3.14)	<.001
Trend test	1.58	(1.46, 1.71)	<.001	1.63	(1.49, 1.78)	<.001	1.59	(1.45, 1.74)	<.001
Per 10 μg/m^3	3.02	(2.57, 3.55)	<.001	2.67	(2.26, 3.16)	<.001	3.36	(2.86, 3.94)	<.001
CKD development									
Stratified by $PM_{2.5}$	Low-$PM_{2.5}$			Moderate-$PM_{2.5}$			High-$PM_{2.5}$		
	HR (95% CI)		*p*	HR (95% CI)		*p*	HR (95% CI)		*p*
Habitual exercise									
Moderate	0.96	(0.83, 1.10)	.55	0.86	(0.75, 0.98)	.03	1.00	(0.87, 1.13)	.94
High	0.88	(0.78, 1.01)	0.07	0.76	(0.67, 0.87)	<.001	0.76	(0.67, 0.87)	.001
\	0.94	(0.88, 1.00)	0.05	0.87	(0.82, 0.93)	<.001	0.87	(0.81, 0.92)	<.001
Stratified by exercise	Inactive-exercise			Moderate-exercise			High-exercise		
	HR (95% CI)		*p*	HR (95% CI)		*p*	HR (95% CI)		*p*
$PM_{2.5}$									
Moderate	1.47	(1.26, 1.70)	<.001	1.28	(1.12, 1.47)	<.001	1.37	(1.23, 1.53)	<.001
High	1.87	(1.54, 2.27)	<.001	2.03	(1.70, 2.42)	<.001	1.96	(1.68, 2.29)	<.001
Trend test	1.39	(1.26, 1.53)	<.001	1.39	(1.27, 1.51)	<.001	1.39	(1.29, 1.50)	<.001
Per 10 μg/m^3	1.88	(1.60, 2.22)	<.001	2.44	(2.08, 2.85)	<.001	2.83	(2.48, 3.23)	<.001

Abbreviations: CKD, chronic kidney disease; HR, hazard ratio.
The low, moderate, and high level of $PM_{2.5}$ was ≤22.40, 22.40–26.01, and >26.01 μg/m^3, respectively.
The inactive, moderate, and high volume of exercise was 0, 0–8.75, and >8.75 MET-h, respectively.
All results were fully adjusted for age, gender, education, season, baseline calendar year, smoking status, alcohol drinking, occupational exposure, physical labour at work, BMI, diabetes, hypertension, dyslipidemia, self-reported cardiovascular disease, self-reported cancer, baseline eGFR, and urinary total protein.

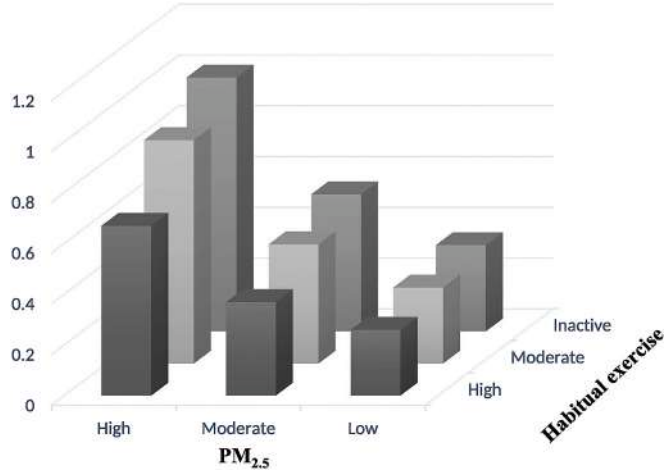

Fig. 2. Combined effects of habitual exercise and $PM_{2.5}$ on eGFR decline ≥30% among adults in Taiwan.
The low, moderate, and high level of $PM_{2.5}$ was ≤22.40, 22.40–26.01 and > 26.01 μg/m^3, respectively. The inactive, moderate, and high volume of exercise was 0, 0–8.75 and > 8.75 MET-h, respectively. All results were fully adjusted for age, gender, education, season, baseline calendar year, smoking status, alcohol drinking, occupational exposure, physical labor at work, BMI, diabetes, hypertension, dyslipidemia, self-reported cardiovascular disease, self-reported cancer, baseline eGFR and urinary total protein. Participants were classified into nine groups according to $PM_{2.5}$ and exercise categories with inactive-exercise exposed to the High-$PM_{2.5}$ comprising the reference group. Chart's data source (Online Resource 8).

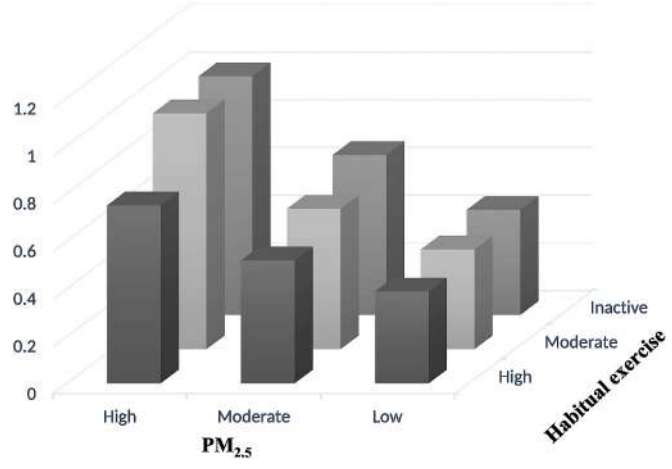

Fig. 3. Combined effects of habitual exercise and $PM_{2.5}$ on incident CKD among adults in Taiwan.
Abbreviations: CKD, chronic kidney disease. The low, moderate and high level of $PM_{2.5}$ was ≤22.40, 22.40–26.01 and > 26.01 μg/m^3, respectively. The inactive, moderate and high volume of exercise was 0, 0–8.75 and > 8.75 MET-h, respectively. All results were fully adjusted for age, gender, education, season, baseline calendar year, smoking status, alcohol drinking, occupational exposure, physical labor at work, BMI, diabetes, hypertension, dyslipidemia, self-reported cardiovascular disease, self-reported cancer, baseline eGFR and urinary total protein. Participants were classified into nine groups according to $PM_{2.5}$ and exercise categories with inactive-exercise exposed to the high-$PM_{2.5}$ comprising the reference group. Chart's data source (Online Resource 9).

Health cohort have also observed no statistically significant interaction between exercise and air pollution on incident asthma/COPD hospitalizations, myocardial infarction and all-cause mortality except for respiratory mortality (Andersen et al., 2015; Fisher et al., 2016; Kubesch et al., 2018) and suggested that participation in exercise may reduce but not reversing the benefits of exercise on respiratory mortality. A cohort study done in Hong Kong elderly reported no interaction effects of exercise and chronic exposure to $PM_{2.5}$ on respiratory mortality while it found some evidence of reducing cardiovascular benefits of walking slowly in higher polluted areas (Sun et al., 2020). Another study including 3,535 children reported that participating in sports was associated with increasing risk of asthma in areas with high level of ozone, but not in those with low-level exposure (McConnell et al., 2002). Our results may not be directly comparable with mentioned studies because of younger or older aged groups and different health outcomes and levels of air pollution in these studies.

The possible reason is that the excess inhaled air pollution results from exercise is only a small fraction of the total inhaled air pollution (Rojas-Rueda et al., 2011). Another potential reason is that acute harmful effects caused by excess dose of inhaled air pollution during sports do not outweigh long-term beneficial health effects of regular exercise (Andersen et al., 2015). In addition, no significant interaction between habitual exercise and $PM_{2.5}$ exposure on systemic inflammation was also reported in our previous study (Zhang et al., 2018b), which indirectly support our findings because systemic inflammation is one of the major pathway of the exercise/PM-renal function association.

Although the measurement of exercise and $PM_{2.5}$ were not directly comparable (i.e., exercise was measured in MET-h and $PM_{2.5}$ in $\mu g/m^3$), our results based on tertile categories indicate that the numerical values of the HRs for the associations with $PM_{2.5}$ were larger than those with exercise. Table 2 shows that each categorical increment in $PM_{2.5}$ was associated with a 65% higher risk of renal function reduction and a 44% higher risk of developing CKD, respectively, whereas each categorical increment in exercise was associated with a 16% lower risk of renal function reduction and a 11% lower risk of developing CKD, respectively. Similar patterns were observed in Table 3 (stratified analysis) and Figs. 2 and 3 (combinations of different exercise and $PM_{2.5}$ categories). Further studies are warranted to investigate whether air pollution mitigation is more effective in kidney health improvement compare with habitual exercise.

This study has several important strengths. Firstly, the large prospective cohort design enabled us to obtain stable and precise estimates. The large sample size also enabled us to conduct a series of subgroup and sensitivity analyses to clarify outcomes' associations with chronic $PM_{2.5}$ exposure and habitual exercise. Second, repeated medical examinations allowed us to use a certain drop in renal function (i.e. eGFR decline $\geq$30%) as one of our health outcomes. This is more meaningful in clinical practice, as a certain drop in eGFR are closely associated with the risk of ESRD and mortality (Coresh et al., 2014). Finally, we used validated spatiotemporal models based on satellite-data for assessment of $PM_{2.5}$. This enabled us to overcome the spatial coverage problems that occur by using data from monitoring stations. This approach also enabled us to monitor the changes of $PM_{2.5}$ exposure over time.

Several limitations in our study should be noted. First, information was not available on whether participants did exercise outdoors or indoors, we could not solely investigate the effects of outdoor exercise. Nevertheless, outdoor exercise was the major mode of exercise for Taiwanese. A national survey shows that around 80% of residents chose outdoor exercise as their most frequent exercise from 2005 to 2016 ("Sports Administration: Report of Active Cities," 2016). Second, instead of direct-measured exercise, information on habitual exercise was collected from a self-administered questionnaire which is commonly used in large-scale epidemiological studies. However, the validity and reliability of the questionnaire have been tested previously (Wen et al., 2008). Third, single measurement of eGFR was used to define incident CKD in this study. According to the clinical practice, diagnosis of CKD needs two separately measurements of eGFR <60 mL/min/1.73 m^2 with an interval of 90 days (KDIGO, 2013). Single measurement of eGFR <60 mL/min/1.73 m^2 indicates that patients might have CKD or acute kidney diseases (including acute kidney injury (AKI)). Fourth, we only evaluated the effects of $PM_{2.5}$ because of the lack of information on other air pollutants, like nitrogen dioxide and ozone (Bowe et al., 2017). However, the collinear issue between pollutants suggests we should analyze each pollutant separately. Finally, our study was conducted in a moderately polluted area. Further studies are warranted in more serious polluted regions to verify our findings.

5. Conclusion

In conclusion, we found that a high habitual exercise combined with a low chronic $PM_{2.5}$ exposure is associated with lower risk of renal function decline and CKD development, whereas a low level of habitual exercise combined a high chronic $PM_{2.5}$ exposure is associated with higher risk of renal function decline and CKD development. Habitual exercise reduces the risk of renal function decline and CKD development regardless of the levels of chronic $PM_{2.5}$ exposure. Chronic $PM_{2.5}$ exposure increased the risk of renal function decline and CKD development regardless of the levels of habitual exercise. Our study suggests that exercise is a safe approach for kidney health improvement for people residing in relatively polluted areas. Our study reinforces the importance of air pollution mitigation for kidney health.

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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.2021.113791.

Author contributions

Dr Lao conceived and designed the study. Drs Chang, Lau, and Lao acquired the data. Yiqian Zeng, Dr Guo, Dr Lin, Yacong Bo, and Dr Yu searched the literature. Yiqian Zeng and Dr Lao did data analysis and interpretation. Yiqian Zeng and Dr Lao drafted the first version of manuscript. All authors critically revised the manuscript. Dr Lao acquired the funding. Drs Chang, Lau, Tam, and Lao supervised this study.

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Comparison of lipid-normalised concentrations of persistent organic pollutants (POPs) between serum and adipose tissue

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ABSTRACT

Human biomonitoring of persistent organic pollutants (POPs) is typically based on serum analysis and for comparison and modelling purposes, data are often normalised to the lipid content of the serum. Such approach assumes a steady state of the compound between the serum lipids and for example lipid-rich adipose tissue. Few published data are available to assess the validity of this assumption. The aim of this study was to measure concentrations of POPs in both serum and adipose tissue samples from 32 volunteers and compare the lipid-normalised concentrations between serum and adipose tissue. For p,p'-DDE, PCB-138, PCB-153 and PCB-180, lipid-normalised adipose tissue concentrations were positively correlated to the respective serum concentrations but generally were more highly concentrated in adipose tissue. These results suggest that the investigated legacy POPs that were consistently found in paired samples may often not be in a steady state between the lipid compartments of the human body. Consequently, the analysis of serum lipids as a surrogate for adipose tissue exposure may more often than not underestimate total body burden of POPs. Further research is warranted to confirm the findings of this study.

1. Introduction

Persistent organic pollutants (POPs) include a range of lipophilic chemicals that are persistent and bioaccumulate in animal and human lipid-rich tissues and fluids (Jones and de Voogt 1999). Examples of POPs are dioxin-like and other polychlorinated biphenyls (PCBs), legacy organochlorine pesticides, and polybrominated diphenyl ethers (PBDEs) (Artacho-Cordón et al., 2015). Most of these POPs are associated with immunologic, teratogenic, reproductive, carcinogenic, and neurological effects, although specific exposure-response relationships vary (Kodavanti et al., 1998).

Despite widespread regulatory bans or use restrictions in most countries, POPs remain detectable in the environment (Syed et al., 2013). The primary route of external exposure for most POPs in the general population is via accumulation in the food chain, including breast milk (Artacho-Cordón et al., 2015). After absorption into the bloodstream, POPs are distributed throughout the body (Lee et al., 2017). Due to their lipophilic character, POPs prefentially partition into lipid-rich tissues and adipose tissue has accordingly been identified as a major storage compartment for these compounds (Patterson et al., 1986).

Biomonitoring for these lipophilic POPs to characterize human body burdens has been conducted using a variety of matrices including adipose tissue samples, blood serum or plasma, and human milk. Due to relative ease of collection, its less invasive nature and/or availability, blood serum or plasma has been preferred to adipose tissue or human milk (Pauwels et al., 2000; Ryan and Mills 1997).

Typically, it is assumed that POPs are in a steady state between the lipid compartments of the human body, such as blood and adipose tissue (Lee et al., 2017; Pauwels et al., 2000; Phillips et al., 1989). Patterson et al. (1988) investigated use of serum lipid as a surrogate for lipid-adjusted concentrations in adipose tissue for measurement of 2,3,7,8-tetrachlorodibenzo-p-dioxin and found a ratio of approximately 1:1, supporting the idea that such highly lipophilic compounds are in a

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steady state in lipid stores throughout the body. For other POPs, such partitioning dynamics have not been clearly demonstrated (Pauwels et al., 2000). Yu et al. (2011) analysed various adipose tissues and serum for various POPs in small set of subjects and found that only <30% of participants had similar concentrations in the various matrices indicating a steady state while the remaining participants' adipose tissue and serum concentrations varied widely. Other work comparing POPs concentrations among human lipid-rich matrices, including serum versus breast milk (LaKind et al., 2009), suggests that the assumption of a consistent relationship between lipid-adjusted POPs concentrations among different matrices is likely too simplistic (Botella et al., 2004; Waliszewski et al., 2003).

While there is a body of work comparing concentrations of lipophilic POPs in different human lipid compartments (see for example Kanja et al., 1992; Stellman et al., 1998; and Pauwels et al., 2000 and more recently Yu et al., 2011), uncertainty remains whether lipid-normalised concentrations of POPs in serum accurately predict lipid-normalised concentrations in other lipid compartments. For example, the analysis of Mannetje et al. (2012) suggests that there is a relationship between increased molecular size and the ratio of concentration in serum versus breast milk which may indicate a kinetic effect.

The aim of this study is to compare lipid-adjusted concentrations between serum and adipose tissue in order to (1) add to the body of evidence assessing the assumption of a steady state of POPs between serum and adipose tissue and to (2) examine the use of serum lipids as a surrogate for body burden. We present the results of analysis of paired adipose tissue and serum samples collected from patients undergoing abdominal surgery.

2. Material and methods

2.1. Sample characteristics

We invited patients undergoing laparoscopic surgery over a four-month period in 2016 to participate in the study. Ethical approval was granted by the Wesley Hospital Ethics Committee (No. 2016.02.180). Participants that consented to participate in the study provided a sample from subcutaneous adipose fat tissue (<1 g) and a serum sample. Adipose tissue samples were obtained during surgery. Blood samples were collected on the same day as the adipose tissue samples. The surgical patients were all fasting for at least 6 h before the operation. Overall, 32 participants (14 females, 18 males) contributed samples. The mean age of all participants was 55 years (52 for females, 57 for males) with a range from 20 to 89 years.

2.2. Lipid extraction of adipose tissue

Between 0.05 and 0.10 g of each adipose tissue sample was accurately weighed (W1), before being homogenised with 1 g of hydromatrix (diatomaceous earth) with a pestle and mortar. The homogenised adipose tissue samples were spiked with internal standards (500 pg each of $^{13}C_{12}$-transChlordane, $^{13}C_{12}$-p,p'-DDE, $^{13}C_{12}$-PCB-81, $^{13}C_{12}$-PCB-123, $^{13}C_{12}$-PCB-167, $^{13}C_{12}$-PCB-189 and 250 pg each of $^{13}C_{12}$-BDE47, $^{13}C_{12}$-BDE99, $^{13}C_{12}$-BDE100, $^{13}C_{12}$-BDE153, $^{13}C_{12}$-BDE154 and $^{13}C_{12}$-BDE183) and transferred to a 100 mL pre-packed Accelerated Solvent Extraction (ASE) cell containing (from the bottom upwards): Two cellulose filters, 30 g anhydrous Na_2SO_4, 10 g hydromatrix. ASE cells were loaded onto an ASE 350 (Thermo Fisher Scientific) and target compounds were extracted using hexane:DCM (3:2 v/v ratio) at 90 °C and 1500 psi (heating time 5 min, static time 5 min, 2 static cycles, rinse volume - 50%, purge time - 120 s). The extract was transferred to a pre-weighed tube (W2) and concentrated under a gentle stream of nitrogen at 40 °C until all traces of solvent were removed. The weight of the tube was recorded again (W3) for lipid determination before crude extracts were reconstituted in 5 mL hexane:DCM 3:2.

2.3. Sample clean-up

Serum samples underwent a combined extraction and clean up where samples (1–5 g) were weighed and spiked with internal standards. The serum samples and the in hexane:DCM 3:2 reconstituted adipose tissue lipid samples were added to the top of 100 mL pre-packed ASE cells containing (from the bottom upwards): two cellulose filters, 5 g silica gel, 2 g hydromatrix, cellulose filter, 12 g of acid impregnated silica (44% sulphuric acid), 5 g florisil and 20 g of hydromatrix. Serum samples were spiked with internal standards and extracted using hexane:DCM (3:2, v/v ratio) at 90 °C and 1500 psi (heating time 5 min, static time 5 min, 3 static cycles, rinse volume - 50%, purge time - 120 s). The extracts were concentrated using a gentle stream of nitrogen at 40 °C to near dryness and then reconstituted to a final volume of approximately 20 μL with *n*-nonane.

2.4. Instrumental analysis

The instrumental analysis methods have previously been described in-depth by He et al. (2018) and Wang et al. (2019). A-chlordane, g-chlordane, p,p'-DDE, PCB-52, PCB-118, PCB-138, PCB-153, PCB-180, BDE28, BDE47, BDE100, BDE99, BDE154, BDE153, BDE183 were determined in all samples using a TRACE GC Ultra equipped with a TriPlus Autosampler, coupled with a TSQ Quantum XLS triple quadrupole mass spectrometer. Separation was achieved using a DB-5MS column (30 m × 0.25 mm i.d.; 0.25 μm film thickness, J&W Scientific) with the following GC programme: initial temperature of 80 °C and held for 2 min, and increased to 180 °C at 20 °C/min, and held for 0.5 min, then increased to 300 °C at 10 °C/min, and held for 5 min. Helium was used as the carrier gas at constant flow rate of 1.0 mL/min. The volume injected was 1.0 μL, in splitless mode. The QqQ mass spectrometer was operated in electron ionization (EI) mode using the multiple reactions monitoring (MRM) mode with an emission current set at 20 μA.

2.5. Determination of lipid content in samples

The lipid content of adipose tissue was determined gravimetrically by applying the weights recorded in the lipid extraction from adipose tissue samples to the following equation:

$$Lipid\ Content\ (\%) = \frac{W3 - W2}{W1} \times 100$$

As blood samples were stored at −20 °C before being spun down to serum, they haemolysed and lipid content for each sample could not be measured serologically. As a result, total serum lipid content was estimated based on measurements of total cholesterol (CHOL) and triglyceride (TG) that were available for some individuals. CHOL and TG data was obtained from independent serology tests that were done by participants shortly before or after their hernia keyhole surgery in which adipose tissue samples were collected. For the lipid content estimation, we employed following formula put forward by Covaci et al. (2006):

$$TL(g/L) = 1.12 \times CHOL + 1.33 \times TG + 1.48$$

For 15 of 32 patients, cholesterol data was available and for 5 of those, there was available triglyceride data. For patients missing CHOL data, we used the mean of the 15 patients with available data (i.e., $\overline{X}_{CHOL} = 1.90\,g/L$) while for patients missing TG data, we used the average of the 5 with available TG data (i.e., $\overline{X}_{TG} = 1.50\,g/L$).

2.6. Quality assurance and quality control (QA/QC)

Laboratory blank samples of hydromatrix were prepared and analysed alongside adipose tissue samples (n = 4), matrix blank (bovine calf serum) samples were analysed alongside serum samples (n = 4). The blank samples were extracted and analysed in each batch of samples.

Method detection limits (MDLs) were defined as the average blank concentrations plus three times their standard deviations (SDs). When concentrations of an analyte were not detected in blank samples, a value of 5 pg/sample were used to calculate the MDL, as this was four times lower than the lowest calibration standard used. The MDLs for the individual chemicals in each experiment are listed in Table 1. Lipid-adjusted limits of quantification (LOQs) for each sample were calculated from:

$$LOQ(ng/g\ lipid) = \frac{0.001 \times MDL}{sample\ volume \times lipid\ content}$$

LOQs in Table 1 are expressed as a range as the MDL differed from sample to sample due to varying lipid contents. Accordingly, the samples with the minimum and maximum lipid content within each matrix correspond to the minimum and maximum LOQ for each chemical, respectively. Duplicate serum sample pairs (n = 5) were included in the analysis to assess the reproducibility of the analytical methods. For replicates A and B and with both replicates above the MDL ($n = 2$), the mean normalised difference (expressed in %) was calculated according to $[value(A - B)/(value(A + B)/2)] \times 100$. The average normalised difference ranged from 15 to 56% (see Table 1).

2.7. Statistical analysis

For the calculation of the geometric mean and determination of the median (see Table 2), non-detected sample concentrations were substituted as ½ the sample-specific limit of quantification (LOQ) when the detection frequency (DF) for that compound was >50%. DF cut-off values for geometric mean and median calculation were chosen at <60% and <50%, respectively. For the rank-order correlation analysis, the regression analysis toolkit included in Excel (2016) was used. A p-value of <0.05 served as the criteria for statistical significance while a p-value of <0.001 was interpreted as high statistical significance.

3. Results

3.1. Summary statistics and detection frequencies

POPs were detected in all serum and adipose tissue samples. An overview of the lipid-adjusted concentrations of detected POPs are presented in Table 2.

For organochlorine pesticides, we report high detection rates for p, p'-DDE at 97% in both adipose tissue and serum. In contrast, g-chlordane and a-chlordane could be detected in 78% and 75%, respectively, of all samples in adipose tissue while both were detectable in less than 15% of all serum samples. PCBs 138, 153, and 180 were detected consistently (i.e., in more than two thirds of all samples in both matrices) whereas PCBs 52 and 118 were less frequently detected and quantified. While all BDEs were detected in adipose tissue, with a frequency ranging from 50% to 100%, detection frequency of BDEs in serum was much lower. Only BDE47, BDE99, and BDE153 could be quantified in any of the serum samples. This limits the usefulness of BDE results when comparing data obtained for samples from adipose tissue with those from serum.

3.2. Correlations between concentrations of POPs in adipose tissue and serum

Rank-order (i.e., Spearman) correlations between quantifiable adipose tissue and serum concentrations were assessed for each analyte with at least 10 available paired measurements (see Table 2). Adipose tissue and serum concentrations of p,p'-DDE were strongly and positively correlated ($R_S = 0.82; p < 0.001$). For PCBs, we found moderate positive rank-order correlations for PCBs 138 and 153, while PCB-180 concentrations were highly correlated between adipose and serum ($R_S = 0.75$, $p < 0.001$).

3.3. Ratios of lipid-adjusted adipose tissue and serum concentrations

Evaluation of the ratios of lipid-adjusted concentrations in adipose tissue and serum samples allows an analysis of the relative distribution among different lipid compartments. Fig. 1 illustrates the distribution of concentration ratios for p,p'-DDE, PCB-138, PCB-153 and PCB-180 based on the ratios calculated from paired samples for each individual (Table 3). We limited our overall ratio presentation to these compounds as they have the highest frequency of paired, quantifiable adipose tissue and serum concentrations. While the ranges of individual ratios encompass 1 for all four of these analytes, the overall picture does not

Table 1
Summary of minimum detection limit (MDL) for the adipose tissue and calf serum blanks and lipid-adjusted limit of quantification (LOQ) ranges, as well as serum duplicates. Blank cells indicate that the analyte was not above the MDL in any duplicate samples.

	Adipose Tissue		Serum		Serum
	4 Laboratory Blanks		4 Matrix Blanks		5 Duplicate Samples
	MDL	LOQ	MDL	LOQ	ND
	pg/sample	ng/g lipid	pg/sample	ng/g lipid	%
g-chlordane	5	0.01–0.13	31	1.2–6.5	
a-chlordane	9.4	0.02–0.25	8.1	0.32–1.7	
p,p'-DDE	960	1.5–26	24	0.95–5.0	21[d]
PCB-52	81	0.13–2.1	5	0.20–1.1	
PCB-118	140	0.22–3.7	5	0.20–1.1	
PCB-138	150	0.23–3.9	27	1.1–5.6	15[b]
PCB-153	100	0.16–2.7	30	1.2–6.3	47[c]
PCB-180	74	0.12–2.0	5	0.20–1.1	56[b]
BDE28	6.8	0.01–0.18	5	0.20–1.1	
BDE47	69	0.11–1.8	5	0.20–1.1	
BDE100	5	0.01–0.13	5	0.20–1.1	
BDE99	26	0.04–0.70	55	2.2–12	
BDE154	5	0.01–0.13	5.6	0.22–1.2	
BDE153	5	0.01–0.13	5	0.20–1.1	44[a]
BDE183	5	0.01–0.13	5	0.20–1.1	

[a] One set of duplicate samples was above MDL for the analyte.
[b] Two sets of duplicate samples were above MDL for the analyte.
[c] Three sets of duplicates were above MDL for the analyte.
[d] Four sets of duplicate samples were above MDL for the analyte.

Table 2
Geometric mean (GM), standard deviation (SD) and median in parentheses, range and percentage of samples above LOQ (%>LOQ) for POPs concentrations (ng/g lipid) within adipose tissue and serum samples of 32 individuals, as well as Spearman (R_S) correlation coefficients between both matrices for statistically significant correlations.

	Adipose Tissue			Serum			Correlation
	GM (SD) [median]	Range	%>LOQ	GM (SD) [median]	Range	%>LOQ	R_S
g-chlordane	0.16 (0.69) [0.16]	<LOQ-3.5	78	NC	<LOQ-10	9	NA
a-chlordane	0.26 (0.82) [0.21]	<LOQ-3.7	75	NC	<LOQ-11	13	NA
p,p'-DDE	190 (810) [180]	<LOQ-3900	97	96 (340) [85]	<LOQ-1500	97	0.82
PCB-52	NC	<LOQ-4.5	13	NC	<LOQ-13	6	NA
PCB-118	NC (34) [1.7]	<LOQ-190	53	NC	<LOQ-7.3	9	NA
PCB-138	12 (290) [13]	<LOQ-1700	94	4.2 (7.7) [4.9]	<LOQ-33	75	0.61
PCB-153	16 (390) [17]	<LOQ-2200	94	6.0 (11) [6.3]	<LOQ-51	88	0.57
PCB-180	13 (280) [15]	<LOQ-1600	94	3.0 (9.2) [4.8]	<LOQ-39	69	0.75
BDE28	NC (0.44) [0.08]	<LOQ-0.88	50	NC	–	0	NA
BDE47	1.9 (3.1) [2.2]	<LOQ-16	78	NC	<LOQ-11	22	NA
BDE100	0.56 (0.71) [0.55]	<LOQ-3.4	97	NC	–	0	NA
BDE99	0.71 (3.0) [0.76]	<LOQ-17	81	NC	<LOQ-9.6	6	NA
BDE154	NC (0.21) [0.06]	<LOQ-0.96	50	NC	–	0	NA
BDE153	4.4 (11) [4.4]	1.0–64	100	NC	<LOQ-6.6	13	NA
BDE183	NC (0.53) [0.08]	<LOQ-2.8	56	NC	–	0	NA

NC: Not calculated due to low rate of detections. NA: Not assessed due to limited number of paired samples with detected concentrations. NS: Not significant.

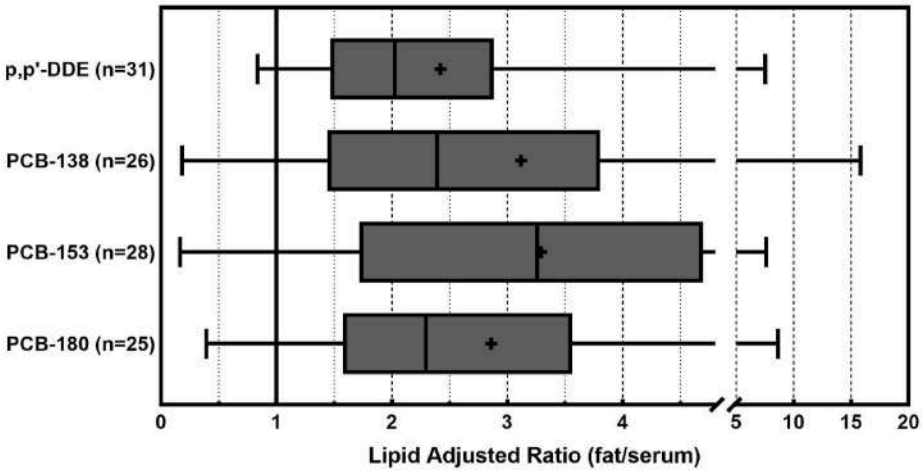

Fig. 1. Ratios of adipose tissue to serum concentrations for selected frequently-detected POPs. Boxes extend from the median to the 25th and 75th percentiles. Whiskers extend to the minimum and maximum. The arithmetic mean is represented by crosses. The black line at X = 1 represents perfect steady state between adipose tissue and serum concentrations.

support a generic assumption that these compounds are in a steady state between lipid stores in the body.

The majority of paired samples for p,p'-DDE and PCB congeners 138, 153 and 180 showed adipose tissue lipid-adjusted concentrations more than two-fold higher than in serum lipid. Furthermore, adipose:serum concentration ratios for the four analytes showed no significant correlation with age ($p > 0.3$, data not shown). It should be noted that Fig. 1 excludes one patient for better graphical representation that had extraordinarily high adipose:serum ratios, ranging from 31 for p,p'-DDE, 42 for PCB-180, 60 for PCB-153 to 63 for PCB-138. Minima for p,p'-DDE and PCB-180 stem from one individual while the minima for PCB-138 and PCB-153 come from another.

3.4. Individual data

As only the four analytes p,p'-DDE and PCB congeners 138, 153 and 180 were detected frequently in both matrices, this section will focus these four analytes. Given the limitations of serum lipid analysis, data for the group of individuals for which serum lipid was estimated from both CHOL and TG measurements rather than from averages are highlighted where appropriate.

Our data show that adipose:serum ratios for different legacy POPs were relatively consistent for a given individual, with relatively low standard deviation (see Table 3). Moreover, we observed very strong Spearman correlations ($R_S = 0.99$) among the individual adipose:serum distribution ratios across the four compounds, each with high statistical significance ($p < 0.001$), indicating that an adipose:serum distribution ratio for one of the four legacy POPS is indicative of the adipose:serum ratios of the other compounds.

4. Discussion

By analysing a range of legacy and emerging POPs in 32 paired serum and subcutaneous adipose tissue samples, this study aims to investigate the assumption that POPs are in a steady state between different human lipid compartments and whether lipid-normalised serum concentrations of POPS can reliably be used as a surrogate for body burden.

In this study, p,p'-DDE and PCB congeners 138, 153 and 180 were detected most frequently and lipid-adjusted adipose tissue and serum concentrations of those four compounds were positively correlated (see Table 2). Furthermore, our data showed that lipid-normalised adipose:serum concentration ratios for different POPs for a given individual were

Table 3
Lipid-normalised adipose:serum concentration ratios for all participants for the legacy POPs p,p'-DDE and PCBs 138, 153, together with the age and sex of the individual. The five individuals with the serum lipid estimation from CHOL and TG measurements as well as the ten individuals with serum lipid estimation from CHOL measurement and TG average are highlighted distinctly from the 17 individuals for which no serum lipid data was available. Furthermore, arithmetic mean and coefficient of variation (CV), calculated as standard deviation divided by mean and displayed as percentage, are provided.

Sample information			Serum lipid data[a]		Adipose:serum ratio				Statistics	
Patient	Age	Sex	CHOL	TG	p,p'-DDE	PCB-138	PCB-153	PCB-180	Mean	CV
Patients with both CHOL and TG measurements										
FAB_002	63	F	2.3	1.5	1.6	NA	2.2	1.3	1.7	27
FAB_008	51	M	2.4	1.4	1.8	2.3	2.4	3.5	2.5	30
FAB_010	58	F	2.7	1.5	1.5	0.18	0.16	1.1	0.8	91
FAB_012	57	M	2.4	0.5	2.0	2.1	2.0	2.2	2.1	5.5
FAB_032	74	M	1.3	2.0	2.6	4.0	3.4	2.4	3.1	24
Patients with CHOL measurement and imputed average TG										
FAB_001	82	F	2.1	NM	3.2	4.4	5.0	4.2	4.2	18
FAB_005	63	F	1.6	NM	1.5	2.3	2.7	2.2	2.2	24
FAB_009	32	F	2.0	NM	2.1	1.4	NA	NA	1.8	27
FAB_014	71	M	1.7	NM	3.2	NA	6.6	5.4	5.1	34
FAB_018	60	M	1.8	NM	2.1	4.0	2.9	1.9	2.7	35
FAB_023	41	F	2.0	NM	NA	0.78	0.68	1.1	0.85	26
FAB_025	37	M	1.4	NM	1.6	1.1	1.7	1.5	1.5	17
FAB_026	74	F	1.9	NM	2.8	3.4	3.5	2.4	3.0	17
FAB_028	42	F	1.8	NM	0.84	0.61	0.98	0.39	0.70	37
FAB_031	40	M	1.6	NM	3.1	2.3	2.2	NA	2.5	21
Patients with both CHOL and TG imputed with average values										
FAB_003	89	M	NM	NM	2.5	2.8	3.5	2.0	2.7	23
FAB_004	63	M	NM	NM	1.9	3.1	4.0	3.0	3.0	29
FAB_006	50	F	NM	NM	1.3	3.3	3.3	5.5	3.3	50
FAB_007	68	M	NM	NM	2.3	3.6	3.6	3.3	3.2	19
FAB_011	59	F	NM	NM	3.9	NA	6.0	3.3	4.4	32
FAB_013	33	F	NM	NM	2.0	2.4	4.4	3.6	3.1	36
FAB_015	55	M	NM	NM	2.4	2.5	5.6	NA	3.5	53
FAB_016	59	F	NM	NM	1.0	1.7	1.4	1.8	1.5	23
FAB_017	79	M	NM	NM	3.8	4.6	4.7	5.3	4.6	13
FAB_019	70	M	NM	NM	31	63	61	42	49	32
FAB_020	49	M	NM	NM	7.2	16	7.6	8.7	9.8	41
FAB_021	41	F	NM	NM	1.4	7.5	6.4	2.1	4.4	70
FAB_022	35	M	NM	NM	1.5	1.5	1.5	NA	1.5	1.9
FAB_024	20	M	NM	NM	1.4	NA	NA	NA	NC	NC
FAB_027	29	F	NM	NM	0.95	NA	NA	NA	NC	NC
FAB_029	60	M	NM	NM	1.5	0.46	0.54	0.78	0.81	56
FAB_030	40	M	NM	NM	7.5	NA	NA	NA	NC	NC

NM = no measurement available; imputed with average values from those with measurements (CHOL: 1.90 g/L; TG: 1.50 g/L).
NA = no ratio available due to a non-detect either in adipose tissue or serum.
NC = not calculated as only one adipose:serum ratio is available.
[a] Serum lipid data is presented in g/L.

relatively similar (see Table 3). These findings are in agreement with the broader literature (Artacho-Cordón et al., 2015; Pauwels et al., 2000; Whitcomb et al., 2005; Arrebola et al., 2012a; Mussalo-Rauhamaa 1991).

Between individuals, the median lipid-normalised adipose:serum ratios were greater than 2 for the same four legacy POPs (see Fig. 1). These results indicate that p,p'-DDE and PCB congeners 138, 153 and 180 are two times more concentrated in adipose tissue than in serum for the majority of participants. The findings from this study are generally consistent with previous observations in the literature, with several studies reporting ranges of lipid-normalised adipose:serum concentration ratios from 1 to 4 (Artacho-Cordón et al., 2015; Arrebola et al. 2012a, 2012b; Mussalo-Rauhamaa 1991; Whitcomb et al., 2005). Since a large part of the body lipids are associated with adipose tissue, an estimation of exposure based on lipid-normalised serum in the assumption of a steady state between serum and adipose tissue which is commonly used in exposure modelling (Czub and McLachlan 2004; Ritter et al., 2009; Quinn and Wania 2012) may result in an underestimation of the amount of POPs that have accumulated in a body and thus may also underestimate overall past exposure.

Notably, the range of lipid-normalised adipose:serum ratios we report spans over more than two orders of magnitude (see Fig. 1). This substantial variance of concentration ratios between individuals poses questions about what could affect such inter-individual differences. One partial explanation may lie in continuously decreasing human exposure to POPs that is demonstrated in temporal trend studies in Australia and globally (Mueller and Toms 2010; Croes et al., 2014; Stubleski et al., 2018; Hardell et al., 2010; Nøst et al., 2013; Zietz et al., 2008). As a result, higher adipose tissue concentrations may reflect stored POPs while low serum concentrations arise from low external exposure. However, more work is needed to fully understand the toxicokinetics of POPs in the human body.

The dataset presented here has several limitations. The number of participants (n = 32) was a reasonable sample size to assess patterns in analyte concentrations. However, LOQs for many analytes in serum samples were quite high compared to the LOQs for adipose tissue samples, resulting in relatively fewer detected and quantified samples for serum than adipose. As a result, the number of samples available for assessing paired correlations and ratios were limited for most analytes. A further limitation to this study was the serum lipid content determination. Only for approximately 15% of participants, we could estimate serum lipid content based on measurements of both serum cholesterol and serum triglycerides with a formula retrieved from the literature (Covaci et al., 2006). For more than half of the participants for which no measurement of either serum cholesterol or serum triglycerides was available, average lipid content was ascribed to samples due to a lack of

sample-specific data on serum lipids. In the future, blood samples must not be frozen before being spun down to serum as this makes lipid content determination difficult. Moreover, the large age range of participants (20–89 years) may present a further limitation.

In conclusion, there has been a general assumption that measuring POPs in serum lipids is a good surrogate for concentrations in lipid compartments throughout the body. Our preliminary results show that lipid-normalised concentrations of legacy POPs such as p,p'-DDE and several PCBs are in some individuals more than a factor of 2, and in few individuals more than a factor of 4, higher in adipose tissue than in serum. Even though the findings from this study cannot fully rule out the possibility of a lipid-normalised serum concentration overestimating lipid-normalised adipose tissue levels, given the large variance of two orders of magnitude shown in Fig. 1, our dataset indicates that the lipid-normalised concentrations in serum for these POPS may more often than not underestimate the lipid-normalised concentrations in adipose tissue in individuals. As a result, serum measurements normalised to serum lipid content may underestimate the actual body burden of POPs in the population. Further research is warranted to confirm the findings of this study.

Declaration of competing interest

The authors declare that no known conflicts of interest have impacted the quality and validity of the conducted research.

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Concentrations of perfluoroalkyl substances in donor breast milk in Southern Spain and their potential determinants

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ABSTRACT

Background: Breast milk is considered to offer the best nutrition to infants; however, it may be a source of exposure to environmental chemicals such as perfluoroalkyl compounds (PFAS) for breastfeeding infants. PFAS are a complex group of synthetic chemicals whose high stability has led to their ubiquitous contamination of the environment.

Objective: To assess the concentrations and profiles of PFAS in breast milk from donors to a human milk bank and explore factors potentially related to this exposure.

Methods: Pooled milk samples were collected from 82 donors to the Human Milk Bank of the Virgen de las Nieves University Hospital (Granada, Spain). Ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) was applied to determine milk concentrations of 11 PFAS, including long-chain and short-chain compounds. A questionnaire was used to collect information on donors' socio-demographic characteristics, lifestyle, diet, and use of personal care products (PCPs). Factors related to individual and total PFAS concentrations were evaluated by multivariate regression analysis.

Results: PFAS were detected in 24–100% of breast milk samples. PFHpA was detected in 100% of samples, followed by PFOA (84%), PFNA (71%), PFHxA (66%), and PFTrDA (62%). Perfluorooctane sulfonate (PFOS) was detected in only 34% of donors. The median concentrations ranged from <0.66 ng/dL (perfluorohexane sulfonic acid [PFHxS]) to 19.39 ng/L (PFHpA). The median of the sum of PFAS concentrations was 87.67 ng/L and was higher for short-chain than long-chain PFAS. Factors most frequently associated with increased PFAS concentrations included intake of creatin animal food items and use of PCPs such as skin care and makeup products.

Conclusions: Several PFAS, including short-chain compounds, are detected in pooled donor milk samples. Breast milk may be an important pathway for the PFAS exposure of breastfed infants, including preterm infants in NICUs. Despite the reduced sample size, these data suggest that various lifestyle factors influence PFAS concentrations, highlighting the use of PCPs.

Abbreviations: PFCAs, Perfluoroalkyl carboxylic acids; PFSAs, Perfluoroalkane sulfonic acids; SC PFAS, Short-chain PFAS; LC PFAS, Long-chain PFAS; NICU, Neonatal intensive care unit; PCPs, Personal care products.

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1. Introduction

Breast milk is considered the best food for infants in general and for high-risk premature infants in particular, offering proper nutrition, immunological benefits, and growth-promoting components and reducing the risk of complications (American Academy of Pediatrics [AAP], 2012). When preterm infants cannot receive breast milk from their mothers, included those admitted to a neonatal intensive care unit (NICU), the World Health Organization (WHO) and AAP recommend the administration of pasteurized human milk from a milk bank rather than artificial infant formula (AAP, 2012; WHO United Nations Children's Fund [UNICEF], 2003). Donated breast milk delivers essential nutrients and therapeutic benefits to the preterm infant but also has the potential to transmit infectious diseases and transfer toxic chemicals from exposed mothers (Carroll, 2014; Lehmann et al., 2018). Consequently, the European Human Milk Banking Association (Weaver et al., 2019) and other international milk banks have established guidelines for donor selection to ensure the safety of the milk (Clifford et al., 2020). These take account of pathogenic microorganisms and certain toxic substances (*e.g.*, tobacco, alcohol, medications, caffeine, and drugs of abuse) but do not consider occupational or environmental exposure to hazardous chemicals.

Per- and polyfluoroalkyl substances (PFAS) are a group of thousands of synthetic chemicals that are widely used in commercial and industrial products. They serve as polymerization aids in the production of fluoropolymers, as surfactants in fire-fighting foams, as anti-mist agents in chromium plating, and as water and oil repellents in textiles, leather, food contact materials, and cosmetics. PFAS are also employed in the production of semiconductors, medical devices, plant protection products, biocides, feed additives, pharmaceuticals, and paints (Glüge et al., 2020). Hydrogen atoms are entirely or partially replaced by fluorine atoms in these aliphatic substances (Buck et al., 2011) and the bond between carbon and fluorine is extremely strong and stable; hence, PFAS are highly resistant to thermal, chemical, and biological degradation and can accumulate in living organisms and biomagnify in food webs (Pérez et al., 2013). The degree of bioaccumulation generally increases with greater length of perfluoroalkyl carbon chain, and the elimination kinetics are highly species-dependent, with humans showing the longest PFAS half-lives, reaching 8.5 years for perfluorohexane sulfonic acid (PFHxS) (Olsen et al., 2009). Over the past decade, exposure to certain PFAS has been associated with lipid and insulin dysregulation (Sinisalu et al., 2020; Sun et al., 2018), infertility (Bach et al., 2016), reduced fetal growth (Kashino et al., 2020), increased miscarriage risk (Liew et al., 2020), obesity (Braun, 2017), impaired cognitive development (Vuong et al., 2019), and altered thyroid (López-Espinosa et al., 2012; Preston et al., 2020) and immune (Abraham et al., 2020; Grandjean et al., 2012) functions. These associations are supported by animal studies indicating that some PFAS are endocrine and metabolic disruptors, immunotoxic, reproductively toxic, and/or carcinogenic (ATSDR, 2018; Fenton et al., 2020; Street et al., 2018).

The most widespread PFAS in the environment are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), long-chain PFAS that are frequently detected in sera from populations worldwide (Bartolomé et al., 2017; Calafat et al., 2007; Kannan et al., 2004; Lewis et al., 2015; Thépaut et al., 2021). Current regulations in the European Union (EU) and elsewhere mainly address PFOS and PFOA, which are listed under the Stockholm Convention on Persistent Organic Pollutants (POPs) (Regulation (EU) 2019/1021; UNEP, 2009) and have been phased out in the EU since 2008 (European Directive, 2006/112/EC). Restrictions are also in place or planned under EU chemical legislation for other PFAS, including short-chain compounds such as PFHxS and perfluorohexanoic acid (PFHxA) (ECHA, 2019). These are less bioaccumulative than long-chain PFAS but are equally persistent in the environment and may exert similar toxicity (Nian et al., 2020).

Food, drinking water, and the indoor environment are considered to be the principal sources of human exposure to PFAS (Cornelis et al., 2012; Haug et al., 2011), which also include cosmetics and all-weather textiles, among other products made from PFAS (EFSA, 2020; Schultes et al., 2018). Seafood, meat, and dairy products may be the major sources of dietary exposure, especially to PFOA and PFOS (Domingo and Nadal, 2017; Titlemier et al., 2007). Socio-demographic factors have also been related to a greater internal PFAS burden, including occupation, male sex, higher age, and low parity (Bartolomé et al., 2017; Colles et al., 2020; Guzman et al., 2016).

Several PFAS have been detected in umbilical cord blood, placenta, breast milk, and plasma samples from breastfed infants, indicating that placental transfer and breastfeeding are both potential routes of PFAS exposure (Abraham et al., 2020; Cariou et al., 2015; Lien et al., 2013; Vela-Soria et al., 2021). It has been reported that a substantial proportion of PFAS in the mother is transferred to the infant during breastfeeding, which may contribute to reduce maternal serum and breast milk concentrations over the lactation period (Bartolomé et al., 2017; Macheka-Tendenguwo et al., 2018; Mondal et al., 2014; Thomsen et al., 2010). Identification of breastfeeding as an important pathway for the exposure to PFAS of breastfed infants (Haug et al., 2011) has been supported by findings of their wide presence in breast milk samples from mothers worldwide (Hu et al., 2021; Lee et al., 2018; Macheka--Tendenguwo et al., 2018).

Human milk banks provide milk for very premature, fragile, and sometimes medically compromised infants who are especially vulnerable to the effects of toxic chemicals. The present study is part of a wider project that aims to assess the potential adverse health impact on neonates in a NICU of exposure to endocrine-disrupting chemicals (EDCs) from their medical care, diet, and environment (Iribarne-Duran et al., 2019). The purpose of this study was to evaluate the concentrations and profiles of eleven long-and short-chain PFAS in milk samples from donors to a human milk bank and to explore factors that influence their concentrations.

2. Material and methods

2.1. Study population

Between 2015 and 2018, 82 donor mothers were recruited from the Regional Human Milk Bank of the Virgen de las Nieves University Hospital in Granada (Southern Spain). In general, donor women are registered at the milk bank after breastfeeding is well-established (*i.e.*, 2–3 weeks post-delivery). Exclusion criteria for donor milk selection include: positive serology for HIV, syphilis, or hepatitis B or C; risk factor for sexual transmitted disease (*e.g.,* unstable partner, non-utilization of condom, tattooing/piercing in previous three months, acupuncture, and blood transfusion); transplantation in previous 6 months; current smoking or drug habit; and high consumption of alcohol (>2 drinks/day or >20 g/day) or caffeine-containing drinks (>3 cups/day or >30 g/day). All local donors supplying the milk bank between 2015 and 2018 (n = 446) were invited to participate in the study and were fully informed of its nature and purpose. Donors who agreed to participate (18.4%) were asked to donate a milk sample for the analysis of environmental chemicals and to complete a structured questionnaire on socio-demographic and reproductive characteristics, lifestyle, diet, and use of personal care products (PCPs). Information on dietary habits and PCP use was available for a subsample of 77 donors. An informed consent form was signed by the donors before collecting personal information and biological samples. The research protocol was approved by the Biomedical Research Ethics Committee of Granada.

2.2. Milk sample collection

Participating donors were asked by the milk bank to collect mature milk over a minimum of 1 week and a maximum of 4 weeks by manual expression and/or breast pump and to keep them frozen (−20 °C) until delivery to the bank. On their arrival at the bank, samples were stored at

−30 °C without breaking the cold chain at any time. Before their pasteurization (done within 2 weeks), samples from each donor were thawed and pooled, obtaining an aliquot of 5–30 mL of the pooled milk. This was then stored at −20 °C until analysis at the "UNETE research unit" of the Centro de Investigación Biomédica (University of Granada). The day of pasteurization was recorded as the donation date. Hence, the interval between the start of milk collection by the mother and the donation date never exceeded 6 weeks.

2.3. Laboratory analysis

A modification of a validated ultra-high performance liquid chromatography-with tandem mass spectrometry method (Vela-Soria et al., 2020) was used (see Supplementary material) to determine the concentrations of eleven PFAS in pooled milk samples, including: seven long-chain PFAS, *i.e.*, six perfluoroalkyl carboxylic acids (PFCAs) with >7 perfluorinated carbons (PFOA, perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], perfluoroundecanoic acid [PFUnDA], perfluorododecanoic acid [PFDoDA] and perfluorotridecanoic acid [PFTrDA]), and one perfluoroalkane sulfonic acid (PFSA) with ≥6 perfluorinated carbons (PFOS); and four short-chain PFAS, *i.e.*, two PFCAs (PFHxA and perfluoroheptanoic acid [PFHpA]), and two PFSAs (perfluorobutane sulfonic acid [PFBS] and PFHxS) (Buck et al., 2011).

Milk aliquots used for the determination of PFAS had not been analyzed before. Quality control (QC) procedures included the use of blanks, low and high-concentration QC materials prepared from a fortified breast milk pool, analytical standards, and reagent and matrix blanks to ensure the accuracy and precision of the data. We also performed repeated measurements of breast milk QC pools, reflecting inter- and intraday variations. Relative standard deviation (%RSD) values were calculated as a measure of the precision of the method. Table S2 summarizes the mean accuracy and %RSD values obtained. The accuracy of the method was also verified by injecting QCs of different concentrations every 20 samples. Limits of detection (LD) ranged between 0.66 and 0.86 ng/L and limits of quantification (LQ) between 2.19 and 2.87 ng/L (Table S2).

2.4. Explanatory variables

The questionnaire administered by the milk bank to prospective milk donors and an *ad hoc* questionnaire were used to gather the following socio-demographic, reproductive, and lifestyle data: age (years), parity (multiparous or primiparous), lifetime duration of breastfeeding, either exclusive or mixed (<1, 1–10, or >10 months), birth weight and length and gestational age of the most recent newborn, schooling (university education or not), current occupation (unemployed, manual worker, or non-manual worker), area of residence (urban, sub-urban, or rural), smoking habit (ever smoked in the past or not), and current body mass index (BMI, kg/m^2) categorized as underweight/normal (<25 kg/m^2) or overweight/obese (≥25 kg/m^2). Women were also asked about their weight gain during the most recent pregnancy (kg) and weight change from before pregnancy (gain, loss, or no change). The number of days post-delivery was calculated as the difference between milk donation and birth dates. Dietary information was collected on the main origin of drinking water and the average consumption frequency (servings per day or week) in the previous 12 months of seafood, fish (oily and lean fish), dairy products (yoghurt, milk, butter, cheese), meat (red meat and cold meats), pulses, eggs, bread, chocolate, cereals, rice, pasta, fruit, vegetables (raw and cooked), fried food, canned food, coffee, and alcoholic beverages (Table 2). Data were also gathered on the frequency with which the women used sun screen, lip protector, face treatments (cream, tonic, milk), body lotion, hand cream, hair mask, makeup products (foundation, lipstick, eyeliner, and eye shadow), nail polish, hair dye, shampoo, shower cream, deodorant, hairspray/mousse/gel, perfume, toothpaste, and mouth wash and received manicure and pedicure treatments in the previous 12 months (Table 3).

Table 1
General characteristics of milk donors (n = 82).

Variables	n (%)	Median	Range
Age (years)		33	19–42
Year of sample collection			
2015	25 (30.5)		
2016	27 (32.9)		
2017	23 (28.0)		
2018	7 (8.5)		
Multiparous	37 (45.1)		
Lifetime duration of breastfeeding (months)			
<1	41 (50.0)		
1–10	22 (26.8)		
>10	19 (23.2)		
Time since delivery (days)		71	20–273
Length of gestation (weeks)		39	26–41
Birth weight (g)		3130	840-4500
Birth length (cm)		50	16–56
Current BMI (kg/m^2)		22.86	17.30–36.09
Overweight/obese	26 (33.8)		
Weight gain during pregnancy (kg)		12	1–36
Weight change from before pregnancy			
Weight loss	19 (22.1)		
Weight gain	39 (49.4)		
No weight change	24 (28.6)		
Area of residence			
Rural	26 (31.2)		
Sub-urban	24 (29.9)		
Urban	32 (39.0)		
Maternal university education	51 (66.2)		
Occupation			
Unemployed	6 (6.3)		
Manual worker	22 (26.3)		
Non-manual worker	54 (67.5)		
Ex-smoker	39 (47.6)		

BMI: Body mass index.

In addition, the protein content of unpasteurized pooled milk samples (g/100 mL) was determined as a potential explanatory variable, given evidence that perfluorinated compounds are mainly transported bound to human serum albumin (Luo et al., 2012) and their lactational transfer is produced by binding to milk protein (Fromme et al., 2010). The total lipid, lactose (g/100 mL), and caloric (kcal/100 mL) contents of samples were also measured as independent variables.

2.5. Statistical analysis

The detection frequency of PFAS in milk samples and 50th, 75th, and 95th percentiles of their concentrations were calculated, including the total concentration of all PFAS (∑PFAS), the most abundant PFAS commonly found in human blood samples (∑4 PFAS = [PFOA + PFOS + PFNA + PFHxS] and ∑5 PFAS = [∑4 PFAS + PFHpA]) (Cousins et al., 2020; EFSA, 2020), long-chain PFAS (∑LC PFAS), short-chain PFAS (∑SC PFAS), PFSAs (∑PFSAs), and PFCAs (∑PFCAs). Total concentrations were calculated as the sum of molar concentrations of the compounds based on molecular weight and were expressed as PFOA (∑PFAS, ∑4 PFAS, ∑5 PFAS, ∑LC PFAS, ∑PFCAs), PFOS (∑PFSAs), or PFHpA (∑SC PFAS). When PFAS were detected in at least 70% of samples, concentrations below the LD were assigned a value of LD/$\sqrt{2}$ and were treated as continuous variables, as were the sums of the different PFAS groups. PFAS detected in less than 70% of the milk samples were categorized as detected or non-detected (binary variables). Spearman's correlation test was used to assess relationships between PFAS concentrations (Fig. 1).

Multivariate regression analyses were performed with natural-logarithm-transformed continuous (linear regression) or binary (logistic regression) PFAS concentrations as dependent variables. A forward stepwise procedure was used to enter independent variables in the models. All variables described in section 2.4, and the year of sample collection (2015, 2016, 2017, or 2018), were tested as potential

Table 2
Food intake frequency of milk donors (n = 77).

Variables	n (%)	Variables	n (%)
Coffee intake = 1 cup/day	17 (20.7)	**Pulse**	
Alcohol intake ≥ 1 drink/ month	4 (4.9)	1 sv/week	13 (16.9)
Origin of drinking water		2 sv/week	29 (37.7)
Tap water	53 (68.8)	>2 sv/week	35 (45.5)
Bottled water	24 (31.2)	**Eggs**	
Seafood		1 sv/week	16 (20.8)
<1 sv/week	11 (14.3)	2 sv/week	28 (36.4)
1 sv/week	19 (24.7)	>2 sv/week	33 (42.9)
>1 sv/week	47 (57.3)	**Bread**	
Lean fish		<1 sv/day	15 (19.5)
<1 sv/week	18 (23.4)	1 sv/day	25 (32.5)
1 sv/week	37 (48.1)	>1 sv/day	37 (48.1)
>1 sv/week	22 (28.6)	**Chocolate**	
Oily fish		Never	10 (13.0)
<1 sv/week	29 (37.7)	<1 sv/day	44 (57.1)
1 sv/week	34 (44.2)	≥1 sv/day	23 (28.0)
>1 sv/week	14 (18.2)	**Cereals**	
Yoghurt		Never	27 (35.1)
<1 sv/day	31 (40.3)	<1 sv/day	35 (45.5)
≥1 sv/day	46 (59.7)	≥1 sv/day	15 (19.5)
Milk		**Rice**	
<1 glass/day	14 (18.2)	1 sv/week	66 (85.7)
≥1 glass/day	63 (81.8)	>1 sv/week	11 (14.3)
Cheese		**Pasta**	
Never/rarely	22 (28.6)	1 sv/week	66 (85.7)
>2 sv/week	34 (44.2)	>1 sv/week	11 (14.3)
≥1 sv/day	21 (27.3)	**Fruit**	
Butter		≤2 sv/week	12 (15.6)
Never	23 (29.9)	>2 sv/week	65 (84.4)
1 sv/week	36 (46.8)	**Raw vegetables**	
>1 sv/week	18 (23.4)	≤2 sv/week	15 (19.5)
Meat		>2 sv/week	62 (80.5)
≤1 sv/week	11 (14.3)	**Cooked vegetables**	
2 sv/week	13 (16.9)	≤2 sv/week	17 (22.1)
>2 sv/week	53 (68.8)	>2 sv/week	60 (77.9)
Cold meat		**Fried food**	
<2 sv/week	43 (55.8)	<1 sv/week	37 (48.1)
2 sv/week	34 (44.2)	1 sv/week	25 (32.5)
Red meat		>1 sv/week	15 (19.5)
Never	20 (26.0)	**Canned food (ever)**	62 (80.5)
<1 sv/week	33 (42.9)		
≥1 sv/week	34 (31.2)		
sv: serving			

Table 3
Use of personal care products among milk donors (n = 77).

Variables	**n (%)**	**Variables**	**n (%)**
Sunscreen (ever)	37 (48.1)	**Eye shadow**	
Sunscreen application		Rarely/never	49 (63.6)
None	40 (51.9)	<once a day	18 (23.4)
Face	26 (33.8)	≥once a day	10 (13.0)
Entire body	11 (14.3)	**Nail polish (traditional)**	
Sunscreen protection factor		Rarely/never	65 (84.4)
None	40 (51.9)	≥once a week	12 (15.6)
<50	12 (15.6)	**Acrylic nail polish**	
50	25 (32.5)	<once a month	70 (90.9)
Lip protector (ever)	30 (39.0)	once a month	7 (9.1)
Face cream		**Manicure**	
<once a day	24 (31.2)	<once a month	67 (87.0)
once a day	31 (40.3)	once a month	10 (13.0)
>once a day	22 (28.6)	**Pedicure**	
Face tonic		<once a month	66 (85.7)
Rarely/never	60 (77.9)	once a month	11 (14.3)
≥once a week	17 (22.1)	**Hair dye**	
Face milk		Never	36 (46.8)
Rarely/never	70 (90.9)	<once a month	23 (29.9)
≥once a week	7 (9.1)	once a month	18 (23.4)
Face treatment		**Shampoo**	
Never	59 (76.6)	<3 times/week	25 (32.5)
<once a month	12 (15.6)	≥3 times/week	52 (67.5)
Once a month	6 (7.8)	**Shower cream**	
Body lotion		<once a day	6 (7.8)
Rarely/never	28 (36.4)	≥once a day	71 (92.2)
<once a day	15 (19.5)	**Hairspray/mousse/gel**	
≥once a day	34 (44.2)	Rarely/never	58 (75.3)
Hand cream		≥once a day	19 (24.7)
<once a day	43 (55.8)	**Deodorant**	
once a day	19 (24.7)	<once a day	8 (10.4)
>once a day	15 (19.5)	once a day	52 (67.5)
Hair mask		>once a day	17 (22.1)
Rarely/never	38 (49.4)	**Perfume**	
≥once a week	39 (50.6)	Rarely/never	12 (15.6)
Foundation makeup		<once a day	24 (31.2)
Rarely/never	41 (53.2)	≥once a day	41 (53.2)
<once a day	19 (24.7)	**Toothpaste**	
≥once a day	17 (22.1)	≤once a day	17 (22.1)
Lipstick		>once a day	60 (77.9)
Rarely/never	39 (50.6)	**Mouthwash**	
<once a day	24 (31.2)	Rarely/never	47 (61.0)
≥once a day	14 (18.2)	<once a day	11 (14.3)
Eyeliner		≥once a day	18 (23.4)
Rarely/never	36 (46.8)		
<once a day	21 (27.3)		
≥once a day	20 (26.0)		

explanatory variables). Given the modest sample size, the p-value threshold of 0.10 was selected to retain explanatory variables in the model. Associations were expressed as exponentiated regression coefficients (exp [β]) or odds ratios (OR) with 95% confidence intervals (CI). The overall R-squared for each model was calculated to determine the percent variability in exposure explained by explanatory variables. R version 4.0.4 (SAS Institute Inc., Cary, NC, USA) was used for data analyses.

3. Results

General characteristics of the study participants are displayed in Table 1. Donors had a median age of 33 years and 45% were multiparous (33 mothers had 1 previous birth). Most of the milk samples were collected in 2015–2017, with only 8% being collected in 2018. The lifetime breastfeeding duration was <1 month for 50% and >10 months for 23%. The median interval between delivery and milk donation was 98 days (3.3 months), ranging from 20 days (<1 month) to 273 days (9 months). In their most recent pregnancy, the birth was preterm (<37 weeks) in 23% of deliveries and the infant had low birth weight (<2500 g) in 18%. Around one-third of donors were overweight or obese; 49% gained weight from before pregnancy and 22% lost weight. More than one-third of the donors resided in the metropolitan urban area of Granada, 66% had completed university education, 26% were manual workers, and 48% were ex-smokers. Most donors drank tap water and consumed >2 servings/week of meat and >1 serving/week of seafood (44% consumed >1 serving/week of oily fish) (Table 2). More than half of donors reported the daily use of face cream, hand cream, shower cream, deodorant, and toothpaste and the frequent use of shampoo (≥3 times/week) and perfume (≥once a day) (Table 3).

The median protein content of milk samples was 1.10 g/100 mL (range = 0.20–6.80 g/100 mL), their median fat content was 3.70 g/100 mL (range = 1.16–8.30 g/100 mL), median lactose content was 7.36 g/100 mL (range = 6.54–8.00 g/100 mL), and median energy content was 68 kcal/100 mL (range = 44–110 kcal/100 mL).

Table 4 shows that detection frequencies (DF) of PFAS ranged from 24.4 to 100%, with PFHpA being detected in all samples (median concentration = 19.39 ng/L), followed by PFOA (DF = 84.1%, median = 7.17 ng/L), PFNA (DF = 70.7%, median = 2.59 ng/L), PFHxA (DF = 65.9%, median = 1.58 ng/L), and PFTrDA (DF = 62.2%, median = 1.69 ng/L). Remaining compounds were detected in less than 40% of samples. The median sum of PFAS concentrations was 87.67 ng/L (range = 7.57–1899 ng/L) and was higher for short-chain than for long-chain PFAS (median = 52.69 [range = 2.74–1168] ng/L *vs.* 20.01 [range = 3.06–571.1] ng/L, respectively) and for PFCAs than for PFSAs (median = 74.97 [range = 5.65–1399] ng/L *vs.* 2.45 [range = 0.72–223.2] ng/L,

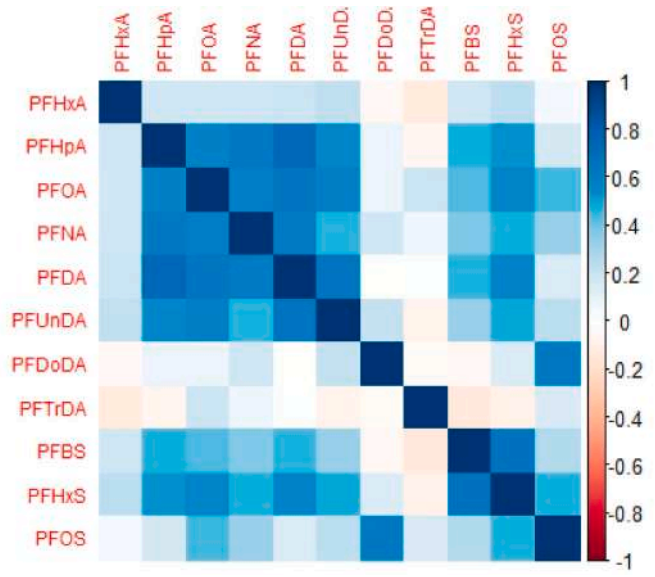

Fig. 1. Correlation heatmap for PFAS concentrations in breast milk.

respectively). At least seven PFAS compounds were detected in the breast milk of 24 donors (29%), 5–6 were detected in 38 (46%), and 2–4 in 20 (24%). Positive correlations were observed between all PFCA compounds except for PFDoDA and PFTrDA, while PFSA concentrations were positively correlated with PFHpA, PFOA, PFNA, and PFDA concentrations (Fig. 1).

Explanatory variables that were associated with PFAS concentrations are exhibited in Table 5 (linear regression models) and Tables S3–S10 (logistic regression models). The R-squared value of models ranged from 14% (∑LC PFAS) to 61% (PFDoDA). Milk samples collected in 2016 or 2017 had lower PFNA, PFDoDA, and ∑LC PFAS but higher PFHxA and ∑SC PFAS concentrations in comparisons to 2015. Multiparous donors had significantly higher concentrations of PFHpA in their milk, while lifetime duration of breastfeeding was associated with higher concentrations of PFOA, PFDA, ∑5 PFAS, ∑PFCAs, ∑LC PFAS, and ∑PFAS. Weight change from before pregnancy (gain or loss) was associated with higher PFDA, PFOS, ∑4 PFAS, and ∑5 PFAS concentrations, and residing in an urban area with higher PFHpA, ∑5 PFAS, and ∑PFAS concentrations.

With regard to food intake, red meat was associated with higher concentrations of ∑5 PFAS; oily fish, milk, and cold meat with higher PFHxA; yoghurt with higher PFDoDA and ∑PFSAs; cheese with higher PFTrDA; butter with higher PFBS; pulses with higher ∑4 PFAS; chocolate with higher PFHpA; and fried food with higher PFOS concentrations. The PCPs most frequently related to increased PFAS concentrations were hand cream, whose use was related to higher PFNA, PFDA, PFBS, PFHxS, PFOS, ∑4 PFAS, ∑SC PFAS, ∑PFCAs, and ∑PFAS; followed by face treatment, associated with higher PFHpA, PFNA, ∑5 PFAS, and ∑PFCAs; and lipstick use, associated with higher PFOA, PFUnDA and PFDoDA. Face cream and body lotion were associated with higher PFUnDA; foundation makeup with higher ∑4 PFAS; eyeliner with higher PFHxA and PFHxS; eye shadow with higher PFOA and PFDA; hair dye with higher PFOA and PFOS; shampoo with higher PFOA; hair mask with higher PFHxS; deodorant with higher ∑PFSAs; and perfume with higher ∑SC PFAS concentrations.

On the other hand, certain factors such as a higher intake of cheese, eggs, cereals, and fish, and a more frequent use of deodorants were associated with a decrease in the milk concentrations of some individual PFAS or total PFAS (Table 5 and Tables S3–S10).

4. Discussion

The concentrations of eleven PFAS were measured in milk samples from 82 donors to a human milk bank in Spain in 2015–2018. More than two-thirds of milk samples had detectable concentrations of PFHpA (100%), PFOA (84%), and PFNA (71%), and almost one-third showed the presence of at least seven PFAS; also, concentrations of short-chain PFAS were higher than of long-chain PFAS, especially in more recently collected samples. These results suggest that breast milk may be an important pathway of PFAS exposure for breastfed infants. Given that donated milk is used for premature newborns with low or very low birth weight (<1500 g) in NICUs, it appears crucial to monitor concentrations of environmental chemicals such as PFAS in human milk banks. Despite the small sample size, these findings suggest that PFAS concentrations in human milk are influenced by various lifestyle factors, such as intake of

Table 4
Concentrations (ng/L) of PFAS in donor breast milk (n = 82).

Compound		LOD	DF (%)	Median	P75	P95	Max.
PFHxA	Perfluorohexanoic acid	0.73	65.9	1.58	26.15	152.3	322.4
PFHpA	Perfluoroheptanoic acid	0.79	100	19.39	55.71	232.3	743.9
PFOA	Perfluorooctanoic acid	0.86	84.1	7.17	23.86	55.12	251.8
PFNA	Perfluorononanoic acid	0.69	70.7	2.59	10.69	25.48	136.5
PFDA	Perfluorodecanoic acid	0.72	24.4	<0.72	1.57	23.01	210.3
PFUnDA	Perfluoroundecanoic acid	0.74	39.0	<0.74	1.60	3.29	14.01
PFDoDA	Perfluorododecanoic acid	0.77	35.4	<0.77	1.66	1.66	131.8
PFTrDA	Perfluorotridecanoic acid	0.78	62.2	1.69	1.69	8.84	13.34
PFBS	Perfluorobutane sulfonic acid	0.80	35.4	<0.80	1.73	66.35	195.0
PFHxS	Perfluorohexane sulfonic acid	0.66	24.4	<0.66	0.74	16.01	45.45
PFOS	Perfluorooctane sulfonic acid	0.86	34.1	<0.86	6.26	26.01	64.75
Sum of PFAS		**P5**	**P25**	**Median**	**P75**	**P95**	**Max.**
∑4 PFAS[a]	PFOA + PFOS + PFNA + PFHxS[b]	<2.04	5.51	14.66	39.69	104.7	437.8
∑5 PFAS[a]	PFOA + PFOS + PFNA + PFHxS + PFHpA[c]	2.19	5.88	53.31	103.9	280.5	1284
∑LC PFAS[a]	PFOA + PFOS + PFNA + PFDA+ PFUnDA + PFDoDA + PFTrDA[d]	3.81	7.32	20.01	47.03	155.1	571.1
∑SC PFAS[a]	PFHpA + PFHxA + PFBS + PFHxS[e]	<2.74	13.75	52.69	125.1	398.4	1168
∑PFSA[a]	PFBS + PFHxS + PFOS	<0.72	<0.72	2.45	8.09	136.6	223.2
∑PFCA[a]	PFHxA + PFHpA + PFOA + PFNA+ PFDA + PFUnDA + PFDoDA + PFTrDA	7.11	30.16	74.97	141.8	450.1	1399
∑PFAS[a]	Sum of all 11 PFAS	11.46	44.58	87.67	208.2	475.4	1899

LOD: Limit of detection; DF: Detection frequency; P75, P95: 75th and 95th percentiles.
LC: long-chain PFAS; SC: short-chain PFAS; PFSAs: Perfluoroalkyl sulfonic acids; PFCAs: Perfluoroalkyl carboxylic acids.
[a] Weighted molar sum of PFAS concentrations (sum of molar concentrations of PFAS based on molecular weight; [b]Most abundant PFAS in human serum (EFSA, 2020); [c]Most abundant PFAS in human serum including PFHpA (Cousins et al., 2020); [d]Long-chain PFAS; [e]Short-chain PFAS.

Table 5
Significant explanatory variables for breast milk concentrations of most prevalent PFAS and summed concentrations of PFAS groups (n = 77).

Predictors	PFHpA	PFOA	PFNA	∑4PFAS	∑5PFAS	∑PFSAs	∑PFCAs	∑Long-chain PFAS	∑Short-chain PFAS	∑PFAS
Year of sample collection (ref: 2015)										
2016			**0.23 (0.10–0.53)**					**0.39 (0.21–0.73)**	**2.91 (1.36–6.17)**	
2017			0.65 (0.27–1.53)					0.64 (0.34–1.18)	**2.75 (1.22–6.22)**	
2018			0.32 (0.09–1.05)					0.55 (0.22–1.38)	0.73 (0.24–2.23)	
Multiparous *vs.* primiparous	2.12 (0.96–4.72)									
Total breastfeeding (ref: <1 month)										
1–10 months		**2.75 (1.31–5.79)**			**2.99 (1.72–5.21)**		**2.27 (1.26–4.11)**	**1.80 (1.01–3.19)**		**2.61 (1.49–4.96)**
>10 months		1.11 (0.51–2.42)			1.01 (0.54–1.89)		0.90 (0.48–1.67)	1.04 (0.58–1.89)		1.31 (0.75–2.29)
BMI (kg/m^2)	0.91 (0.82–1.01)									
Weight change from pre-conception										
Weight gain				**2.22 (1.02–4.81)**	1.66 (0.89–3.09)					
Weight loss				**3.03 (1.58–5.78)**	**1.71 (1.01–2.89)**					
Area of residence (ref: rural)										
Sub-urban	1.20 (0.44–3.30)				1.32 (0.72–2.42)				**0.48 (0.23–0.99)**	0.62 (0.34–1.12)
Urban	**4.04 (1.51–10.8)**				**3.08 (1.77–5.36)**				1.68 (0.80–3.55)	**1.82 (1.06–3.11)**
Ex-smoker									**0.44 (0.22–0.87)**	
Coffee intake: 1 *vs.* <1 cup/day				**0.44 (0.21–0.90)**						
Lean fish intake (ref: <1 sv/week)										
1 sv/week									**0.29 (0.13–0.63)**	**0.46 (0.26–0.84)**
>1 sv/week									0.48 (0.21–1.12)	0.64 (0.34–1.19)
Oily fish intake (ref: <1 sv/week)										
1 sv/week										**0.56 (0.34–0.93)**
>1 sv/week										0.99 (0.52–1.56)
Yoghurt intake: ≥1 *vs.* <1 sv/day						**2.09 (1.02–4.29)**				
Cheese intake (ref: rarely/never)										
>2 sv/week	**0.30 (0.11–0.76)**				**0.46 (0.26–0.81)**	0.46 (0.20–1.08)				**0.51 (0.29–0.86)**
≥1 sv/day	**0.32 (0.10–0.99)**				**0.51 (0.27–0.95)**	0.40 (0.15–1.05)				**0.50 (0.27–0.93)**
Red meat intake (ref: ≤1 sv/week)										
2 sv/week					0.94 (0.52–1.69)					
>2 sv/week					**1.85 (1.07–3.21)**					
Pulses intake (ref: 1 sv/week)										
2 sv/week				1.33 (0.60–2.93)						
>2 sv/week				**2.53 (1.17–5.45)**						

(*continued on next page*)

Table 5 (*continued*)

Predictors	PFHpA	PFOA	PFNA	∑4PFAS	∑5PFAS	∑PFSAs	∑PFCAs	∑Long-chain PFAS	∑Short-chain PFAS	∑PFAS
Egg intake (ref: 1 sv/week)										
2 s/week	**0.30 (0.10–0.92)**									
>2 sv/week	0.46 (0.15–1.38)									
Chocolate intake (ref: never)										
<1 sv/day	3.27 (0.93–11.5)									
≥1 sv/day	2.98 (0.74–12.0)									
Cereal intake (ref: never)										
<1 sv/day				**0.38 (0.21–0.70)**						**0.53 (0.32–0.89)**
≥1 sv/day				**0.36 (0.16–0.80)**						0.65 (0.35–1.22)
Face treatment (ref: never)										
<once a month	1.99 (0.98–2.38)		2.12 (0.85–5.26)		1.65 (0.89–3.06)		1.57 (0.78–3.14)			
once a month	**3.13 (1.26–7.80)**		**4.26 (1.26–14.42)**		**4.02 (1.71–9.47)**		**3.59 (1.38–9.36)**			
Body lotion use (ref: rarely/never)										
<once a day							0.95 (0.48–1.89)			
≥once a day							**0.53 (0.29–0.98)**			
Hand cream use (ref: <once a day)										
once a day			2.09 (0.95–4.61)	**2.05 (1.07–3.92)**			1.77 (0.95–3.29)		**2.03 (1.00–4.19)**	**2.07 (1.23–3.49)**
>once a day			1.46 (0.60–3.56)	1.74 (0.86–3.52)			1.62 (0.79–3.33)		1.30 (0.58–2.91)	1.20 (0.69–2.07)
Foundation makeup use (ref: never)										
<once a day				1.55 (0.80–2.97)						
≥once a day				1.96 (0.98–3.95)						
Lip protector use: ever *vs.* never				**0.46 (0.27–0.80)**						
Lipstick use (ref: rarely/never)										
<once a day		**3.42 (1.50–7.84)**								
≥once a day		0.47 (0.18–1.25)								
Eye shadow use (ref: rarely/never)										
<once a day		0.60 (0.25–1.44)								
≥once a day		**6.39 (2.32–17.6)**								
Hair dye use (ref: never)										
<once a month		1.03 (0.46–2.32)								
once a month		**2.24 (1.01–4.98)**								
Shampoo use: ≥ *vs.* <3 times/ week		**2.01 (1.01–4.00)**								

(*continued on next page*)

Table 5 (*continued*)

Predictors	PFHpA	PFOA	PFNA	∑4PFAS	∑5PFAS	∑PFSAs	∑PFCAs	∑Long-chain PFAS	∑Short-chain PFAS	∑PFAS
Shower cream use: ≥ *vs.* <once a day					**0.37 (0.16–0.89)**					
Deodorant use (ref: <once a day)										
once a day		**0.24 (0.10–0.65)**			**0.40 (0.19–0.86)**	1.17 (0.36–3.86)				
>once a day		0.85 (0.26–2.77)			0.81 (0.35–1.87)	**4.30 (1.13–16.3)**				
Perfume use (ref: rarely/never)										
<once a day									**3.35 (1.25–8.95)**	
≥once a day									1.81 (0.75–4.39)	
R^2	24%	35%	21%	26%	43%	16%	15%	14%	40%	34%

PFSAs: Perfluoroalkyl sulfonic acids; PFCAs: Perfluoroalkyl carboxylic acids.
Associations are reported as exponentiated regression coefficients (exp[β]) with 95% confidence intervals (CI).
Bold: p-value<0.05.

certain animal food items and the use of PCPs. Some of these factors were previously reported, as discussed below.

4.1. PFAS concentrations in milk

There has been increasing research into the presence of PFAS in human breast milk over the past decade (Hu et al., 2021; Macheka-Tendenguwo et al., 2018; Supplementary material, Table S11). Studies have indicated a wide variation in the geographical distribution of PFAS concentrations and profiles, revealing a global decline in the concentration of some PFAS congeners, especially in countries where their production and utilization have been restricted, such as the USA and Germany (Bjerregaard-Oelsen et al., 2016; Černá et al., 2020; Macheka-Tendenguwo et al., 2018). In this regard, the Stockholm Convention (UNEP, 2016) and EU regulations (Commission Regulations [EU] 2017/1000; 207/2011) have contributed to a gradual decline in levels of PFOS and PFOA. In the present study, PFAS concentrations in the donor milk samples collected in 2015–2018 were generally several times lower than in samples collected between 2012 and 2015 in hospitals or primary health care centers in other Spanish regions (Beser et al., 2019; Lorenzo et al., 2016; Motas Guzmán et al., 2016). In the most recent Spanish study by Beser et al. (2019), PFOA, PFOS, and PFNA concentrations in milk samples collected in 2015 were higher than in the present samples, while they did not detect any of the remaining nine PFAS analyzed (Table S11). Likewise, concentrations of PFOS, PFOA, PFNA, and PFHxS in breast milk samples gathered in Catalonia in 2007–2008 were higher in comparison to the present findings (Kärrman et al., 2010; Llorca et al., 2010), although the presence of other PFAS such as PFDA and PFUnDA was not detected (Kärrman et al., 2010). The lower concentrations of PFAS found in donor milk samples from Granada *versus* other Spanish regions may be attributable to the lower level of economic development in the South of Spain, as indicated by the results of a Spanish biomonitoring study of PFAS concentrations in serum samples from adults in 2009–2010 (Bartolomé et al., 2017).

PFAS concentrations in the present samples are generally comparable or in the lower range of those observed in breast milk from other countries, although the majority of previous studies only measured the most abundant PFAS, *i.e.,* PFOS, PFOA, PFNA, PFDA, and PFHxS (Macheka-Tendenguwo et al., 2018; Table S11). A recent Chinese study of the same eleven PFAS as in the present study reported a higher detection frequency of PFOA, PFOS, and PFDA but a much lower detection frequency of the remaining PFAS in breast milk samples (n = 174) than in the present samples (Jin et al., 2020); for instance, PFHpA was not detected in any sample but was found in all of the present samples. In the same way, Lee et al. (2018) reported higher concentrations of PFOA, PFOS, and PFHxS but lower concentrations of the remaining PFAS in milk from 293 Korean mothers in comparison to the present donors. Overall, the present results suggest a decline in breast milk concentrations of PFOS (detected in only one out of three donors), a continued exposure to PFOA, and widespread exposure to short-chain PFAS such as PFHpA and PFHxA, whose concentrations were higher than previously reported in breast milk (Macheka-Tendenguwo et al., 2018; Table S11).

Our findings are in line with studies indicating the predominance of short-chain *versus* long-chain PFAS in breast milk (Fujii et al., 2012; Kang et al., 2016; Kim et al., 2011; Lorenzo et al., 2016). Short-chain PFAS are more soluble and have a lower molecular weight, facilitating their passage through the mammary epithelial membrane and their contamination of breast milk. In addition, the widespread and growing use of alternative short-chain PFAS over the last years would have increased human exposure (Kang et al., 2016; Lorenzo et al., 2016). It has also been suggested that the transfer of sulphonates (PFSAs) to human milk is easier than that of carboxylates (PFCAs) (Roosens et al., 2010); however, the latter were more abundant than the former in the present study.

4.2. Determinants of PFAS concentrations in breast milk

PFAS concentrations were not associated with the age of milk donors. The relationship of breast milk PFAS with age is not clear, with some studies describing higher PFAS concentrations with increasing age (Lee et al., 2018) and others showing no such association (Antignac et al., 2013; Llorca et al., 2010; Motas Guzmán et al., 2016; Nyberg et al., 2018). The BMI of donors was not related to breast milk PFAS concentrations in the present study, except for a suggestive inverse association with PFHpA. Previous reports have been contradictory, showing both positive and negative associations (Berg et al., 2014; Brantsaeter et al., 2013; Cariou et al., 2015; Jensen et al., 2015; Lee et al., 2018; Lorenzo et al., 2016). On the other hand, weight change from before pre-conception appeared to influence the increase the concentrations of PFDA, PFOS, ∑4 PFAS, and ∑5 PFAS. This finding is not easy to explain, given that weight change may be an indicator of changes in diet or lifestyle that could lead to increased PFAS exposure, as previously suggested (Lee et al., 2018).

Lifetime breastfeeding was associated with higher PFDA, PFOA, ∑PFCAs, ∑long-chain PFAS, and total PFAS concentrations, while multiparous status was associated with lower PFBS but higher PFHpA concentrations. In contrast, various studies reported lower PFAS concentrations in the milk of multiparous mothers in comparison to those who were breastfeeding for the first time (Awad et al., 2020; Barbarossa et al., 2013; Croes et al., 2012; Motas Guzmán et al., 2016; Thomsen et al., 2010), suggesting a greater transfer (either placental or via breastfeeding) of PFAS to the first newborn. Thus, Thomsen et al. (2010) reported a reduction rate of 7.7 and 3.1% per month in breast milk concentrations for PFOA and PFOS, respectively, while Mondal et al. (2014) estimated that breastfeeding was associated with monthly decrease of 1–3% in maternal serum concentrations of PFOA, PFOS, PFHxS, and PFNA and of 1–8% in breast milk concentrations of PFOA and PFOS. However, is still not well established that breast milk concentrations of PFAS decrease over the lactation period. In line with our results, a Korean study found higher PFOS, PFOA, PFNA, and total PFAS concentrations in breast milk collected at 30 *versus* 6 days after the delivery, which were attributed to changes in the dietary and lifestyle patterns of mothers throughout the lactational period (Lee et al., 2018). It has also been proposed that the interval between pregnancies may have an impact on the body burden of PFAS, with a longer interval being associated with breast milk concentrations that may be as high as observed for the first breastfeeding episode (Whitworth et al., 2012). Nevertheless, the associations with breastfeeding duration observed in this study remain poorly understood.

Some previous studies observed higher PFAS concentrations in the breast milk of women residing in urban or semi-urban *versus* rural areas (Abdallah et al., 2020; Liu et al., 2010; Tao et al., 2008a, b). In the same line, urban donors in this study showed higher concentrations of PFHpA, ∑5 PFAS, and total PFAS concentrations, while their education and occupation did not appear to influence PFAS concentrations.

Dietary intake has been identified as a substantial source of PFAS exposure (Domingo and Nadal, 2017). The intake of fish and seafood has been associated with higher internal concentrations of PFAS in several studies (Berg et al., 2014; Rylander et al., 2010; Thépaut et al., 2021; Tyrrell et al., 2013), including reports of adult serum and breast milk samples (Bartolomé et al., 2017; Motas Guzmán et al., 2016). In addition, research on the presence of PFAS in food marketed in Spain found that fish and shellfish were the most contaminated groups, showing the highest concentrations of PFOS, PFOA, PFHpA, and PFHxS (Domingo et al., 2012a, b). However, no positive relationship was found between fish/seafood intake and PFAS concentrationss in the present study, although meat consumption was associated with increased total PFAS concentrations, consistent with observations of a higher PFAS content in foods of animal *versus* non-animal origin (Tittlemier et al., 2007). The intake of other food items did not show a clear trend towards an increase in PFAS exposure. Notably, the consumption of fried food was associated with higher PFOS concentrations. This may in part be explained by a greater use of non-stick cookware or PFOS-contaminated oil for frying or by a higher intake of fried processed food contaminated with PFOS. However, no data are available to support these propositions.

Higher concentrations of several PFAS, including long- and short-chain compounds, were found in the milk from women who more frequently used various PCPs, suggesting that PCPs might be a potential source of PFAS exposure. Few data are available on the presence of PFAS in PCPs; however, nine PFAS, including PFOA and PFNA, were detected in foundation, nail polish, and sunscreen products sold in Japan (Fujii et al., 2013), and twenty-five PFAS, most frequently PFHpA and PFHxA, in foundation and cosmetic powder products sold in Sweden (Schultes et al., 2018). In the present study, the use of foundation was positively associated with the sum of PFOA, PFOS, PFNA, and PFHxS concentrations, and the use of skin care and hair products, cosmetics, perfume, and deodorant was associated with higher concentrations of long-chain and short-chain PFAS, PFCAs, and PFSAs. These results support a previous study of 264 Korean women that found the utilization of cosmetics and skin care products to be associated with breast milk concentrations of PFHpA and PFOS, respectively (Kang et al., 2016). In the same line, recent biomonitoring study of adults in Belgium and Norway reported associations between the use of cosmetics (*e.g.*, sunscreen, mouthwash, and lip balm) and serum concentrations of PFAS (Colles et al., 2020; Thépaut et al., 2021). Dermal exposure to PFAS has been considered negligible in comparison to exposure from diet, drinking water, and ingestion of house dust (Trudel et al., 2008; Vestergren et al., 2008). However, exposure assessment studies have not considered the potential contribution of PCPs to dermal uptake due to the lack of adequate dosage data. Dermal permeability studies have also shown that the skin may be a relevant route of PFAS exposure under certain conditions, underscoring the need to re-assess the potential contribution of dermal exposure (Franco et al., 2012).

4.3. Implications for newborn exposure and health

Literature reports suggest that breast milk is an important pathway for the exposure of breastfed infants to PFAS, while also acts as a route for PFAS progressive elimination from the mother's body. In general, PFAS concentrations in the present milk samples were lower than described in these studies; however, it should be taken into account that: 1) exposure may start during the fetal period via placental transfer, meaning that the infant would already have a body burden of PFAS at birth; 2) although epidemiological evidence on the effects of postnatal exposure to PFAS, particularly short-chain PFAS, remains limited, potential effects include thyroid hormone imbalances, altered postnatal growth, and a decreased antibody response to vaccines (Abraham et al., 2020; Grandjean, 2018; Jin et al., 2020; Lopez-Espinosa et al., 2012); 3) there is a lack of knowledge on the toxicological properties of many PFAS in current use and on the combined adverse effects of this complex group of synthetic chemicals; and 4) most importantly, milk donated to the human milk bank is given to highly vulnerable preterm infants in NICUs, for whom the acceptable level of risk should be zero. Interestingly, based on findings of an association between plasma PFAS concentrations and antibodies against diphtheria and tetanus in one-year-olds (Abraham et al., 2020), the EFSA estimated that critical levels in breast milk would be 60 ng/L for PFOA and PFNA, 73 ng/L for PFHxS and PFOS, and 133 ng/L for the sum of the 4 PFAS (EFSA, 2020). These values are comparable to the upper concentrations observed in the present study. Moreover, a recent study reported that the exposure of preterm infants to PFAS through human breast milk might exceed reference values for older and healthier infants (Aceti et al., 2021).

4.4. Strengths and limitations

The main limitation of this study is the small sample size, which reduced the capacity to detect possible determinants of PFAS exposure,

particularly for compounds with a low detection frequency and therefore modeled as binary variables. Nevertheless, similar or even smaller sample sizes were used by most published studies on PFAS in breast milk (Macheka-Tendenguwo et al., 2018; Table S11). In addition, extrapolation of the study findings to lactating women in general is limited, because milk donors tend to be more educated and have higher incomes in comparison to non-donor lactating women (Osbaldiston and Mingle, 2007). Indeed, most donors in this study had a university education and were non-manual workers. Moreover, data were not available to establish whether the socio-demographic profile differed between participating and non-participating donors. However, neither education nor occupation was associated with milk PFAS concentrations. A further limitation was the use of a questionnaire not specifically designed for an exhaustive investigation of sources of PFAS exposure, and the lack of more detailed data on dietary patterns, occupations, and other potential sources of exposure prevented the identification of additional exposure pathways. Further, the considerable number of explanatory factors assessed may have led to some spurious statistically significant associations. It is also possible that bias may have resulted from the misreporting of dietary intakes and other factors. Nevertheless, misclassification is unlikely to be driven by exposure levels. Finally, the wide time frame for sample collection (ranging from 20 days to 9 months since delivery) may hamper comparisons with other studies on PFAS breast milk concentrations, given that internal PFAS exposure may vary over the lactation period due to toxicokinetics and/or lifestyle changes. In fact, our research group is currently investigating time-dependent variations in breast milk PFAS concentrations over the lactation period.

The main strength of this study is the assessment of pooled milk samples (over a maximum of 4 weeks) rather than spot samples. It is well established that lactational transfer of PFAS occurs by binding to milk protein. Protein levels decrease linearly in human milk over the first year of lactation, particularly over the first 6 weeks post-partum (Ballard and Morrow, 2013). Hence, PFAS assessments in spot breast milk samples may increase the risk of exposure misclassification in comparison to the assessment of pooled samples. Moreover, some of the eleven PFAS measured (*e.g.,* PFUnDA, PFDoDA, and PFTrDA) have been less well studied in breast milk samples and human biomonitoring studies. To our knowledge, this is the first report on the presence of PFAS in breast milk samples supplied by donor mothers to a human milk bank. The results suggest that requirements for donor selection may not be sufficient to minimize the exposure of breastfed infants to environmental chemicals.

5. Conclusions

This study of the concentrations of eleven PFAS in donor breast milk demonstrated the wide presence of these compounds in milk samples, especially short-chain PFAS such as PFHpA and PFHxA. PFOA and PFNA showed lower concentrations than observed in previous studies but were still detected in a large proportion of samples, whereas the findings for PFOS suggest a decrease in exposure levels. The data also suggest that certain lifestyle patterns, such as the use of PCPs, may have an influence on the presence of PFAS in breast milk; however, these data should be interpreted with caution given the limited sample size. Further studies are required to elucidate the main factors contributing to the increase in PFAS concentrations in breast milk and to determine changes in exposure levels over the lactation period This issue is especially urgent in relation to the supply of human milk to preterm infants and the need to limit their exposure to harmful chemicals.

Declaration of competing interest

The authors declare no actual or potential competing financial interests.

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Appendix A. Supplementary data

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Detection of SARS-CoV-2 RNA on contact surfaces within shared sanitation facilities

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ABSTRACT

Contamination of contact surfaces with SARS-CoV-2 has been reported as a potential route for the transmission of COVID-19. This could be a major issue in developing countries where access to basic sanitation is poor, leading to the sharing of toilet facilities. In this study, we report SARS-CoV-2 contamination of key contact surfaces in shared toilets and the probabilistic risks of COVID-19 infections based on detection and quantification of the nucleic acid on the surfaces. We observed that 54–69% of the contact surfaces were contaminated, with SARS-CoV-2 loads ranging from 28.1 to 132.7 gene copies per cm^2. Toilet seats had the highest contamination, which could be attributed to shedding of the virus in feces and urine. We observed a significant reduction in viral loads on the contaminated surfaces after cleaning, showing the potential of effective cleaning on the reduction of contamination. The pattern of contamination indicates that the most contaminated surfaces are those that are either commonly touched by users of the shared toilets or easily contaminated with feces and urine. These surfaces were the toilet seats, cistern handles and tap handles. The likelihood (probability) of infection with COVID-19 on these surfaces was highest on the toilet seat (1.76×10^{-4}(1.58×10^{-6})) for one time use of the toilet. These findings highlight the potential risks for COVID-19 infections in the event that intact infectious viral particles are deposited on these contact surfaces. Therefore, this study shows that shared toilet facilities in densely populated areas could lead to an increase in risks of COVID-19 infections. This calls for the implementation of risk reduction measures, such as regular washing of hands with soap, strict adherence to wearing face masks, and effective and regular cleaning of shared facilities.

1. Introduction

The current COVID-19 pandemic has claimed over 3.9 million lives and infected another 184 million globally, as at 7th July 2021 (WHO, 2021). The primary mode of transmission of the SARS-CoV-2 virus, the causative agent for COVID-19, is through respiratory droplets (Chan et al., 2020; Cai et al., 2020; Bahl et al., 2020; Morawska and Milton, 2020). This has led to the implementation of mitigation measures, such as social distancing and the use of face masks (Liu and Zhang, 2020; WHO, 2020; Howard et al., 2020; Dalton et al., 2020; Viner et al., 2020). Additionally, transmission of the virus through contaminated contact surfaces has been postulated (Qu et al., 2020; Zoran et al., 2020; Jones, 2020). These are of concern due to the stability/survival of this virus on surfaces such as plastic, steel, wood and aluminium (Van Doremalen et al., 2020; Pastorino et al., 2020). Their survival on contact surfaces is dependent on the material and environmental conditions, for instance, it is reported to persist on plastics for 3–4 days at 65% relative humidity (RH) and 21–23 °C (Van Doremalen et al., 2020), aluminium for 2–3 h at 19 °C−21 °C temperature range (Pastorino et al., 2020), stainless steel for four days and on glass for two days (Chin et al., 2020), all at room temperature. However, Goldman (2020) posited that most of these studies reporting on the survival of SARS-CoV-2 or surrogate viruses on fomites exaggerate the potential risks due to the use of unrealistic viral titre. Despite in-depth information on the potential transmission routes of the virus, there is a lack of data on the role of shared sanitation facilities as a possible route of transmission. Although this has been studied within hospital settings (Ye et al., 2020; Ong et al., 2020), the risks posed by shared sanitation facilities outside of the hospital

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environment has been neglected.

The reported shedding of viral particles in feces and urine, by both symptomatic and asymptomatic individuals, highlights the increased risks from the use of shared sanitation. The World Health Organization (WHO) reports that between 2 and 27% of COVID-19 patients have diarrhoea (WHO, 2020b), which may result in the shedding of this virus in feces. SARS-CoV-2 viral loads of 1.7×10^6–4.1×10^7 gc/mL have been reported by Han et al. (2020), and 6.3×10^6–1.26×10^8 gc/g of stool by Lescure et al. (2020). Additionally, although not common, the detection of SARS-CoV-2 concentrations of 3.2×10^2 gc/ml (Peng et al., 2020) and 6.1×10^5 gc/ml (Yoon et al., 2020) have been reported in urine. These results show that in circumstances where fecal and urine contamination of surfaces could occur, such as shared sanitation facilities, the risks of COVID-19 infections could be high. This is especially important in slums or informal settlements in developing countries such as South Africa, where a lack of basic sanitation facilities is a significant concern. The World Bank reported that living in cramped conditions within cities has a significant contribution to a high risk of infections with COVID-19 (WBG, 2020).

The risks associated with shared sanitation could be due to the contamination of contact surfaces by infected individuals either via deposition of aerosols or faecal matter contaminations. Additionally, several studies have shown strong evidence in support of the indoor airborne transmission of viruses, especially in crowded and poorly ventilated areas (Nishiura et al., 2020; Coleman et al., 2018; Knibbs et al., 2012), such as shared toilets. For instance, SARS-CoV-2 is reported to survive in aerosols for up to 3 h (Kumar et al., 2020), meaning the sharing of toilet facilities could be a major risk factor.

Therefore, by detecting and quantifying the concentration of SARS-CoV-2 on key contact surfaces within these shared sanitation facilities, the risks of infection could be estimated. The quantitative microbial risks assessment (QMRA) approach has been encouraged as a tool to assess risks associated with bioaerosols, drinking water, reclaimed water and irrigation water (Carducci et al., 2016; Petterson and Ashbolt, 2016; Girardi et al., 2019; Gularte et al., 2019; Ezzat, 2020). This approach has been used in estimation of the risks for COVID-19 infections for wastewater treatment workers (Zaneti et al., 2020; Dada & Gyawali, 2020), exposure in a market setting (Zhang et al., 2020) and most recently via contact surfaces (Pitol and Julian, 2021). According to Haas et al. (2014) the QMRA approach involves a sequence of four interrelated steps: a) hazard identification; b) exposure assessment; c) dose-response assessment and d) risk characterization. This is the first study using QMRA for assessing risks from the use of shared sanitation facilities outside the clinical setting, focusing on contact surfaces despite the widespread understanding that sanitation facilities may facilitate its spread. This could therefore provide background information on the contamination of such surfaces and could be used in developing risk reduction measures aimed at reducing the potential spread of COVID-19 (and possibly other similar outbreaks) via the use of shared toilet facilities.

2. Methodology

2.1. Study area and sampling

Two peri-urban informal settlements located within the eThekwini Municipality (Durban) of South Africa were selected for this study. These two settlements are located approximately 1.5 km apart, with an approximate total population of 16 500. This study was done at a time the reported active clinical cases were low in South Africa, with about 600 000 active cases of COVID-19 in South Africa, specifically the KwaZulu-Natal province had over 100 000 active cases.

A total of eight (8) shared toilets, referred to as community ablution blocks (CABs), were investigated, four in each settlement. It is worth noting that the CABs are categorized into males and females, however, this study focused on the difference in contamination within the vaiorus CABs irrespective of gender. The contact surfaces selected included the following: cistern handle, toilet seat, floor surface in front of the toilet, internal pull latch of cubicle door and tap in handwash basin (Fig. 1). These were selected based on recommendations made in previous studies (Park et al., 2017; Bohnert et al., 2016; Mpotane et al., 2013). A total of 68 swab samples were taken. Sampling was done twice (two weeks apart) in September 2020. On each sampling event, samples were taken in the morning before the toilets are cleaned and approximately 30 min after cleaning by trained caretakers. Cleaning was done with antiseptic detergents and water. The swab samples were taken according to the methodology proposed by Park et al. (2017). Briefly, the swab was moistened with PCR grade nuclease free water moved across the sampling area horizontally, vertically and diagonally. An area of approximately, 50 cm^2 was swabbed for the toilet seat and toilet floors, 20 cm^2 for the cistern handle and internal latch and 30 cm^2 for the tap handle. The swab area was determined based on the available area of these contact surfaces. Swabs were placed in a 400 µL PCR-grade nuclease free water and transported to the laboratory on ice. The personnel carrying out the sampling were fully clothed in personal protective equipment (face masks, shields, lab coats, gloves and face shields).

2.2. Molecular detection of SARS-CoV-2

Upon arrival at the laboratory, each tube containing the swab was vortexed for 10 s and the swab carefully removed from the tube, pressing gently against the side of the tube to remove excess water. The swab was then discarded and disposed of as biohazard waste. Two approaches were used in the detection of the viral RNA in the samples. This include direct quantification without the RNA extraction step, using 5 µL of the initial sample as a template for the molecular analysis. The second approach involved extraction of RNA from the swab samples using the extraction kit followed by quantification of the RNA copy numbers as described below.

2.3. RNA extraction

Nucleic acid (RNA) was extracted directly from 140 µl of swab solution using the QiAmp Viral RNA MiniKit (Qiagen, Hilden, Germany), according to manufacturer's instructions. RNA was eluted in 80 µl of sterile nuclease free water and then quantified using the Implen Nanophotometer® NP 80 (Implen GmbH, Munich, Germany). The quality of the extracted RNA was determined based on the Nanophotometer® NP 80 results prior to amplification. The extracted RNA was then stored at −80 °C for further analysis. The second detection and quantification approach did not require RNA extraction, therefore the swab samples were vortexed vigourously and these samples were used for droplet

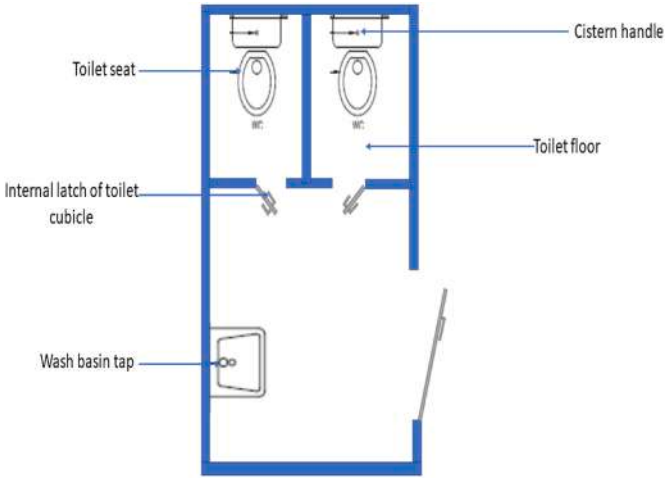

Fig. 1. Key contact surface areas within the internal surfaces of CABs that were considered in this study.

digital Polymerase Chain Reaction (ddPCR) amplification using the protocol described below (Section 2.4).

2.4. Viral detection and quantification using droplet digital PCR

RNA, which was stored for not more than 24 h at −80 °C, was thawed at room temperature and quantitified using the Implen Nanophotometer® NP 80 (Implen GmbH, Munich, Germany). All RNA samples were then diluted and standardized to 1 ng using sterile nuclease free water. Additoinally, direct detection without RNA extraction was also done to determine the suitability of this approach in detection and quantification of viral loads on the surfaces. For the detection of SARS-CoV-2, the 2019-nCoV CDC ddPCR Triplex Probe Assay (Biorad, USA), which simultaneously targets the N1 (FAM labelled) and N2 (FAM and HEX labelled) region of the SARS-CoV-2 genome was used. The assay also targets the human RPP30 (HEX labelled) gene for use as an internal control. Amplification was achieved using the One-Step RT-ddPCR Advanced Kit for Probes Supermix (Biorad, USA), which contains reverse transcriptase and 300 mM Dithiothreitol (DTT). Each ddPCR reaction mix contained 5.5 µl supermix, 2.2 µl reverse transcriptase, 1.1 µl of 300 mM DTT, 1.1 µl of 20X 2019-nCoV CDC ddPCR Triplex Probe Assay, 6.6 µl of sterile DNase free water and 5 µl of the standardized RNA template to get a final volume of 22 µl. All sample plates contained positive, negative and no template control wells. The SARS-CoV-2 positive control (Exact Diagnostics) contained synthetic RNA transcripts of 5 gene targets (E, N, ORF1ab, RdRP and S) while the negative control (Exact Diagnostics) contained human genomic DNA and RNA spiked into a synthetic matrix. Sterile nuclease-free water was used in place of RNA for the no template control. Sample plates were sealed and vortexed for 20 s. Thereafter, droplet generation was carried out using the QXDx Automated Droplet Generator (Biorad, USA), and the plates were then heat sealed with a pierceable foil. A C1000 Touch Thermal Cycler (Biorad, USA) was then used to perform PCR under the following conditions: Reverse transcription at 50 °C for 1 h, enzyme activation at 95 °C for 10 min, 40 cycles of denaturation at 94 °C for 30 s and annealing at 55 °C for 60 s. This was followed by enzyme deactivation at 98 °C for 10 min and droplet stabilization at 4 °C for 30 min with a ramp rate of 2 °C/second. The sealed droplet plate was then transferred to the QX200 Droplet Reader (Biorad, USA). The distribution of positive and negative droplets in each well was read using the QuantaSoft 1.7 software (Biorad, USA) while data analysis was carried out using the QuantaSoft Analysis Pro 1.0 software (Biorad, USA). The results were interpreted as follows: a sample is considered positive if it has any or both of the SARS-CoV-2 markers even in the absence of the RPP30 gene. Similarly, a sample is considered negative if it does not contain any of the SARS-CoV-2 markers even if it contains RPP30. Presence of the RPP30 gene is not mandatory for the presence of SARS-CoV-2. A sample run was considered invalid if there are positives in the negative and no template control wells.

2.5. Probability of COVID-19 infection from the use of shared sanitation: A case of the community ablution blocks

The four interrelated steps used in assessing the potential risks of COVID-19 infections are described below:

***Hazard Identification*:** The SARS-CoV-2 virus is the hazard of choice for this assessment. The concentration of this virus determined based on the extracted RNA was used for the risk assessment.

***Exposure assessment*:** Contact surfaces are recognized as important routes for the spread of infectious diseases, mainly through surface-hand interactions. These surfaces sometimes referred to as fomites, have been associated with different outbreaks in cruise ships, restaurants, nursing homes, schools, daycare centres and gyms (Bures et al., 2000; Aitken and Jeffries, 2001; Barker et al., 2004; Boone and Gerba, 2005). Therefore, the main exposure scenario considered in this study is hand contamination as a result of contact with the surfaces monitored. To assess the dose of the SARS-CoV-2 virus ingested via this route Fig. 2 presents the process flow.

Dose−Response Model: The dose-response relation adopted for this study is the exponential model expressed as;

$$p(d) = 1 - \exp\left(-\frac{d}{k}\right) \quad (1)$$

Where p(d) is the infection risk at a dose of d in units of PFU and k is a pathogen dependent parameter, referred to as the infectivity constant. The k was taken as 4.1×10^2 PFU for SARS-CoV. The dose response model and k were determined based on data for the infection of transgenic mice susceptible to SARS-CoV (Watanabe et al., 2010). These are adopted for the SARS-CoV-2 because SARS-CoV-2 and SARS-CoV have the same cell receptor (angiotensin-converting enzyme 2 (ACE2)) and a similar cellular tropism (Chu et al., 2020; Hoffmann et al., 2020). These dose-response parameters have been used in assessing the risks of COVID-19 infections for workers in wastewater treatment plants (Zaneti et al., 2020).

The dose d was based on the concentration of the viral RNA detected by the ddPCR analysis. This accounted for the fraction of the viral particles that are transferred from the contact surfaces to the mouth/lips or eyes. A two-step process was used to calculate the dose;

1. The efficiency of viral transfer from the contact surface to the hand was accounted for by assuming that 2 cm^2 of the surface will be touched with a transfer efficiency as presented in Table 1.
2. The potential of transfer of the viral particle on the hands to the mouth/lips or eyes.

Table 1 presents the information used to ascertain the concentration of the SARS-CoV-2 virus transferred from the contact surface to the hands and subsequently from the hands to the mouth/lips or eyes.The dose (d) also took into account the ratio of genome copies to viable SARS-CoV-2 viral particles. For this study we assumed a uniform distribution ratio between 1:100 to 1:1000 for genome copy to viable SARS-CoV-2 viral particle (Pitol and Julian, 2021). Additionally, we

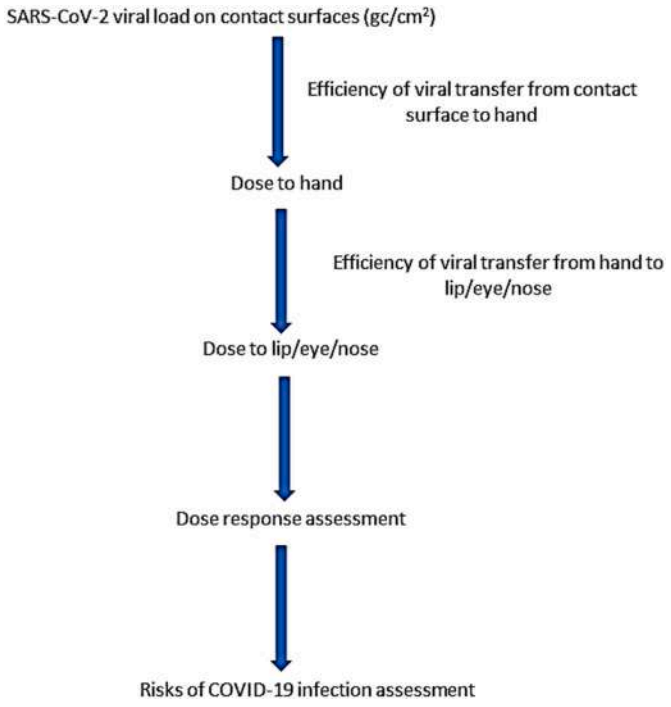

Fig. 2. Scenario for assessing the exposure and possible risks associated with contamination of the contact surfaces (Adapted from Ryan et al., 2014).

Table 1
Transfer efficiencies for determination of dose of SARS-CoV-2 transferred from contact surfaces to mouth/lips or eyes.

Parameter	Input value	Reference
Viral transfer from contact surface to hands	Uniform distribution (0.33; 0.68)	Ryan et al. (2014)
Viral transfer from hands to mouth/lips or eyes	Median value of 0.34	

factored the prevalence of contamination of the various contact surfaces into the risk assessment. This accounts for the likelihood that the contact surfaces will be contaminated at the time when a user comes into contact.

***Risk characterization*:** The outcome of the previous steps were combined to determine the risks of infection for users of the shared toilet facilities. Risk of infection from multiple exposures within a day were assessed by assuming that inhabitants use the toilet facilities between two to three times daily. Therefore the number of times of exposure per day was assumed to be uniformly distributed between 2 and 3. This was used in assessing the daily risks as well as yearly risks based on exposures for everyday in the year. This was determined due to the fact that these shared toilet facilities are the only source of sanitation access in the study area. The risks from multiple exposures was therefore determined using the following formula:

$$p(n) = 1 - (1 - p(d))^n \quad (2)$$

Where *p(n)* is the risks of infection after *n* times of exposure; and *p(d)* is the risk of infection from a single exposure. To determine the annual risks of infection, *p(d)* refers to the daily risks of infection.

2.6. Sensitivity analysis of QMRA inputs

To determine the impact of the various inputs in the QMRA analysis, the following parameters were considered, concentration of the SARS-CoV-2 (gc/cm^2), gene copy to infective viral particle ratio, the transfer efficiency of the viral particles from the surface to the hand and the number of times of exposure within a day. These parameters were varied from their minimum to maximum values. For the purpose of the sensitivity analysis only the risk of infection from exposure to the uncleaned toilet seats was considered. To determine the impact of these parameters, the calculated median infection risks were averaged and used to calculate the factor sensitivity coefficients (*FSi*), using the equation:

$$FSi = P_{i,x}/P_{baseline} \quad (3)$$

Where $P_{i,x}$ is the calculated averaged median risks per parameter, after varying the input values *x*, and $P_{baseline}$ is the baseline median infection risks.

2.7. Statistical analysis

Descriptive statistics to represent the mean and standard deviation were performed with Excel (Microsoft Corporation, USA). Comparison of viral load between the different contact surfaces was performed using the Kruskal-Wallis Test, comparison between two data categories (such as comparing viral load on cleaned and uncleaned surfaces) was done using the Mann Whitney Test. Comparative statistical analysis were all performed with GraphPad Prism Version 7 (GraphPad Software, CA, USA).

3. Results

3.1. Prevalence of contamination using extracted RNA

The chance/likelihood of contamination on the contact surfaces varied over the two sampling events. The highest prevalence of contamination of 68.8 (±20.6) % was observed for the tap handle, followed by the toilet floor with the internal latch giving the lowest prevalence of contamination (54.1 (±16.2) %) among the studied contact surfaces (Fig. 3). Despite the observed difference, there was no statistically significant difference in the prevalence (p value ≥ 0.05). This information on likelihood of contamination was used in estimating the probable risk of infection due to contact with these surfaces.

3.2. Concentration of SARS-CoV-2 on contact surfaces before and after cleaning based on extracted RNA

Per cm^2 swabbed, the mean concentration of SARS-CoV-2 was highest on the toilet seats (132.9(±39.8) gc/cm^2), followed by the cistern handle (69.1(±21.6) gc/cm^2) and internal latch (60.1(±14.5) gc/cm^2). The differences in the concentration between the different contact surfaces were statistically significant (p value ≤ 0.05).

Cleaning reduced the concentration of SARS-CoV-2 RNA on these contact surfaces, with significant (p value ≤ 0.05) reduction on the toilet seat, cistern handle, internal latch and toilet floors. For instance, after cleaning, the mean viral load on the toilet seats was reduced to 2.1 (±0.21) gc/cm^2 from the initial 132.9(±39.8) gc/cm^2 (Fig. 4). However, there was no significant reduction observed on the tap handles after cleaning (p value ≥ 0.05), as shown in Fig. 4.

3.3. Comparison of direct quantification vs quantification via extracted RNA

Detection of the SARS-CoV-2 on the swab without the initial RNA extraction step presented higher prevalence compared with the prevalence observed using the extracted RNA. For instance, via direct sample analysis, the highest prevalence was observed for cistern handle (83.3 (±29.2) %) with a corresponding prevalence of 59.3(±17.8) % when the viral RNA was extracted first before analysis. Similar trends were observed, where prevalence was consistently lower when the RNA was extracted. The only exception were swab samples from the floor, where prevalence via analysis of extracted RNA was higher (59.7(±19.3) %) compared to direct detection (50(±17.5) %) (Fig. 5A).

There was a similar trend in the viral load difference when these two approaches (direct quantification and quantification via extracted RNA) were used. For instance, via direct quantification without RNA extraction, 244.9(±85.7) g/cm^2 was recorded on the toilet seats, however when the RNA was extracted, the concentrations was reduced to 132.7 (±39.8) gc/cm^2. These differences are statistically significant (p value ≤ 0.05), indicating consistently lower concentrations when the RNA was extracted from the samples prior to analysis. However, as observed with the prevalence, the only exceptions were the floor and cistern handle

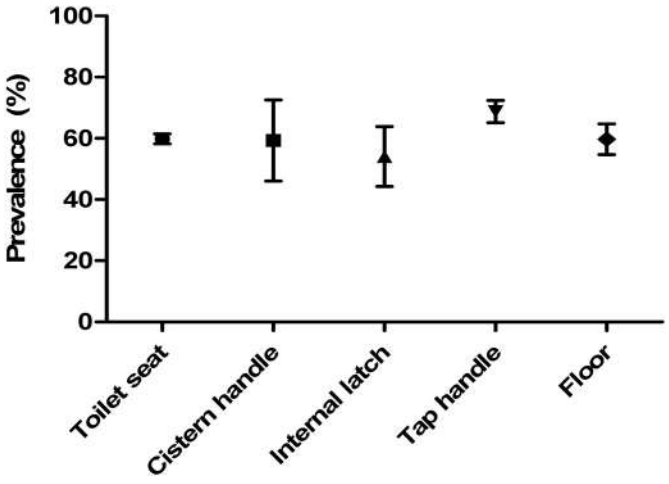

Fig. 3. Percentage of contact surfaces contaminated with SARS-CoV-2 (n = 16).

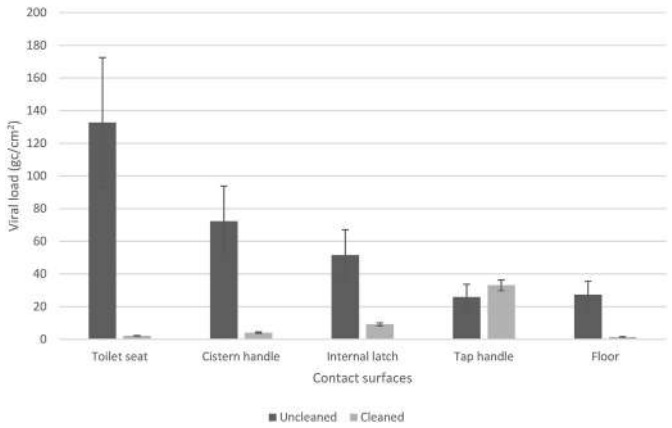

Fig. 4. Concentration of SARS-CoV-2 on key contact surfaces in the shared toilets (n = 16). *Error bars representing standard deviation.

swab samples (Fig. 5B). The RPP30 gene was present in all extracted and unextracted RNA samples regardless of whether or not they contained any of the SARS-CoV-2 genetic markers. Presence of the RPP30 gene is indicative of sufficient cellular material and proper nucleic acid extraction.

3.4. Probability of infection with COVID-19 from use of the shared toilets

The probability of infection with COVID-19 as a result of exposure to the SARS-CoV-2 virus particles on the contact surfaces varied considerably, driven mainly by the difference in the viral loads described above and the prevalence/likelihood of contamination of these surfaces. The magnitude of the risks after single exposure was similar for contact with almost all the surfaces (10^{-5}), however the highest median risks were observed for contact with the uncleaned toilet seats. It was estimated that approximately two people out of every 10 000 people using the toilet who touch the toilet seat could potentially be infected with COVID-19 (1.76×10^{-4} ($\pm 1.58 \times 10^{-6}$) per person). These estimates were made based on a single exposure event. However, considering that these toilet facilities are the only source of sanitation services within the communities studied, providing both access to potable water and sanitation, multiple exposures within a day were considered. Use of the toilet facilities twice or three times in a day was observed to increase the risks of infections with COVID-19. For instance, multiple contacts with the toilet seat within a day (daily risks) resulted in an increase in the median risks from 1.76×10^{-4} ($\pm 1.58 \times 10^{-6}$) per person for a single exposure to 4.33×10^{-4} (4.03×10^{-6}) per person for daily risks (multiple exposures in a day). This means that for every 10 000 people who use the toilet facility between two or three times in a day, about four of them may be infected. Similar significantly increased risks (p value ≤ 0.05) were observed for all the other contact surfaces (Table 2). We further observed an increase in the risks of infection with COVID-19 when exposure over the course of a year (yearly risk) is considered (Table 2), relying on the fact that these shared sanitation facilities are the only source of sanitation in the studied areas.

The risks of infection were reduced, considering exposure after the toilets have been cleaned, although not statistically significant for most of the surfaces (Table 2). Most notable reductions were exposure via contact with the toilet seat, internal latch and toilet floor. For instance, the probability of infection reduced from about two people out of 10 000 exposed people potentially been infected to about two people out of one million being infected (2.34×10^{-6} ($\pm 2.09 \times 10^{-8}$)). Similar significant reduction in probable risks were recorded after cleaning for contact with the other contact surfaces mentioned previously (p value ≤ 0.05), except the tap handle and internal latch (Table 2). As observed for multiple exposures to the uncleaned surfaces, multiple exposures to the cleaned surfaces could also increase the risks of infection, as reported in Table 2.

3.5. Parameter sensitivity in the infection risk calculation

The sensitivity analysis for the various input parameters based on their minimum and maximum input ranges showed that these values had an impact on the risk estimates calculated. However, their impact varied depending on the parameter. The gene copies of SARS-CoV-2 measured on the various contact surfaces was determined to have the highest impact on the risks estimates, with an *FSi* of 2.88 (Fig. 6). Among the four parameters chosen for the sensitivity analysis, varying the number of times of exposure within a day between twice or three times had the least impact on the risk estimates, with an *FSi* of 1.01. These results therefore, shows that the concentration of the viral particles measured could be the main parameter affecting the risks estimates.

4. Discussion

Contact surface contamination within the toilet facilities was widespread (Fig. 3), with a high prevalence of contamination on the tap handles, the floor of these toilets and the internal latch of the toilet cubicles. Several studies have reported similar findings in relation to the most contaminated surfaces in toilet facilities (McGinnis et al., 2019; Abiose, 2019; Verani et al., 2014; Sabra, 2013; De Alwis et al., 2012; Flores et al., 2011; Fankem et al., 2006). Notably, Fankem et al. (2006)

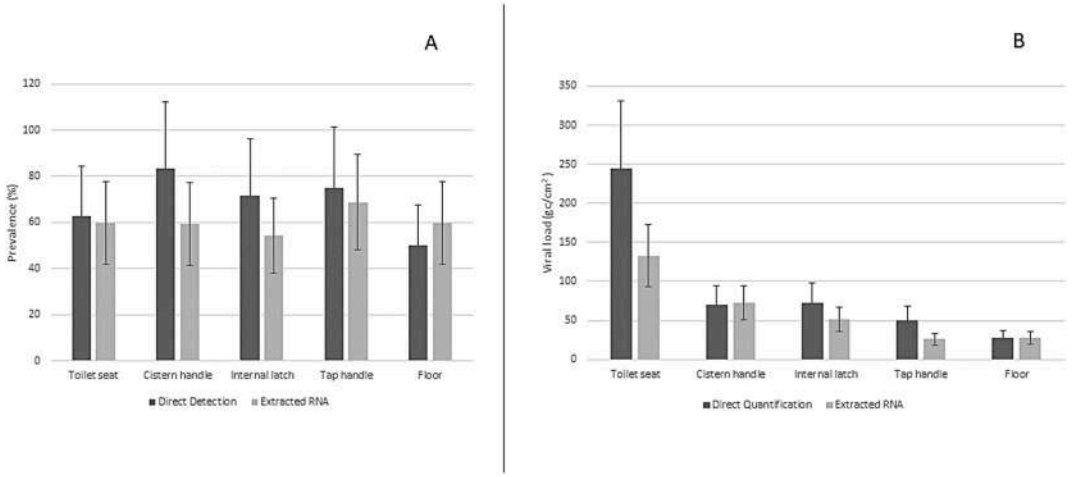

Fig. 5. Difference in the detection and quantification of SARS-CoV-2 via direct analysis and RNA extraction (n = 16): (A) comparison on prevalence and (B) viral loads. *Error bars representing standard deviation.

Table 2
Median risks (±90% CI) of infection with COVID-19 due to contact with surfaces within shared toilets.

Exposure frequency	Toilet seat		Cistern handle		Internal Latch		Tap handle		Floor	
	Uncleaned	Cleaned	Uncleaned	Cleaned	Uncleaned	Cleaned	Uncleaned	Cleaned	Uncleaned	Cleaned
One-time risk	1.76×10^{-4} ($\pm 1.58 \times 10^{-6}$)	2.34×10^{-6} ($\pm 2.09 \times 10^{-8}$)	9.16×10^{-5} ($\pm 8.20 \times 10^{-7}$)	9.00×10^{-6} ($\pm 8.07 \times 10^{-8}$)	6.10×10^{-5} ($\pm 5.47 \times 10^{-7}$)	2.03×10^{-5} ($\pm 1.82 \times 10^{-7}$)	3.95×10^{-5} ($\pm 3.54 \times 10^{-7}$)	3.67×10^{-5} ($\pm 3.29 \times 10^{-7}$)	3.79×10^{-5} ($\pm 3.39 \times 10^{-7}$)	3.13×10^{-6} ($\pm 2.80 \times 10^{-8}$)
Daily risk	4.33×10^{-4} ($\pm 4.03 \times 10^{-6}$)	5.73×10^{-6} ($\pm 5.33 \times 10^{-8}$)	2.24×10^{-4} ($\pm 2.09 \times 10^{-6}$)	2.21×10^{-5} ($\pm 2.06 \times 10^{-7}$)	1.49×10^{-4} ($\pm 1.58 \times 10^{-6}$)	4.98×10^{-5} ($\pm 4.63 \times 10^{-7}$)	9.69×10^{-5} ($\pm 9.02 \times 10^{-7}$)	8.99×10^{-5} ($\pm 8.37 \times 10^{-7}$)	9.29×10^{-5} ($\pm 8.65 \times 10^{-7}$)	7.67×10^{-6} ($\pm 7.14 \times 10^{-8}$)
Annual risks	6.03×10^{-2} ($\pm 5.22 \times 10^{-4}$)	8.22×10^{-4} ($\pm 7.41 \times 10^{-6}$)	3.17×10^{-2} ($\pm 2.80 \times 10^{-4}$)	3.16×10^{-3} ($\pm 2.84 \times 10^{-5}$)	2.12×10^{-2} ($\pm 1.89 \times 10^{-4}$)	7.12×10^{-3} ($\pm 6.39 \times 10^{-5}$)	1.38×10^{-2} ($\pm 1.23 \times 10^{-4}$)	1.28×10^{-2} ($\pm 1.15 \times 10^{-4}$)	1.32×10^{-2} ($\pm 1.18 \times 10^{-4}$)	1.10×10^{-3} ($\pm 9.91 \times 10^{-6}$)

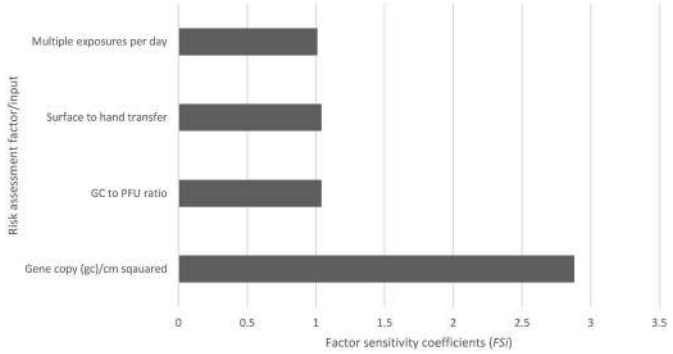

Fig. 6. Sensitivity ranking of the infection risks calculation input parameters for exposure to the uncleaned toilet seat.

observed that the most contaminated surfaces in public toilets found in airports, bus terminals, and universities were the sanitary napkin dispensers, toilet seats, sinks, and floors. However, in those studies, the frequency of contamination on these surfaces was much lower (3–21%) compared to the frequency observed in our study. Our prevalence of contamination was in accordance with the observations reported by Sabra (2013), where 91.3% toilet handles, 73% of toilet doors, 53% of toilet sink and 50% of tap handles were reportedly contaminated with bacteria. It must be noted that these findings were observed for bacterial contamination; therefore, the difference could further be due to the difference in organisms. When using a human adenovirus virus (HAdV), Verani et al. (2014) found 135 out of 172 surfaces within toilet facilities in a health care setting to be contaminated. Contamination of contact surfaces outside the sanitation setting has also been reported in hospitals (Chia et al., 2020; Ryu et al., 2020; Peyrony et al., 2020; Lei et al., 2020), home settings (Xie et al., 2020a,b; Fernández-de-Mera et al., 2020; Döhla et al., 2020) and public spaces (Fernández-de-Mera et al., 2020).

Contamination of the contact surfaces could be as a result of direct contact with feces or urine, unclean hands or even through cough or sneeze. For instance, the high frequency of contamination on the cistern handle, the tap handle and internal latch could be as a result of this direct contact with uncleaned hands. Contamination of the toilet seat and the toilet floor could also be from contaminated fecal matter and urine. The frequency of contact has been proposed as the most critical factor in the direct contamination of contact surfaces within public toilets (Fankem et al., 2006). The higher frequency of contact could therefore be responsible for the high prevalence of contamination on these contact surfaces. In addition to the frequency of use, the contamination of these contact surfaces could be an indication of hygiene. De Alwis et al. (2012) reported a high bacterial contamination on door handles used by males, whereby 50% of the users of these toilets did not wash their hands with soap. The contamination of toilet floors has been attributed to a high frequency of contact with the bottom of shoes (Flores et al., 2011). This could potentially be a significant source of contamination for other contact surfaces, such as cistern handles. A study by Flores et al. (2011) observed that the bacterial community on toilet floors was similar to those found on toilet flush/cistern handles. They attributed this to the use of foot in operating these cistern/flush handles by some of the users. This is a common practice in shared sanitation facilities.

Contamination of the toilet seat and the floors could be via indirect contact. For instance, flushing of toilets could be a significant source of contamination. Flushing results in the generation of droplets and aerosols that could be deposited on theses surfaces (Flores et al., 2011). Using modelling approaches, Li et al. (2020) postulated that massive upward transport of viral particles is observed with over 40–60% of the particles potentially deposited on the toilet seat. Contamination of the toilet seat up to 24 flushes after initial shedding in feces and urine could still occur, although the concentrations could reduce with each flush (Johnson et al., 2017). Using bacterial indicators, Johnson et al. (2017) observed $3\log_{10}$ reduction after the first flush, 1–2 $\log_{10}$ after the second and thereafter less than 1 $\log_{10}$ reduction with each flush. Therefore, SARS-CoV-2 viral particles shed in feces and urine could be deposited on the toilet seat during flushing, this could potentially be the main source of the contamination of the toilet seats. Additionally, contamination of the floor could be due to accidental urination on the floor, which could be a common phenomenon in the male toilets, although this study did not specifically measure the difference in contamination within the male and female toilets.

We observed that direct detection and quantification of SARS-CoV-2 in swab solutions gave higher prevalence of contamination and viral load (Fig. 5). The lower numbers recorded for analysis done using the RNA extraction approach, could be attributed to losses during the RNA extraction process. The higher frequency of contamination and viral load on the floor swabs determined via the RNA extraction approach as compared with the direct estimation approach could be due to the elimination of PCR inhibitors during RNA extraction as compared to other surfaces. It is worth noting that the toilet floor was constantly soiled, as a result without RNA extraction, several PCR inhibitors inherent in soil could be transferred to the amplification stage resulting in interferences. Therefore, although direct quantification of SARS-CoV-2 on contact surfaces without RNA extraction is possible and gives higher concentrations, we do not recommend it for surfaces with high solid contents, such as floors. However, direct quantification is an important approach to consider for the estimation of risks from contact with contaminated surfaces with less solids.

The difference in concentration of SARS-CoV-2 observed in this study (Fig. 4) could also be attributed to the same factors responsible for the frequency of contamination, which are fecal matter contamination, unclean hands and cough or sneeze. However, the viral load on the toilet seats per cm^2 were significantly higher (p value ≤ 0.05) than any of the

other contact surfaces. This could be attributed to the phenomenon of droplet and aerosol generation during flushing. Shedding of SARS-CoV-2 in feces and urine of both symptomatic and asymptomatic patients is well reported (Jones et al., 2020; Amirian, 2020; Bowser, 2020; Pan et al., 2020; Xie et al., 2020a,b; Peng et al., 2020; Yoon et al., 2020), therefore higher viral load on the toilet seats is to be expected. The concentrations on the other contact surfaces points towards direct contamination via uncleaned hands. Hand transmission of COVID-19 is one of the main routes of transmission, leading to hand washing as a major intervention to reduce infections (Gupta and Lipner, 2020; Lin et al., 2020; Beiu et al., 2020). The toilets are cleaned once a day, which resulted in a significant reduction of viral load on almost all the contact surfaces, except for the tap handle (Fig. 4). The viral loads detected on the internal latch and tap handle indicates that cleaning does not usually focus on these surfaces, despite a high contact frequency. The findings, therefore, show that cleaning of shared sanitation facilities should consider surfaces with high contact frequency and small crevices, such as the toilet seat, tap handle and internal latch.

The viral contamination of key contact surfaces within shared toilets could potentially result in COVID-19 infections. The estimated risks show that the highest probability of infection from a one-time use of the toilets is the contact with the toilet seat (Table 2). A manageable risk of 1.17×10^{-3} has been recommended by Zhang et al. (2020), meaning 1 person out of a thousand being infected is acceptable. In contrast Zaneti et al. (2020) derived a tolerable risk of infection for SARS-CoV-2 to be 5.5×10^{-4} per person per year (pppy), setting a very high tolerable/acceptable risk figure. Considering one-time exposures, the risks estimates from our study are lower than these recommended tolerable/acceptable risks figures. However, with multiple exposure within a day or over a year, the risks of infection with COVID-19 within our study area were higher than these tolerable or acceptable risks estimates published (Table 2). Comparatively, the risks estimated from this study are lower compared to the risks published by Zaneti et al. (2020) for workers in wastewater treatment plants (2.6×10^{-3} to 1.3×10^{-2}) per exposure. Furthermore, Pitol and Julian (2021) reported median risks of 1.6×10^{-4} to 5.6×10^{-9} when they modelled the risks of infection with COVID-19 based on surface contamination, similar to our findings. The application of QMRA to measure the potential risks of infection via surfaces, therefore shows that this may not be a significant route of infection. This could be due to the conversion ratio of the gc/cm^2 to PFU/cm^{2}of 1:100 and 1:1000 of gc/cm^2 to PFU/cm^2 which was used both in our study and the study by Pitol and Julian (2021). Reports have shown that SARS-CoV-2 viral particles shed in feces may still be infectious (Zhang et al., 2020; Wang et al., 2020; Xiao et al., 2020), however this is inconclusive due to the varying reports on their survival in the environment. It is also important to consider that the potential risk can be high due to the frequent use of these facilities by the communities. The contact time is very short due to a high population that rely on these facilities and the SARS-CoV-2 virus is reported to survive on surfaces from a few hours (Chin et al., 2020), to four days (Chin et al., 2020; Van Doremalen et al., 2020).

Cleaning could potentially reduce the risks of infection, however, in our study, we observed that despite the significant reduction in viral load after cleaning on almost all the surfaces, the potential of infections with COVID-19 was still high. Tuladhar et al.,(2012) found residual bacterial and viral contamination on surfaces after cleaning, which means the detection of the SARS-CoV-2 on the contact surfaces after cleaning could be residual viral particles. Therefore, the estimated risks on the contact surfaces after cleaning could be much lower. However, to ensure maximum protection for users of these shared toilets and other facilities with similar characteristics, other risks reduction interventions should be considered.

5. Limitation of the study

The risk or probability of infection with COVID-19 was based on the assumption of a worst-case scenario where a gene copy is considered an infectious viral particle. By using the ratio of genome copies to viable SARS-CoV-2 viral particles of 1:100 to 1:1000 (Pitol and Julian, 2021), this was addressed. However, the risk assessment based on SARS-CoV-2 viral RNA concentration could potentially result in over estimation of the associated risks, because the detection and quantification of viral RNA and inactivated viruses may still yield positive results.

6. Conclusions

We established in this study that key contact surfaces within shared toilets investigated in this study were contaminated with SARS-CoV-2, with the highest prevalence of contamination on the floor, tap and cistern handles. This shows areas of high hand contact had the highest possibility of being contaminated, indicating that uncleaned hands may be the main source of contamination. However, based on viral load per cm^2, the most contaminated surface is the toilet seat, the shedding of SARS-CoV-2 virus in feces and urine could be the main reason for this high concentration. We also showed that the presence and quantity of SARS-CoV-2 on contact surfaces could be determined directly without an RNA extraction step using ddPCR, which can potentially reduce the cost associated with such analysis. However, this is not recommended for surfaces with high solid contents, such as floors. Cleaned contact surfaces had significantly lower viral load compared to the uncleaned surfaces except for the tap handle, this shows that the potential risks of infection with COVID-19 due to contact with these surfaces could be reduced with effective and regular cleaning.

7. Recommendation/risk reduction interventions

The calculated risks of infections associated with the use of the shared toilets call for the introduction of additional measures to protect public health, especially in developing countries where large proportion of the population may rely on shared toilet facilities. Some of these risk reduction measures are:

1. **Frequent and effective cleaning:** Cleaning of the shared toiles is currently done once a day, due to the high contamination found on the key contact surfaces we recommend that cleaning be carried out at least twice. For instance, Tuladhar et al. (2012) observed that a second wipe of a contaminated surface with chlorine resulted in an extra 1–3 $\log_{10}$ reduction in concentration of various pathogens including influenza virus.
2. **Close of water closet lid during flushing:** The viral concentration on the toilet seats was the highest, this could be attributed to the shedding of SARS-CoV-2 in feces and urine. These could have been dispersed unto the toilet seat and possibly the floor during flushing. Therefore, by closing the water closet lid, the spread of the droplets or aerosols generated could be reduced, therefore limiting exposure.
3. **Hand washing with soap:** To reduce the possibility of transmission and contamination of the contact surfaces, frequent washing of hands with soap, as recommended, should be encouraged. This provide a two-way protection, firstly limits contamination of contact surfaces and secondly, reduces the possibility of infection from contaminated hands.
4. **Face masks:** Aerosols are easily generated during flushing and these may remain suspended for a while, therefore the use of face masks could provide an additional layer of protection.

Author contributions statement

All authors were involved in the conceptualization of the manuscript, data collection was performed by I.D.Amoah, L. Pillay, N. Deepnarian, O. Awolusi, K. Pillay and P. Ramlal. Writing of the original draft manuscript was done by I.D.Amoah, L. Pillay, N. Deepnarian, O. Awolusi and K. Pillay under the supervision of S. Kumari and F. Bux. Initial

reviewing and editing of the manuscript was done by I.D.Amoah, S. Kumari and F. Bux. Final revision of the and approval was done by all authors.

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Effect of operational strategies on microbial water quality in small scale intermittent water supply systems: The case of Moamba, Mozambique

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ABSTRACT

Intermittent drinking water supply affects the health of over 300 million people globally. In Mozambique, it is largely practiced in cities and small towns. This results in frequent microbial contamination of the supplied drinking water posing a health risk to consumers. In Moamba, a small town in Southern Mozambique with 2,500 water connections, the impact of changes in operational strategies, namely increased chlorine dosage, increased supply duration and first-flush, on the microbial water quality was studied to determine best practices. To that aim, water quality monitoring was enhanced to provide sufficient data on the microbial contamination from 452 samples under the different strategies. The water at the outlet of the water treatment plant during all strategies was free of *E. coli* complying to the national standards. However, *E. coli* could be detected at household level. By increasing the chlorine dosage, the number of samples that showed *E. coli* absence increased at the two sampling locations in the distribution network: in Cimento from 72% to 83% and in Matadouro from 52% to 86%. Modifying the number and duration of supply cycles showed a different impact on the water quality at both locations in the distribution network. A positive effect was shown in Cimento, where the mean concentrations decreased slightly from 0.54 to 0.23 CFU/100 mL and 16.7 to 7.3 CFU/100 mL for *E. coli* and total coliforms respectively. The percentage of samples positive for bacteria was, however, similar. In contrast, a negative effect was shown in Matadouro where the percentage of positive samples increased and the mean bacterial concentrations increased slightly: *E. coli* from 0.9 to 1.5 CFU/100 mL and total coliforms 17.6 to 23.0 CFU/100 mL. Enhanced water quality monitoring improved operational strategies safeguarding the microbial water quality. The *E. coli* contamination of the drinking water at household level could point at recontamination in the distribution or unsafe hygienic practices at household level. Presence of faecal contamination at household level indicates potential presence of pathogens posing a health risk to consumers. Increasing chlorine dosage ensured good microbiological drinking water quality but changing the number of supply cycles had no such effect.

1. Introduction

Safe drinking water is acknowledged as a basic human right (UN, 2010) and the Sustainable Development Goal (SDG) 6, target 6.1, aims to achieve "a universal and equitable access to safe and affordable drinking water for all by 2030" (UN, 2016). It is widely known that drinking unsafe water may cause exposure to pathogens, which can result in waterborne diseases, such as cholera, gastroenteritis or hepatitis E (Howard and Bartram, 2003). However, inadequate water, sanitation and hygiene still caused 829,000 diarrhoeal deaths worldwide in 2016, which corresponds to about 60% of total diarrhoeal-related mortality rates (Prüss-Ustün et al., 2019). Progress on SDG 6 is monitored using indicator 6.1.1, which is the percentage of population using "safely managed" water supplies, i.e. whether water sources are improved, accessible on premises, available when needed (for more than 12 h per day), and free from microbial contamination.

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According to the WHO/UNICEF Joint Monitoring Programme (JMP), 29% of the world population does not have access to safely managed drinking water (WHO and UNICEF, 2017). In Mozambique, diarrheal diseases play an important role in deaths and disability, and are strongly associated with precipitation (Horn et al., 2018). Several studies describe the prevalence of infections with waterborne pathogens, such as *Vibrio cholerae, Cryptosporidium* and rotavirus in Mozambique (Casmo et al., 2018; Deus et al., 2018; Semá Baltazar et al., 2017).

Over 300 million people globally rely on intermittent water supply (IWS), piped water delivered for less than 24 h per day (Kumpel and Nelson, 2016). Numerous countries in Africa, Asia and Latin America practice IWS as a normal operational strategy because water supply companies are not able to supply water continuously and sustain a positive operating pressure within the distribution network. This is also due to high levels of leakage in distribution networks (Agathokleous and Christodoulou, 2016; Galaitsi et al., 2016; Klingel, 2012). Various studies noted that IWS is multi-faceted and co-produced by lack of water resources, infrastructure deficits and the ever increasing non-revenue water (Galaitsi et al., 2016; Kumpel and Nelson, 2016). IWS can lead to the risk of waterborne diseases due to microbial contamination through ingress of pathogens in non- or low pressurized pipes through cracks or fittings, release of microbial biofilms formed under stagnant conditions during re-pressurization, recontamination during household storage, use of unsafe alternative water sources, or limited water availability for hygiene practices (Coelho et al., 2003; Kumpel and Nelson, 2016). Cases of waterborne illnesses due to IWS continue to be documented and Bivins et al. (2017) suggested that, globally, IWS may account for 17.2 million infections causing 4.5 million cases of diarrhoea and 1560 deaths each year. Which feature of IWS increases the growth of opportunistic pathogens still needs to be investigated (Bautista-de los Santos et al., 2019). When the drinking water supply is turned on after a period without supply, drinking water may contain elevated turbidity, and high concentrations of indicator bacteria can be flushed out of the pipes (Kumpel and Nelson, 2014). Pathogens may also enter the drinking water and upon consumption may cause infections (Skraber et al., 2005).

Despite the high prevalence of IWS in the world, the literature published to date on water quality in IWS systems is limited to a few studies in large urban areas (Kumpel and Nelson, 2016), refugee camps (Alazzeh et al., 2019) and one small town in Central America (Erickson et al., 2017). In particular, small towns in sub-Saharan Africa are experiencing an increase in water demand due to population growth, while the development and appropriate management of water infrastructure and services is lagging behind (Matsinhe et al., 2008). This may lead to water shortage resulting in an increase in IWS in these towns. These towns are not only heterogeneous among themselves, but are diverse within the administrative boundaries as they often have both urban and rural areas, has implications for infrastructure planning and resource allocation (Marks et al., 2020). In Mozambique, water supply is intermittent due to old transport and distribution networks, high levels of leakage, limited hydraulic capacity and increased city demand and population growth. As of 2015, small towns represent 15% of the total Mozambican population, and this share is projected to increase to 18% (about 6.5 million people) by 2030 (World Bank, 2018). The majority of the cities in Mozambique experience intermittent supply with variable water supply duration (Gumbo et al., 2003). Therefore, the aim of this study was to evaluate how different operational strategies at full scale can improve drinking water quality in an IWS system in a small town of Mozambique. We studied the impact of increased disinfectant dosage, increased supply duration and first-flush. To the authors' knowledge this is the first study to investigate the effect of operational strategies on drinking water quality in small scale IWS systems in sub-Saharan Africa. The results will be of interest for practitioners and researchers that focus on small water systems, particularly in low-resources settings.

2. Material and methods

2.1. Study area

Moamba district is located in Mozambique, in the southern part of the Maputo province and has an area of 4,628 km^2. The district consists of four towns and has a population of 83,876 inhabitants (Instituto Nacional de Estatistica, 2018). Vila de Moamba, one of the four towns of the province, has a population of 24,650 inhabitants and 83% of the population is supplied with piped drinking water.

The Water Treatment Plant (WTP) of Moamba has a capacity of 3,000 m^3/day. The source for the production of drinking water is the Incomáti river; water is abstracted 3.5 km from the WTP. After infiltration the water is pumped into a buffer tank (80 m^3), which is connected to the WTP with a pipeline. At the WTP, the river water is subjected to:

- coagulation-flocculation based on dosing of aluminium sulphate
- rapid sand filtration by six pressure filters with a capacity of 40 m^3/h each
- disinfection by dosing chlorine solution with a calculated dose of 1.8 mg CL_2/L

The WTP is operational in two shifts: from 6:00–12:00 (morning cycle) and 15:00–19:00 (afternoon cycle). The disinfected water is stored in a 500 m^3 reservoir and 150 m^3 water tower before distribution into the network. The water supply system of the WTP covers the areas of the District of Moamba and the Administrative Post of Pessene (14 km from Moamba). The distribution network has a total length of 45 km with approximately 3,336 connections. The distribution network is made of class 9 PVC with diameters ranging from 50 mm to 250 mm. The treated water is intermittently supplied to Moamba from approximately 6:00–10:00 (morning cycle) and 15:00–18:00 (afternoon cycle), whereas Pessene receives drinking water from 10:00–15:00 and 18:00–19:00.

2.2. Experimental design

2.2.1. Chlorine dosing

To assess the effect of chlorine dosing on drinking water quality, different dosages of granular high test hypochlorite ($Ca(OCl)_2$) with 65% of active chlorine were applied. A chlorine solution was prepared by diluting $Ca(OCl)_2$ in a 200 L tank and then dosed via an injector chlorinator for 48 h. The tank was fitted with a stirrer and a positive displacement diaphragm dosing pump (Grundfos DMX 14-10, Denmark). The chlorine solution was added to the filtered water to achieve a calculated dosage of 1.8 and 2.2 mg Cl_2/L, respectively. The dosing rate of the injector chlorinator was kept constant throughout the experiments. Samples were taken every hour during supply. All experiments were performed in duplicate.

During the different dosing experiments, the concentration of the chlorine dosing suspension was adjusted to achieve the desired chlorine dosage in the different experiments.

2.2.2. Daily supply cycles

During standard operations of the WTP, water is supplied to Moamba for approximately 7 to 9 h in two daily cycles. In between those two cycles the WTP continues operating and water is supplied to the village of Pessene located about 14 km from Moamba. To investigate an effect of supply duration, water was supplied continuously for 10 h and 12 h (one cycle) to Moamba only and compared with normal operation (two cycles).

2.2.3. First flush

To assess the water quality during restart of the drinking water supply after an idle time of not supplying (first flush), samples were taken every 10 min during at least 50 min at two locations in the distribution network. The first flush was studied during standard operations with two supply cycles per day, resulting in a first flush in the morning and one in the afternoon. The effect of the first flush was examined by pairwise comparison of the results of t = 0 and t = 10 min.

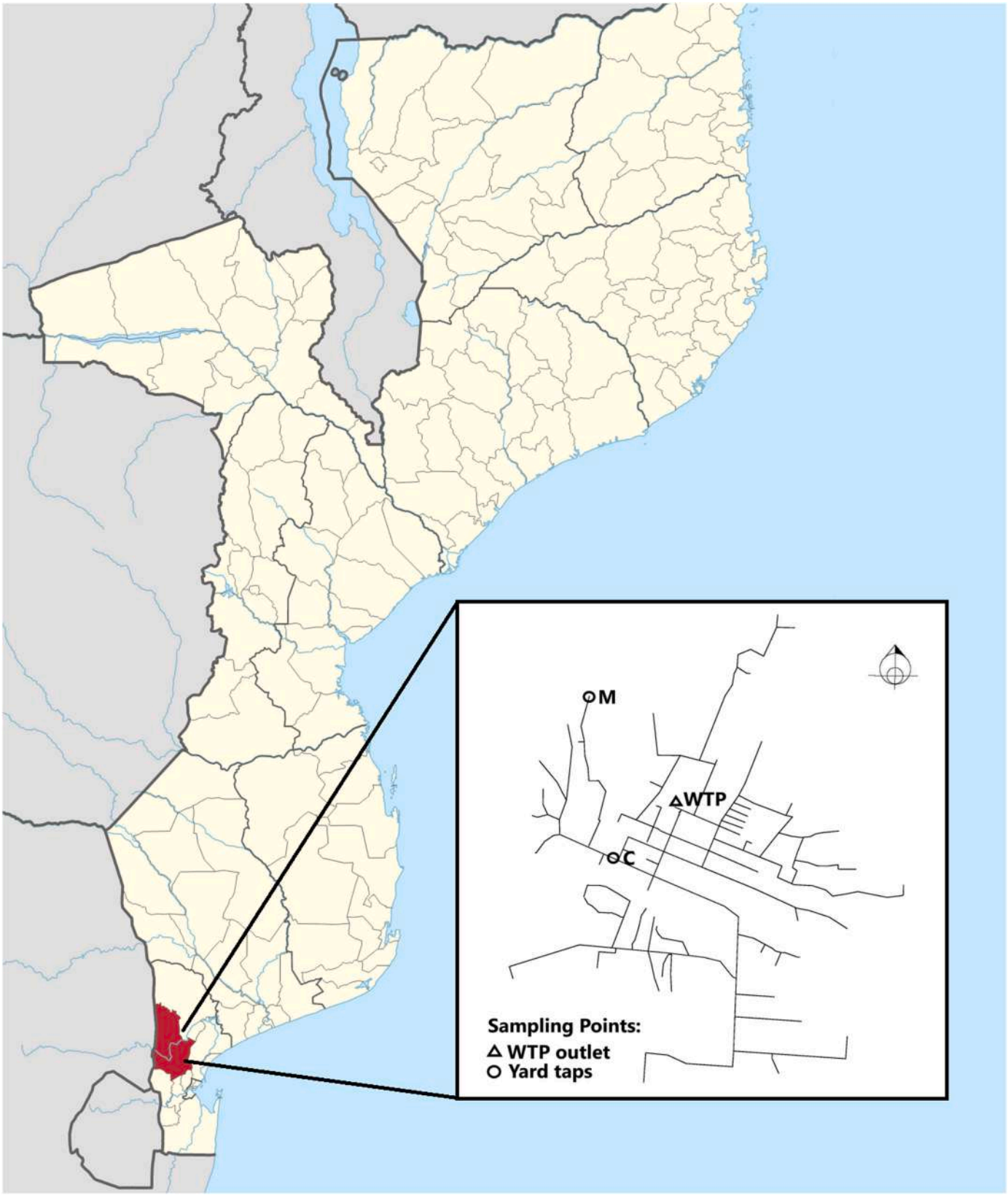

Fig. 1. Distribution network of Moamba and location of the WTP and sampling points in Matadouro (M) and Cimento (C).

2.3. Selection of sampling points

Three sampling points were selected: one at the outlet of the WTP and two household yard taps in different neighbourhoods, namely Bairro Cimento and Bairro Matadouro. The two neighbourhoods were selected based on the distance from the WTP (800 and 2,200 m, respectively) and spatial patterns of the neighbourhoods (Matadouro is a densely populated neighbourhood with lack of formal spatial planning whereas Bairro Cimento is a less dense and planned neighbourhood). The sampling points are shown in Fig. 1.

2.4. Sampling

The experiments were conducted between November 2017 and October 2018. During these experiments, samples were taken every 10 min for the first hour after starting the supply cycle and then hourly from the following locations: outlet WTP, Cimento household yard tap (C) and Matadouro household yard tap (M). The tap at the sampling points was cleaned with a clean tissue soaked with ethanol 70% and flamed before samples were taken. Samples for microbiological analyses were collected in 100 mL sterile whirl-pak thio-bags® containing sodium thiosulfate for neutralizing the residual chlorine and directly put in a cooling box for transport. The samples were stored at most 24 h prior to microbiological analyses. Samples for physico-chemical analyses were collected in 75 mL plastic cups and directly analysed in the field. In total, 717 samples were collected in this study (Table 1).

2.5. Methods

2.5.1. Physico-chemical analyses

Water temperature and pH (PT115 pH meter, Palintest, United Kingdom), free and total chlorine (PTH7100, Palintest, United Kingdom), conductivity (PT157, Palintest, United Kingdom), and turbidity (PTH092, Palintest, United Kingdom) were measured on site for 655 samples.

2.5.2. Microbiological analyses

In 452 samples enumeration of total coliforms and *Escherichia coli* was based on ISO 9308-1 (ISO, 2014), using the membrane filtration method and incubation on chromocult agar nutrient pad sets (Sartorius Stedim Biotech, Germany) for 24 h at 37 °C in a portable incubator (Aquagenx, United States), according to the manufacturer's instructions. For each sample, 100 mL was tested in duplicate. Enumeration of all dark blue to violet colonies provided the presumptive amount of *E. coli* in the filtered water volume. Salmon red colonies were coliform bacteria colonies other than *E. coli* as indicated in the suppliers' documentation.

2.5.3. Statistical analyses

Concentrations of *E. coli* and total coliforms, free chlorine concentrations and turbidity were logarithmically (base 10) transformed. Time of sampling was registered in minutes from starting drinking water supply. Water supply was from 7:00 and lasted 9, 10 or 11 h. Or, water supply was stopped after 5 h, and started again 4 h later for a duration of 4 h. Multivariate linear regression analyses of the relationship of the concentrations of *E. coli* and total coliforms respectively with the total distance from the WTP (m), time of sampling, free chlorine concentration, temperature (°C), pH, turbidity (NTU) and conductivity (micro-Siemens/cm) were conducted using R (version 3.5.2 (2018-12-20) - "Eggshell Igloo") and lm (Chambers, 1992; Wilkinson and Rogers, 1973). The model with the lowest Akaike information criterion was selected using the step-function (parameter k = 3.84). For graphical presentation of the data package ggplot2 was used (Wickham, 2016). Relations for *E. coli* and total coliforms were analysed separately, but also for the joint bacteria concentration, whereby the factor bacteria with values "*E. coli*" and "Total coliforms" was included. Similarly, a relation between both bacteria groups was analysed, as well as effects of the environmental factors, such as temperature, pH and conductivity, on free chlorine concentration and turbidity.

Table 1
Number of samples taken per experiment.

Supply duration	Calculated chlorine dosing concentration (mg Cl_2/L)		Total number of samples
	1.8	2.2	
Standard operations	275	230	505
10 h	56	54	110
12 h	64	38	102
Total number of samples	*395*	*322*	*717*

3. Results

3.1. Effect of increased chlorine dosing

Under standard operations (two supply cycles per day), increasing the calculated chlorine dosage from 1.8 to 2.2 mg Cl_2/L resulted in an increase of the mean concentration of chlorine at the outlet of the WTP by 30% from 0.79 mg/L to 1.03 mg/L, in Cimento by 77% from 0.52 mg/L to 0.92 mg/L, and in Matadouro by 75% from 0.36 mg/L to 0.63 mg/L (Fig. 2). The concentration of free chlorine observed at the same sampling points using a higher chlorine dosing concentration complied with the national Mozambican standard of 0.2–0.5 mg/L (MISAU, 2004) and the number of compliant samples at the WTP outlet increased from 90% to 100%, in Cimento from 81% to 100% and in Matadouro from 70% to 82%. The bacterial load at the outlet of the WTP showed absence of *E. coli* in all samples, and the mean concentration of coliforms was 6.5 CFU/100 mL. The concentrations of *E. coli* and total coliforms increased from the outlet of the WTP, through Cimento to Matadouro, but the difference in mean concentration with the two chlorine dosages was minimal (Fig. 2). The main difference is shown by the number of samples that showed *E. coli* absence: in Cimento it increased from 72% to 83% and in Matadouro it increased from 52% to 86% (see Supplementary Table 1). The increased chlorine dosing had an effect on the compliance with the national Mozambican standard of 0.2–0.5 mg/L (MISAU, 2004). The number of non-compliant samples containing <0.2 mg/L free chlorine at the WTP outlet decreased from 10% to 0%, in Cimento from 19% to 0% and in Matadouro from 30% to 18%. However, by increasing the chlorine dosing the number of non-compliant samples containing >0.5 mg/L free chlorine increased at the WTP from 68% to 100%, in Cimento from 45% to 75% and in Matadouro from 22% to 49%.

The free chlorine concentration under standard operations is highly significantly dependent on chlorine dose, distance from WTP and temperature (Supplementary table 2a). The residual concentration of free chlorine increased with dose (1.8–2.2 mg/L) and pH (7.2–9.2), and decreased with distance (0–2,200m) and temperature (4.2–37 °C).

Increased *E. coli* and total coliforms concentrations at higher distances from the WTP were observed (Fig. 2), this effect was statistically insignificant, probably because *E. coli* concentrations were low and the majority of data (77%, n = 232) consisted of non-detects. For more statistical power, *E. coli* and total coliform concentrations were jointly statistically analysed with bacteria as a factor. In this combined analysis, none of the conditions were found to have a significant effect (see Supplementary Table 2b). The turbidity, conductivity, pH and temperature under standard operations are shown in Supplementary Table 3.

3.2. Effect of varying daily supply cycles

Similar results were obtained for the different levels of chlorine when varying the number of daily supply cycles and the overall supply duration with a decrease in the concentration of residual chlorine over the

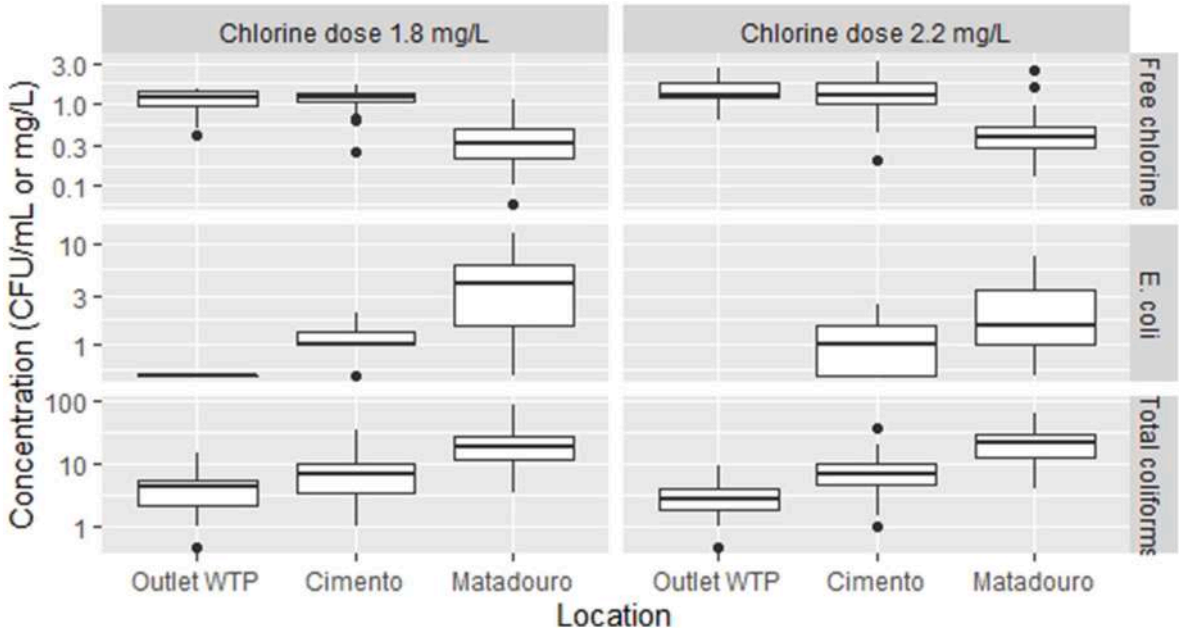

Fig. 2. ox-Whiskerplots of free chlorine, E. coli and total coliforms concentrations according to location. Each grid represents the concentration of the free residual chlorine, E. coli or total coliforms achieved with chlorine doses of 1.8 and 2.2 mg Cl_2/L. The box represents the median and quartiles, the whiskers show the 95%-interval and dots are outliers.

distance from the WTP and higher concentrations of residual chlorine by using higher dosing concentrations. For supply during one or two daily cycles, the percentage of samples positive for microbial contamination with a higher mean concentration of total coliforms and *E. coli* in Matadouro (most distant point from the WTP) than in Cimento (Table 2). Specifically, the number of samples positive for *E. coli* increased with distance from 22% in Cimento to 30% in Matadouro for two daily supply cycles and from 20% in Cimento to 42% in Matadouro for one cycle. Comparing standard operation and modified operation, no clear differences could be identified for bacterial or physico-chemical contamination. In Cimento, the percentage of samples positive for *E. coli* and total coliforms was similar while supplying one or two cycles, whereas the mean concentrations decreased with one cycle: *E. coli* decreased from 0.54 to 0.23 CFU/100 mL and total coliforms from 16.7 to 7.3 CFU/100 mL. At Matadouro the percentage of positive samples and mean concentrations of *E. coli* and total coliforms slightly increased when one supply cycle was applied. The percentage of positive samples increased from 29% to 42% for *E. coli* and from 92% to 100% for total coliforms, respectively. The mean concentration for *E. coli* and total coliforms increased from 0.9 CFU/100 mL to 1.5 CFU/100 mL and from 17.6 CFU/100 mL to 23.0 CFU/100 mL, respectively. These results show that the effect of modifying the operations can differ by location in the same distribution network.

An increase in the median and average residual concentration of free chlorine were observed at Cimento, but not at Matadouro, when changing the supply from two cycles to one cycle. Dosing experiments with 1.8 mg Cl_2/L showed a median concentration of 0.44 mg Cl_2/L and an average concentration of 0.52 mg Cl_2/L using 2 cycles, while supplying with one cycle median and average concentrations were 1.16 and 1.13 mg Cl_2/L, respectively. In Matadouro the mean and average concentration were similar, 0.32 and 0.39 mg Cl_2/L for one supply cycle versus 0.24 and 0.36 mg Cl_2/L for two supply cycles. Similar results were obtained with dosing experiments of 2.2 mg Cl_2/L. No significant change was observed for different supply durations in *E. coli* and total coliform concentrations. The bacterial concentration on $\log_{10}$ scale was highly significantly dependent on the distance, time and conductivity, see Supplementary Table 2c. Bacterial concentrations increased with distance, but decreased with increasing time and pH. Free chlorine, temperature and conductivity did not play a role according to this model. The turbidity, conductivity, pH and temperature under modified operations are shown in Supplementary Table 3.

3.3. *Effect of first flush*

In order to ascertain the effect of first flush, samples were collected every 10 min after re-starting the water supply to Moamba for the first 50 min, both in the morning and afternoon cycles. Fig. 3 shows the results of the concentration *E. coli* and total coliforms measured in the neighbourhood of Cimento (closer to the WTP) and Matadouro (further away from the WTP). The concentration of *E. coli* and total coliforms did not show a considerable increase at the beginning of the supply cycle, during the first 50 min. The mean concentration of total coliforms

Table 2
E. coli and total coliforms mean concentrations for different water supply durations.

Parameter		Standard operation Water supply during 11 h (2 cycles)		Modified operation Water supply during 10 and 12 h (1 cycle)	
		Cimento	**Matadouro**	**Cimento**	**Matadouro**
E. coli	Number of samples	105	103	76	78
	Mean concentration CFU/100 mL (min – max)	0.54 (0–11)	0.9 (0–15.5)	0.23 (0–2.5)	1.5 (0–12.5)
	Number of samples with >1 CFU/100 mL (%)	23 (22%)	31 (30%)	15 (20%)	32 (42%)
Total coliforms	Number of samples	105	103	76	78
	Mean concentration CFU/100 mL (min – max)	16.7 (0–100)	17.6 (0–75)	7.3 (0–36.5)	23.0 (0–89)
	Number of samples with >1 CFU/100 mL (%)	84 (79%)	95 (92%)	62 (80%)	78 (100%)

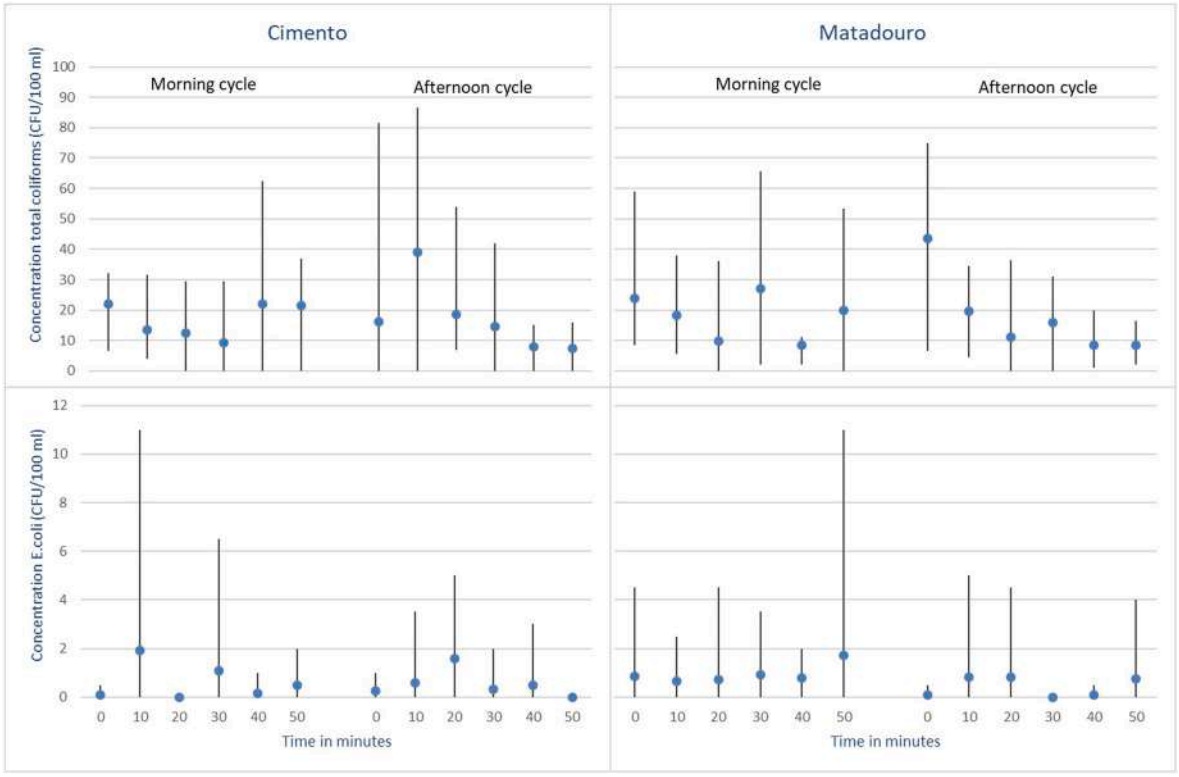

Fig. 3. Bacteriological results of the first flush after starting the distribution of drinking water during the morning and afternoon cycle. Total coliform and E.coli concentrations for Cimento and Matadouro are presented as a function of the time since the beginning of the supply cycle.

fluctuates at both locations in the morning and afternoon cycle. The mean concentration for *E. coli* in Matadouro slightly increased: in the morning cycle from 0.9 CFU/100 mL at t = 0–1.8 CFU/100 mL at t > 50 min and in the afternoon cycle 0.1 at t = 0–1.8 CFU/100 mL at t > 50. No clear correlation was found comparing every pair of measurements at 10 min intervals up to 50 min. The clearest first flush effect was expected directly after re-starting the water supply, especially by comparing t = 0 and t = 10 min. Comparing the bacterial results at t = 0 and t = 10 pairwise, showed that the concentration of coliform bacteria in Matadouro varied between a decrease of 0.47 CFU/100 mL to an increase of 0.26 CFU/100 mL, and in Cimento between a decrease of 0.20 CFU/100 mL and an increase of 0.30 CFU/100 mL. The bacterial concentration was dependent on the free chlorine concentration, but a statistical effect of time (t = 0 versus t = 10 min) was not found.

Turbidity and residual concentration of free chlorine did not show a clear increase or decrease at either of the locations. The deviation of the residual concentration of free chlorine between t = 0 and t = 10 min varied between −0.04 and 0.47 mgCl_2/L for Cimento and −0.17 and 0.43 mgCl_2/L for Matadouro. In 61% and 52% of the samples taken from Cimento and Matadouro respectively, the deviation was less than 0.1 mg Cl_2/L. The deviation in turbidity between t = 0 and t = 10 min varied between −9.4 and 1.8 NTU for Cimento and −7.0 and 2.6 NTU for Matadouro. Turbidity fluctuated between 2.2 and 27.1 NTU in Cimento and 1.2–23.5 NTU in Matadouro during all first flush experiments from t = 0 to t = 50 min, but also in this case no clear increasing or decreasing trend was identified.

4. Discussion

The aim of this study was to understand the effect of increased disinfectant dosage, number and duration of supply cycles, and first-flush on drinking water quality in an IWS system in a small town in Mozambique. When considering the indicator of faecal contamination, *E. coli*, no contamination was detected in treated water leaving the WTP, nevertheless *E. coli* was detected at the point of delivery at household level. Recontamination of the treated drinking water in the distribution could have occurred from ingress in the pipes. If faecal contamination entered the distribution system, some pathogens could have persisted even though faecal indicators were inactivated, which poses a health risk (LeChevallier et al., 2004). Similar results were obtained in large urban centres in, among others, Pakistan, India and Uganda where the water distribution systems were not capable of maintaining high water quality from the water treatment facilities to the end-user (Hashmi et al., 2008; Matsinhe et al., 2014). Based on literature, Bivins et al. (2017) showed that the available evidence suggests large variability in the prevalence of faecal contamination in IWS networks with the proportion of samples positive for *E. coli* ranging from 2% to 32%. In our study the prevalence of *E. coli* was 28% at the sampling point closer to the WTP, but as high as 48% at the furthest sampling point. For research purposes, we recommend detection of waterborne pathogens in the distribution network, such as adenovirus, rotavirus, *Cryptosporidium* and *Vibrio cholerae*, which cause infections in Mozambique (Casmo et al., 2018; Deus et al., 2018; Liu et al., 2016; Semá Baltazar et al., 2017). This information supports the need or improvement of control measures, such as chlorination, and the health risk to consumers.

An increased chlorine dose of 2.2 mg Cl_2/L improved the residual chlorine level in the distribution network by a minimum of 0.2 mg/L, thereby complying with international guidelines (WHO, 2017b) and national standards (MISAU, 2004). The residual concentration of free chlorine decreased with the distance, which is similar to other studies (Egbe and Bassey, 2016; Karikari and Ampofo, 2013; Sakomoto et al., 2020). The percentage of samples with levels of residual chlorine lower than 0.2 mg/L, 19% in Cimento and 30% in Matadouro, was much lower compared to the percentage of samples with *E. coli*, 28% in Cimento and 48% in Matadouro, and total coliforms, 81% in Cimento and 89% in Matadouro. Similar results were obtained in other studies where drinking water samples contained coliforms or *E. coli* even though the concentration of residual free chlorine was above 0.2 mg/L

(Erickson et al., 2017; Sakomoto et al., 2020). Although the number of samples complying with the national Mozambican standard of >0.2 mg/L residual chlorine increased by increasing the chlorine dose, the number of samples with concentration >0.5 mg/L and therefore not complying with the national standards (MISAU, 2004) also increased. In this study, the percentage of samples at the WTP outlet with a residual concentration of free chlorine higher than 0.5 mg/L increased from 68% to 100%, by increasing the chlorine dose from 1.8 mg Cl_2/L to 2.2 mg Cl_2/L. Of all samples from yard taps containing bacteria, 56% contained coliform bacteria even though the residual concentration of free chlorine was higher than 0.5 mg Cl_2/L. Analogously, in water samples from a WTP outlet to the tap in Ethiopia, coliforms could be detected even though containing 0.5 mg Cl_2/L free chlorine (Duressa et al., 2019). By increasing chlorine dosage, the number of samples positive for *E. coli* in the distribution network decreased (see Supplementary Table 1), in line with other studies in which a weak inverse correlation was observed between free chlorine levels and faecal coliforms (Karikari and Ampofo, 2013). If the range of residual chlorine at the WTP outlet is between 0.2 and 0.5 mg/L the concentrations at the tap very distant from the WTP may be less <0.2 mg/L. To ensure higher levels of free chlorine further in the distribution network, booster chlorination might be an option as suggested in a study in Uganda (Sakomoto et al., 2020). However, when faecal contamination of a drinking-water supply is detected, the World Health Organization recommends that the concentration of free chlorine should be increased to greater than 0.5 mg/l throughout the system as a minimum immediate response (WHO, 2017b). As *E. coli* concentrations were low and the majority of data (77%, n = 232) consisted of non-detects. For more statistical power, *E. coli* and total coliform concentrations were jointly statistically analysed with bacteria as a factor. However, no clear inverse relation was shown between increasing chlorine dosing and levels of bacteria.

In the case of Moamba, water is supplied in multiple daily cycles (Silva-Novoa Sanchez et al., 2019). In another study on IWS with multiple daily cycles in rural Nepal, consumers' perception of the level of service in terms of water quality worsens as the duration of supply decreases (Guragai et al., 2017). However, there is no evidence that the duration and number of supply cycles correlate to the water quality. In this study we increased the supply duration to up to 12 h per day, the minimum threshold used by the WHO/UNICEF JMP to track the 'available when needed' factor of target 6.1 of SDG 6. However, no association between increased availability and lower number of daily cycles (one as opposed to two) and microbial water quality was observed. In fact, the effect of modifying the operations in Moamba differed per location within the same distribution network: the bacterial concentration decreased close to the WTP outlet, and increased further in the distribution network. The residual concentrations of free chlorine at the tap closer to the WTP outlet were higher supplying one cycle compared to two cycles, but further in the distribution network the concentrations were similar. In general, microbial growth and public health implications depend on the duration of the stagnation periods, the composition of the microbial community, and disinfectants in IWS (Bautista-de los Santos et al., 2019). Microbial growth due to overnight stagnation has also been reported in continuous water supply (Lautenschlager et al., 2010; Lipphaus et al., 2014). However, our findings have not yet been followed up by further studies to investigate the causes of differentiated water quality outcomes at these specific locations. Research on the composition of the microbial community as described by Bautista-de los Santos et al. (2019) or microbial source tracking can clarify these differences or to identify possible contamination sources (Liu et al., 2018).

In this study, the effect of first flush on the microbiological water quality is not significant, although the bacterial concentrations are slightly higher at t = 0 min and t = 10 min, after starting the operation, compared with other time points. This is similar to the findings of Alabdula'aly and Khan (2017), who showed that stagnation in the distribution network affects the water quality, but not to a degree that would warrant collective actions. In contrast to our findings, other studies showed an effect of first-flush on the drinking water quality. Kumpel and Nelson (2013) showed more contamination during the first flush after the supply re-started and during periods of low pressure. In another study, the water quality was degraded during some first-flush events and after pipe breaks and repairs (Erickson et al., 2017). In the same study, higher concentrations of heterotrophic plate count and spore-forming bacteria were found during many first flush events, even when total coliform and *E. coli* were not detected (Erickson et al., 2017). Stagnation of water in the piping system caused by pressure deficits and intermittent feeding of the system entails that pathogens may enter and grow in the water distribution network (Andey and Kelkar, 2007; Jensen et al., 2002; Lee and Schwab, 2005). This hazard increases at high temperatures by running pipes close to the surface (Klingel, 2012). In this study, the water stagnated at most 14 h, and no significant difference was found between first flush events that occurred after different stagnation times. Future research is needed to better understand the importance of the effect of first-flush on pathogens.

In addition to these risks inherent to IWS, distribution systems in low and middle-income countries often have additional vulnerabilities which may degrade the water quality. Some examples are frequent pipe breaks (Lee and Schwab, 2005), poor quality control of treated water entering the distribution network (Besner et al., 2002; Lee and Schwab, 2005), and unhygienic repair practices (Besner et al., 2002). In general, for coping with the contamination ingress due to backflow through leaky joints, air valves, perforations in IWS, the WHO (2017b) recommends implementing the following control measures, where feasible: maintain positive pressure, provide continuous supply; maintain minimum chlorine residuals in the distribution network and, if necessary, install secondary/booster chlorination; implement a leak detection and repair programme; implement a pipe and fittings replacement programme; and develop design and construction specifications and standards. Climate change affects safe drinking water supply as it is expected to alter the frequency and severity of extreme weather events (WHO, 2017a). As large areas of the country are exposed to cyclones, droughts and flooding, Mozambique is vulnerable for climate change (Arndt et al., 2011). Assuming climate change alters precipitation patterns and subsequently the number of wet days diarrheal cases might increase in Mozambique (Horn et al., 2018). Therefore adaptation of the drinking water supply to climate change is required (WHO, 2017a). Implementation of a systematic risk assessment and risk management approach, such as climate-resilient Water Safety Plans, might support better understanding of possible health risks and how these can be managed, including climate change aspects (WHO, 2017a).

5. Study limitations

The results of this study are subject to a few limitations. First, experiments on the effect of supply duration at the full scale were impossible in Pessene in order not to alter the supply pattern, and water supply with even longer duration was not possible due to the existing work shifts of the utility operators. Second, only two chlorine dosages were included in this publication due to limited skills of the operator working in one of the shifts that arbitrarily decided to bypass the chlorine dosing tank and to add chlorine directly in the reservoir, making it impossible to control chlorine concentration. This episode highlighted once again the issue of limited technical capacities locally available in small towns (Tutusaus et al., 2018). Finally, in this study, only negative controls were used for microbial analyses to exclude false positive results. No positive controls were used to exclude false negatives.

6. Conclusion

The main conclusions of this study are:

- Residual concentration of free chlorine increased with dose and pH, and decreased with distance and temperature.
- No faecal contamination was detected in treated water leaving the WTP, but was assumed to enter in the distribution system. The presence of faecal contamination is indicative of the potential presence of pathogens posing a health risk for consumers.
- Increased chlorine dosage can improve compliance with microbiological water quality standards.
- The presence of chlorine resistant pathogens can still pose a risk for human health.
- The mean concentration of *E. coli* in the two sampling points in the distribution network was nearly unchanged.
- Changing the number and duration of water supply cycles showed a positive impact on microbial water quality in the sampling point closest to the WTP and negative impact in the sampling point furthest from the WTP. Thus, modifying the operations can have different impacts on the different locations in the same distribution network.
- Contrary to published literature, the effect of first flush on the microbiological water quality was not statistically significant in this study.

Declaration of competing interest

The authors declare no conflict of interest.

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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.2021.113794.

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Effects of long-term air pollution exposure on ankle-brachial index and cardio-ankle vascular index: A longitudinal cohort study using data from the Electricity Generating Authority of Thailand study

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ABSTRACT

Background: Ankle-brachial index (ABI) and cardio-ankle vascular index (CAVI) are surrogate measures of atherosclerosis based on the functional performance of vessels, and are highly related to cardiovascular events. However, only a few longitudinal studies have been conducted on their associations with long-term air pollution exposure.

Objective: This study aimed to examine whether long-term air pollution exposure is associated with ABI and CAVI in workers of the Electricity Generating Authority of Thailand (EGAT) in the Bangkok Metropolitan Region (BMR).

Methods: This longitudinal study included 1261 participants (age range, 57–76 years as of 2007) of the EGAT study (2007–2017). ABI and CAVI were measured in 2007, 2012, and 2017. Annual mean concentrations of particulate matter ≤10 μm in diameter (PM_{10}), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), ozone (O_3), and carbon monoxide (CO) were estimated by ordinary kriging using data from 22 background and 7 traffic monitoring stations in BMR between 2002 and 2017. Linear mixed-effects models were used to assess associations between air pollution (expressed as 1-year, 3-year, and 5-year average concentration) and ABI and CAVI (expressed as percent changes per interquartile range (IQR) increase in PM_{10}, O_3, NO_2, SO_2, and CO). We also applied the mixed-effect ordinal logistic models to calculate odds ratios (ORs) of having high or moderate CAVI per an IQR increase in air pollution.

Results: After controlling for potential confounders, 1-year average CO was negatively associated with ABI, but not significantly (−0.48%, 95% CI: −1.03, 0.07). Three-year average NO_2 was positively associated with CAVI (6.67%, 95% CI: 0.21, 13.1). In contrast, 1-year average PM_{10} was inversely associated with CAVI although the association was not significant. Although not significantly, 1-year average NO_2 and CO were positively associated with prevalence of high or moderate CAVI.

Conclusions: Although not statistically significant, long-term NO_2 and CO exposure was associated with ABI and CAVI in the participants of the EGAT study.

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1. Introduction

Atherosclerosis is the primary cause of ischemic heart disease and stroke. It is a chronic process in which lipids and fibrous plaque accumulate in the arteries and, coupled with inflammatory processes, cause lumen occlusion and plaque rupture (Libby and Theroux, 2005; Lusis, 2000; Ross Russell, 1993). Künzli et al. (2011) demonstrated that measures of atherosclerosis can serve as health outcomes in air pollution-related epidemiological research, especially those derived from morphological characteristics of the arterial wall (e.g., carotid intima-media thickness, coronary artery calcification) and functional performance of vessels (e.g., ankle-brachial index (ABI), arterial stiffness).

ABI is a biomarker of the degree of subclinical peripheral atherosclerosis and is measured as a ratio of systolic blood pressure at the ankle to that at the brachial artery in the arm (Heald et al., 2006; Norgren et al., 2007). The diagnosis of peripheral artery disease is indicated by ABI <0.9. Additionally, a high ABI (>1.3) suggests arterial stiffness in the lower extremities, which indicates vessel incompressibility (Aboyans et al., 2012). An increased risk of cardiovascular diseases (CVD) in relation to both low and high ABI has been suggested (Ankle Brachial Index Collaboration, 2008; Heald et al., 2006; Resnick et al., 2004).

There is evidence for relationships between ABI and exposure to air pollutants in North American and Western European countries, albeit inconsistent. A higher prevalence of low and high ABI was associated with long-term exposure to particulate matter ≤2.5 μm in diameter ($PM_{2.5}$), particulate matter ≤10 μm in diameter (PM_{10}), and nitrogen dioxide (NO_2) (Zhang et al., 2018). Long-term exposure to traffic-related NO_2 was also positively associated with high ABI, but not low ABI (Rivera et al., 2013). On the other hand, some studies found no associations between ABI and $PM_{2.5}$ and PM_{10} (Hoffmann et al., 2009; Roux et al., 2008). To the best of our knowledge, no study has been conducted in Asian countries, where air quality is relatively poor.

Cardio-ankle vascular index (CAVI) is a new non-invasive parameter of arterial stiffness which can be measured by electrocardiogram, phonocardiogram, and pulse wave velocity (PWV). It is a measure of overall stiffness of the artery from the aorta to the ankle. Since CAVI is calculated from heart-ankle PWV, it is theoretically independent of blood pressure at the time of measurement (Asmar, 2017; Miyoshi and Ito, 2016; Shirai et al., 2011; Sun, 2013; Yambe et al., 2004) and may thus serve as a better predictor of arterial stiffness (Takaki et al., 2007). Previous studies have also suggested CAVI to be a useful long-term predictor of CVD risk (Mizuguchi et al., 2007; Nakamura et al., 2008; Yingchoncharoen et al., 2012). High CAVI was associated with coronary artery disease (CAD), cerebral artery disease, and chronic kidney disease (Asmar, 2017). However, limited studies exist on CAVI in relation to air pollutants, with only two studies from Taiwan suggesting a possible positive association between CAVI and personal short-term exposure to particulate matter 1.0–2.5 μm in diameter ($PM_{1\text{-}2.5}$), ozone (O_3), and $PM_{1\text{-}2.5}$ components (Wu et al., 2010, 2012). No study has investigated the association between CAVI and long-term air pollution exposure.

The main sources of air pollutants in Bangkok are the city's suffocating traffic and agricultural burning (Chuersuwan et al., 2008). Periodically, the concentrations of PM_{10}, $PM_{2.5}$, and other gaseous pollutants such as O_3, NO_2, sulfur dioxide (SO_2), and carbon monoxide (CO) have intermittently been measured to be over the yearly, or 24-h average values indicated in WHO air quality guidelines (WHO Regional Office for Europe, 2006). Although many epidemiological studies in Thailand have demonstrated significant associations between air pollution and CVD, those studies considered only short-term effects, and long-term effects of air pollution have seldom been examined (Paoin et al., 2021). Therefore, the present study aimed to examine the association between long-term air pollution exposure and ABI and CAVI in a large retrospective cohort study, called the Electricity Generating Authority of Thailand (EGAT) study, which was conducted in workers of EGAT on the chronic disease and incidence of CVD (Vathesatogkit et al., 2012) in the Bangkok Metropolitan Region (BMR).

2. Methods

2.1. Study design and participants

Details of the EGAT study have been described previously (Vathesatogkit et al., 2012). Briefly, the EGAT cohort study consisted of three cohorts (i.e., EGAT1, EGAT2, and EGAT3). EGAT1 and EGAT3 cohorts located at the EGAT's headquarters in BMR, while EGAT2 was located at three separate hydro-electric dams at Western and Northern Thailand. The current study included the data from EGAT1 cohort from 2007 to 2017. This cohort was conducted in 1985 (followed up in 1997, 2002, 2007, 2012, and 2017) among 3499 randomly enrolled workers for CVD risk factors in nutrition and toxicology (Vathesatogkit et al., 2012). The EGAT study was approved by the Ethics Committee of Ramathibodi Hospital.

For the present longitudinal study, we extracted data of 1839 participants (age range, 57–76 years as of 2007) who lived in BMR in 2007 (Figure S1). The following criteria were used: 1) participants who were followed up 2 or 3 times during the period spanning 2007 to 2017; 2) participants who lived in Bangkok, Nonthaburi, Samut Prakarn, or Pathum Thani. We excluded 578 participants who were lost to follow-up or had died or moved out of the study area since 2007. We also excluded 46 participants with ABI <0.9 (32 participants) or >1.3 (14 participants) for the CAVI study because of possible peripheral arterial disease and calcification of ankle arteries (Marumo et al., 2018; Sato et al., 2016), respectively. These participants may give a falsely low CAVI score (Shirai et al., 2006).

ABI and CAVI were measured with a VaSera CAVI instrument (Fukuda Denshi Co., Ltd., Tokyo, Japan) using previously described methods (Shirai et al., 2006). Each measurement was performed for both the right and left ankles, yielding two sets of ABI and CAVI measurements for each participant. For both ABI and CAVI, the average of the left- and right-side measurements was obtained (Yingchoncharoen et al., 2012).

2.2. Data collection and physical examination

Physical examination data and sociodemographic characteristics such as sex, age, blood pressure, heart rate, weight, height, waist and hip circumference, body mass index (BMI), waist/hip ratio, smoking status, alcohol drinking, regular exercise, education level, income, prevalence of diseases (e.g., hypertension, diabetes, and hypercholesterolemia), treatment status, and medication use were retrieved from EGAT1 cohort data (Vathesatogkit et al., 2012).

2.3. Exposure assessment

Hourly air pollution data, including PM_{10}, SO_2, NO_2, CO, and O_3 levels, were extracted from the database of the Pollution Control Department (PCD), a governmental organization responsible for monitoring air pollution in Thailand.

We used ordinary kriging (Geniaux et al., 2017; Leem et al., 2006; Liu et al., 1996) to estimate daily average exposure to PM_{10}, SO_2, NO_2, and CO. For O_3, daily maximum 8-hr average was estimated, as described elsewhere (Paoin et al., 2021). In brief, concentrations of air pollutants measured at 22 background and 7 traffic monitoring sites in BMR from 2002 to 2017 were used to generate a long-term annual average from all discontinuous site-specific measurements. The number of monitoring sites which used to generate ordinary kriging models were varied during study period (Table S1). Grids of 100 × 100 m were generated for the prediction. For each air pollutant, the average concentration was estimated at the sub-district level based on concentrations in grids closest to the centroid of each sub-district. The assignment was based on the sub-district address of each participant (sub-district

levels).

One, three, and five-year average concentrations preceding the measurement of ABI and CAVI were used as indices for long-term exposure to air pollution, as the period of follow-up was 5 years in the present study.

2.4. Covariates

A wide range of covariates were selected from previous reports (Hoffmann et al., 2009; Rivera et al., 2013; Roux et al., 2008; Wu et al., 2010; Zhang et al., 2018). Data including age (years), sex (male/female), BMI (kg/m^2), smoking status (never-smoker/former-smoker/current-smoker), alcohol drinking (non-user/former-user/occasional-user/current-user), regular exercise (<3 times per week/at least 3 times per week), education level (0th-8th grade/9th-12th grade/>12th grade), income (<10,000 Thai baht/10,000–20,000 Thai baht/20,000–50,000 Thai baht/>50,000 Thai baht), hypertension (yes/no), diabetes (yes/no), hypercholesterolemia (yes/no), treatment of hypertension (yes/no), and treatment of diabetes (yes/no).

2.5. Statistical analysis

Linear mixed-effects models were used to analyze associations between long-term air pollution exposure (PM_{10}, O_3, NO_2, SO_2, and CO) and ABI and CAVI. Within-participant variation was treated as a random effect. Time-varying covariates (i.e., age, BMI, hypertension, diabetes, hypercholesterolemia, treatment of hypertension and diabetes, regular exercise, smoking status, alcohol drinking, income) were treated as constant during each 5-year follow-up period (2002–2007, 2007–2012, and 2012–2017). For example, covariates in year 2002 were carried forward for the period of 2002–2007, while covariates in year 2007 were carried forward for the period of 2007–2012. These covariates remained almost constant during all 5-year follow-up periods.

For each air pollutant, three predefined adjustment models were constructed, as follows: the basic model included only age and sex as covariates (Model I); the second model (Model II) included age, sex, BMI, regular exercise, smoking status, alcohol drinking, education level and income as covariates; and the third model (Model III; main model) included covariates from Model II plus hypertension, diabetes, hypercholesterolemia, treatment of hypertension and diabetes as covariates.

The main models were also stratified by different exposure windows using 1-year, 3-year, and 5-year average of air pollution concentrations before examination only for non-movers during 2007–2017. We applied two-pollutant models for each pollutant to evaluate the robustness of the one-pollutant models if more than one pollutant had a significant effect on an outcome. To assess the robustness of the results, we examined the associations using both right- and left-side ABI and CAVI, as well as the maximum and minimum right- and left-side ABI and CAVI. We also restricted analysis in the group of participants with normal ABI (ABI = 0.9–1.3). In addition, the main models were also stratified by income group, including high income (>50,000 Thai baht per month) and low to middle income (≤50,000 Thai baht per month); education levels, including high education (>12th Grade) and low education (≤12th Grade); overweight (BMI ≥ 25 or BMI < 25 kg/m^2); disease prevalence (yes or no), including hypertension, diabetes, and hypercholesterolemia at the baseline in 2007. We estimated percent changes in ABI and CAVI per interquartile range (IQR) increase in PM_{10}, O_3, NO_2, SO_2, and CO, with 95% confidence intervals (CIs).

We also tested for statistical differences between the effect estimates among different subgroups by calculating the 95% CI as shown below:

$$Q_1 - Q_2 \pm 1.96\sqrt{(SE_1)^2 + (SE_2)^2}$$

Where Q_1 and Q_2 are the estimates of two categories, and SE_1 and SE_2 are their respective standard error (Schenker and Gentleman, 2001).

We also applied mixed-effect ordinal logistic models to assess the odds ratios (ORs) per an IQR increase in air pollution on prevalence of high CAVI using the category of normal or a mild risk of atherosclerosis (CAVI<8 (low CAVI), n = 472 CAVI measurement), borderline or a moderate risk of atherosclerosis (8 ≤ CAVI < 9 (moderate CAVI), n = 884 CAVI measurement), and a high risk of atherosclerosis (CAVI ≥ 9 (high CAVI), n = 1464 CAVI measurement) as used by previous studies (Gómez-Marcos et al., 2015; Park et al., 2018; Saiki et al., 2020) per an IQR increase in air pollution. The higher value of the measures between right- and left-sided CAVI values was used for the analysis of having high or moderate CAVI versus low CAVI. All models were adjusted using the variables from the main model (Model III). All statistical analyses were performed using R statistical project version 3.6.1. P < 0.05 was considered statistically significant.

3. Results

Approximately 70% of participants in the EGAT1 cohort were male (age range, 57–76 years as of 2007) (Table 1). In 2007, almost 96% of participants lived in Bangkok and Nonthaburi, almost 40% had a high

Table 1
Basic characteristics of study participants at baseline (in 2007).

Variables	ABI study (N = 1261)	CAVI study (N = 1215)
Sex, n (%)		
Male	901 (71.5)	867 (71.4)
Female	360 (28.5)	348 (28.6)
Age, years		
Mean ± SD	63.6 ± 4.5	63.5 ± 4.5
Range	57–76	57–76
Body mass index, kg/m^2		
Mean ± SD	24.9 ± 3.5	24.8 ± 3.4
Smoking status, n (%)		
Never smoker	721 (57.2)	696 (57.3)
Former smoker	420 (33.3)	406 (33.4)
Current smoker	113 (9.0)	106 (8.7)
Alcohol drinking, n (%)		
Non-user	469 (37.2)	450 (37.0)
Former-user	167 (13.2)	161 (13.3)
Occasional-user (<1 day/week)	379 (30.1)	367 (30.2)
Current-user	234 (18.6)	225 (18.5)
Regular exercise, n (%)		
<3 times/week	941 (74.6)	904 (74.4)
At least 3 times/week	311 (24.7)	302 (24.9)
Education level, n (%)		
0 – 8th Grade	236 (18.7)	227 (18.7)
9th – 12th Grade	400 (31.7)	388 (31.9)
>12th Grade	610 (48.4)	585 (48.1)
Income (monthly), n (%)		
<10,000 Baht	138 (10.9)	134 (11.0)
10,000–20,000 Baht	150 (11.9)	145 (11.9)
20,000–50,000 Baht	338 (26.8)	329 (27.1)
>50,000 Baht	484 (38.4)	464 (38.2)
Prevalence of diseases, n (%)		
Hypertension	684 (54.2)	652 (53.7)
Diabetes	217 (17.2)	205 (16.9)
Hypercholesterolemia	593 (47.0)	569 (46.8)
Treatment status, n (%)		
Hypertension	471 (37.4)	448 (36.9)
Diabetes	201 (15.9)	191 (15.7)
City of residence, n (%)		
Bangkok	615 (48.8)	590 (48.6)
Nonthaburi	593 (47.0)	576 (47.4)
Samut Prakarn	23 (1.8)	22 (1.8)
Phathum Thani	30 (2.4)	27 (2.2)
ABI		
Median ± IQR	1.1 ± 0.07	1.1 ± 0.09
CAVI		
Median ± IQR		8.48 ± 1.35

Abbreviations: ABI, ankle brachial index; CAVI, cardio ankle vascular index; SD, standard deviation; IQR, interquartile range.

income (>50,000 baht/month) and high education level (>12th Grade), all were retired (age >55 years), around 50% had hypertension and hypercholesterolemia, and over 15% had diabetes.

Each participant was followed-up every 5 years in 2007 (1839 participants), 2012 (1189 participants) and 2017 (876 participants). The characteristics of study participants at second follow-up in 2012 were shown in Appendix (Table S2). We included 1261 participants with 2933 ABI measurement for ABI analysis, and 1215 participants with 2820 CAVI measurement for CAVI analysis. Median (range) ABI and CAVI were 1.1 (0.53–1.45) and 8.87 (1.12–14.6), respectively. All ABI and CAVI values, including average values and left- and right-side values, followed a normal distribution. During 10 years of follow-up, there were 46 participants who had abnormal ABI, including 32 participants with ABI <0.9 and 14 participants with ABI >1.3. Additionally, more than a half of CAVI measurements (1464 of 2820 CAVI measurements) had CAVI ≥ 9 which referred to a high risk of atherosclerosis.

Our model showed good model performance from 2002 to 2017; leave-one-out cross validation R^2 values were 0.99 for PM_{10}, O_3, NO_2, and SO_2, and 0.98 for CO. The model predictions also showed low bias values, whereby the cross-validation slopes (predicted vs. observed) were 0.99 for PM_{10}, O_3, NO_2, and SO_2, and 0.98 for CO.

Table 2 shows summary statistics of air pollutant exposure levels with mean, standard deviation (SD), range, and IQR for all participants (1-year average air pollution levels) from 2002 to 2017. The statistics were calculated based on the 1-year average assigned to participants (districts with more participants had more weight). In this study, the average concentration of PM_{10} was 43.3 μg/m^3, which is close to the annual PM_{10} standard in Thailand (50 μg/m^3). The 1-year average PM_{10} (standard deviation) concentrations were 46.1 (9.0), 34.3 (6.0), and 51.1 (5.5) μg/m^3 in 2007, 2012, and 2017, respectively. Annual concentrations of PM_{10} in each sub-district in 2002, 2007, 2012, and 2017 were shown in Appendix (Figure S2). NO_2 and SO_2 concentrations were lower than the respective annual standards in Thailand. There is no annual standard established for O_3 and CO in Thailand. Table 3 shows the positive correlation between the air pollutants.

Table 4 presents percent changes in ABI and CAVI per IQR increase in 1-year average levels of PM_{10}, O_3, NO_2, SO_2, and CO estimated in Models I, II, and III. PM_{10}, SO_2, and CO were negatively associated with ABI in Models I and II. In the main model (i.e., Model III), higher PM_{10}, SO_2, and CO were associated with lower ABI, although the marginally negative association was only for CO [-0.48% (95%CI: −1.03, 0.07) per IQR increment]. We observed a null association between O_3 and NO_2 with ABI in all models.

NO_2 was significantly associated with higher CAVI in Models I and II. After adjusting for history of diseases and treatment status, the result of NO_2 and CAVI became insignificant in Model III [5.73% (95%CI: −1.31, 12.8) per IQR increment]. PM_{10} was inversely associated with CAVI [-5.05% (95%CI: −10.7, 0.59) per IQR increment], although the association was not significant in Model III.

During 10 years of follow-up (2007–2017), there were 85 (for ABI study) and 81 (for CAVI study) participants who moved to other addresses but they still lived in our study area. For the non-movers, associations of 3-year and 5-year average air pollutants with ABI and CAVI were similar to those of 1-year average air pollutants (Table S3).

Table 2
Summary statistics of exposure levels for all participants (1-year average air pollution levels) during the study period.

Environmental variable	Mean ± SD	Range	IQR
PM_{10} (μg/m^3)	43.3 ± 10.0	23.6–111.2	11.9
O_3 (ppb)	31.3 ± 4.1	17.0–40.1	5.3
NO_2 (ppb)	19.4 ± 4.5	7.1–30.2	5.0
SO_2 (ppb)	3.6 ± 1.4	1.0–11.1	2.1
CO (ppm)	0.7 ± 0.1	0.3–1.6	0.2

Abbreviations: SD, standard deviation; IQR, interquartile range; ppb, part per billion; ppm, part per million.

Table 3
Correlation coefficients between air pollutants during the study period.

	PM_{10}	O_3	NO_2	SO_2	CO
O_3	0.32				
NO_2	0.44	0.28			
SO_2	0.14	0.04	0.14		
CO	0.33	0.16	0.49	0.12	

Table 4
Estimated percent change (95% CI) in ABI and CAVI per IQR increase in 1-year average levels of pollutants.

	PM_{10} (μg/m^3)	O_3 (ppb)	NO_2 (ppb)	SO_2 (ppb)	CO (ppm)
ABI					
Model I	−0.53 (−0.83, −0.24)*	0.05 (−0.35, 0.46)	0.1 (−0.2, 0.41)	−0.73 (−1.2, −0.27)*	−0.59 (−1.01, −0.17)*
Model II	−0.47 (−0.85, −0.1)*	0.16 (−0.34, 0.65)	−0.17 (−0.61, 0.27)	−0.66 (−1.2, −0.11)*	−0.77 (−1.24, −0.3)*
Model III	−0.25 (−0.66, 0.17)	−0.32 (−0.89, 0.26)	−0.17 (−0.69, 0.36)	−0.29 (−0.92, 0.34)	−0.48 (−1.03, 0.07)
CAVI					
Model I	−7.38 (−11.5, −3.24)*	−1.22 (−7.03, 4.59)	5.25 (0.89, 9.61)*	−3.87 (−10.5, 2.75)	2.17 (−3.91, 8.25)
Model II	−6.57 (−11.9, −1.24)*	−1.67 (−8.82, 5.47)	6.62 (0.37, 12.9)*	−1.58 (−9.44, 6.28)	2.08 (−4.7, 8.86)
Model III	−5.05 (−10.7, 0.59)	−0.97 (−8.94, 7.01)	5.73 (−1.31, 12.8)	0.5 (−8.08, 9.08)	3.56 (−3.96, 11.1)

Coefficients are expressed as percent change per IQR (11.9 μg/m^3 for PM_{10}, 5.3 ppb for O_3, 5.0 ppb for NO_2, 2.1 ppb for SO_2 and 0.2 ppm for CO). Significance indicated by *P < 0.05. Model I: adjusted for age and sex; Model II: further adjusted for BMI, smoking status, alcohol drinking, regular exercise, education level and income. Model III (main model): further adjusted for hypertension, diabetes, hypercholesterolemia, and treatment of hypertension and diabetes.

Although not statistically significant, 5-year average CO showed a marginally inverse association with ABI [-0.51% (95%CI: −1.13, 0.12) per 0.2 ppm]. CAVI was positively associated with 3-year [6.67% (95% CI: 0.21, 13.1) per 3.4 ppb] and 5-year average NO_2 [5.92% (95%CI: −0.45, 12.3) per 3.3 ppb].

In the sensitivity analyses (Table S4), the results for right- and left-side ABI and CAVI, as well as maximum and minimum right- and left-side ABI and CAVI, followed the same patterns as those observed for average ABI and CAVI. The results of the sub-analysis in the group of participants with normal ABI were not substantially different from those observed in all participants (Table S5).

The associations of PM_{10} with ABI and CAVI were significantly different among participants with different income levels (p-value of 0.02 for ABI and 0.08 for CAVI). Specifically, stronger negative association was observed in low-middle income compared to high income participants (Table S6 and Table S7). In addition, the association between PM_{10} and ABI was significantly different among participants with and without hypercholesterolemia (p-value = 0.03), where stronger negative estimate was observed in participants without hypercholesterolemia (Table S6). We found stronger inverse associations between CO and ABI in participants with high income and education level, and participants without diabetes (Table S6). Besides, NO_2 was significantly stronger associated with higher CAVI in participants with hypertension (p-value = 0.02), and also participants without hypercholesterolemia (p-value = 0.08) (Table S7).

Table 5 presents the ORs of having high or moderate CAVI versus low CAVI per IQR increase in 1-year average levels of PM_{10}, O_3, NO_2, SO_2, and CO estimated in the main model (Model III). Although NO_2 and CO

Table 5
The odd ratios (95%CI) of having high or moderate CAVI versus low CAVI for IQR increase in 1-year average levels of pollutants.

	High CAVI
PM_{10}	0.95 (0.83, 1.07)
O_3	0.97 (0.83, 1.14)
NO_2	1.08 (0.92, 1.26)
SO_2	1.06 (0.88, 1.27)
CO	1.15 (0.98, 1.34)

Coefficients are expressed as ORs per IQR (11.9 μg/m^3 for PM_{10}, 5.3 ppb for O_3, 5.0 ppb for NO_2, 2.1 ppb for SO_2 and 0.2 ppm for CO). Significance indicated by: * P-value < 0.05. All models were 3rd model.

were positively associated with prevalence of high or moderate CAVI, the associations were not significant. We observed no clear association between prevalence of high or moderate CAVI with PM_{10}, O_3, and SO_2.

4. Discussion

In this longitudinal study, we assessed the associations between long-term air pollution exposure and ABI and CAVI in workers of EGAT in BMR, Thailand. We found that higher 1-year average CO exposure was associated with lower ABI, but not significantly. We also observed a positive association between 3-year average NO_2 and CAVI. In contrast, 1-year average PM_{10} showed a marginally inverse association with CAVI. Although not significantly, 1-year average NO_2 and CO were positively associated with prevalence of high or moderate CAVI.

In a cross-sectional study, long-term exposure to $PM_{2.5}$, PM_{10}, and NO_2 was associated with the prevalence of abnormal ABI (ABI <0.9 or >1.3) (Zhang et al., 2018). Another cross-sectional study reported that long-term exposure to traffic-related NO_2 was positively related to a higher prevalence of high ABI (>1.3), but not low ABI (<0.9) (Rivera et al., 2013). In contrast, some recent studies found no clear associations of $PM_{2.5}$ and PM_{10} with ABI (Hoffmann et al., 2009; Roux et al., 2008). In the present study, ABI was negatively associated with long-term exposure to CO, but the association was not significant.

The mean or median of ABI levels of our study and previous studies were around 1.1 (Hoffmann et al., 2009; Rivera et al., 2013; Roux et al., 2008; Zhang et al., 2018). The average PM_{10} (43.3 μg/m^3) and NO_2 (19.4 ppb) levels in our study were much higher than PM_{10} (Roux et al., 2008; Zhang et al., 2018) and NO_2 (Rivera et al., 2013; Zhang et al., 2018) levels in previous studies, respectively.

Based on evidence from animal studies, mechanisms underlying the development of atherosclerosis due to air pollution exposure have been suggested to involve vascular damage through systemic inflammation and oxidative stress (Araujo et al., 2008; Araujo and Nel, 2009; Chen et al., 2010; Chen and Nadziejko, 2005; Niwa et al., 2007; Simkhovich et al., 2008; Sun et al., 2005; Suwa et al., 2002), autonomic imbalance, and endothelial dysfunction (Brook and Rajagopalan, 2010; Rodríguez-Mañas et al., 2009).

To our knowledge, this is the first longitudinal cohort study to examine the association between long-term air pollution exposure and CAVI. We observed a positive association between CAVI and long-term exposure to NO_2 in workers of EGAT, but no associations were observed for other pollutants. Decreased bioavailability of nitric oxide may cause altered vascular autonomic control and endothelial dysfunction, leading to changes in arterial stiffness (Tanaka and Safar, 2005; Unosson et al., 2013).

In previous cross-sectional studies, the time period of exposure to air pollution ranged from 1 year (Hoffmann et al., 2009; Zhang et al., 2018) to 10 (Rivera et al., 2013) or 20 (Roux et al., 2008) years. Two of these studies described associations between ABI and 1-year average PM and NO_2 (Zhang et al., 2018) and 10-year average NO_2 (Rivera et al., 2013), suggesting that the time period of air pollution exposure that affects ABI and CAVI is long (i.e., from 1 year up to 10 years). Furthermore, a previous pilot study detected changes in human carotid atherosclerosis using high-resolution magnetic resonance imaging in individual participants at both 16 and 24 months (Adams et al., 2004). Therefore, the progression of atherosclerosis may be observed more clearly in longer time frames. Although not significantly, we observed associations between long-term NO_2 and CO exposure and ABI and CAVI, i.e., indicators of the progression of atherosclerosis based on the functional performance of vessels.

The information set shows essential data, such as the disease prevalence, BMI, educational attainment, income, exercise habits, alcohol intake, and smoking status. Subsequently, these variables were included in the model. Ordinary kriging was developed to assess the spatial representativeness of monitoring stations and improve the exposure of air pollution estimation accuracy.

This study has several limitations. First, EGAT participants had a higher salary, education level, and socioeconomic status than the general Thai population (Vathesatogkit et al., 2012). Furthermore, this study only involved individuals of older ages, who might be more susceptible to air pollution effects. Second, traffic variables and land use data were excluded from the ordinary kriging system according to the limitation of Thailand's available information. Nevertheless, air pollution data from traffic monitoring stations were used for the assessments of traffic-related air pollution exposure (e.g., NO_2 and CO). Moreover, the limitation of ordinary kriging method is that when the limited number of monitoring stations were used for model, bias might be arisen especially in the area with one or none of monitoring station close by. However, our study area has 29 monitoring stations (22 ambient stations and 7 traffic stations) in 7762 km^2 as shown in Figure 2 of our previous study (Paoin et al., 2021). Therefore, we believe that the current approach is justifiable for this analysis. Lastly, the date of movement for each participant was not available if some participants moved to other addresses but they still lived in our study area. We assumed that they did not move during 1-year prior the ABI and CAVI measurement. However, we added sensitivity analyses using 1-year, 3-year, and 5-year average air pollution concentrations prior the examination for only non-movers.

5. Conclusions

Long-term exposure to NO_2 and CO was associated with ABI and CAVI in participants of the EGAT study, but the associations were not significant. Further longitudinal studies on air pollutants, especially $PM_{2.5}$, and their associations with CAVI and ABI will be needed to understand and elucidate potential underlying mechanisms. Besides, future studies should focus on the variations in exposure levels based on emission sources, mostly driven by the geographical variabilities.

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Ethics and consent

Approval for the study was obtained from the Ethics Committees of Ramathibodi Hospital and the Graduate School of Engineering, Kyoto University.

Declaration of competing interest

The authors declare no conflicts of interest.

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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.2021.113790.

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First nationwide exposure profile of major persistent organic pollutants among Korean adults and their determinants: Korean National Environmental Health Survey Cycle 3 (2015–2017)

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ABSTRACT

Since 2009, Korea has measured the exposure levels of major environmental chemicals and heavy metals among representative adult populations through the Korean National Environmental Health Survey (KoNEHS). However, exposure to persistent organic pollutants (POPs) has never been assessed. This study reports the serum concentrations of twenty-four POPs and their influencing factors for Korean adults (n = 1295) who participated in the KoNEHS Cycle 3 (2015–2017). The POPs included seven organochlorine pesticides (OCPs), eleven polychlorinated biphenyls (PCBs), and six polybrominated diphenyl ethers (PBDEs). Among them, three OCPs (i.e., hexachlorobenzene (HCB), *p,p′*-dichlorodiphenyltrichloroethane (*p,p′*-DDT), and *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE)) and five PCBs (i.e., PCB52, PCB118, PCB138, PCB153, and PCB180) were detected in over 60% of the samples. PBDEs were not detected at a detection frequency of 60% or above. The most frequently detected POPs were *p,p′*-DDE (99.8%, geometric mean of 128.47 ng/g lipid), followed by PCB180 (98.8%, 8.49 ng/g lipid), PCB153 (98.8%, 13.14 ng/g lipid), HCB (96.2%, 67.08 ng/g lipid), PCB138 (95.2%, 8.84 ng/g lipid), PCB118 (89.6%, 2.66 ng/g lipid), *p,p′*-DDT (80.5%, 6.68 ng/g lipid), and PCB52 (71.2%, 1.57 ng/g lipid). The concentrations of most POPs were lower than or similar to concentrations reported in national-scale biomonitoring surveys. The only exception was HCB, whose concentration was up to seven-fold higher than the concentration reported by the Canadian Health Measures Survey. Excluding HCB and PCB52, most POPs showed increasing serum levels among older adults, adults with higher body mass index, adults living in coastal areas, and more frequent fish consumption. Relatively higher POP concentrations were observed in menopausal women. This study provides the first data on POP exposure levels among the representative adult population in Korea, and the results highlight the need to integrate POPs in the national biomonitoring program.

1. Introduction

Persistent organic pollutants (POPs) are not easily dissipated in the natural environment and are prone to long-range environmental transport (Wania and Mackay, 1996). They are highly lipophilic chemicals that tend to bio-accumulate and bio-magnify in food chains (Jones and De Voogt, 1999). Owing to the demonstrated or potential adverse effects of POPs such as reproductive disorders, endocrine system disorders, and carcinogenicity, most countries have banned their use since the early 1970s (Arrebola et al., 2018; Luo et al., 2017). These POPs include toxic substances, such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) (WHO, 2003). In addition to the initial list of twelve legacy POPs, new POPs were recognized by the Stockholm Convention on POPs in 2009, including eight polybrominated diphenyl ethers (PBDEs) (Lohmann et al., 2007; Sharkey et al., 2020; UNEP, 2009). PBDEs were previously extensively used as organic flame retardants and are structurally and toxicologically similar to PCBs (Meeker et al., 2009; Rahman et al., 2001; We et al., 2011).

Following the prohibition or regulation of the production and use of POPs, their concentrations in environmental media, biota and humans have slowly but gradually decreased; however, they are still widely detected in ecosystems and human tissues owing to their persistence and bio-accumulative characteristics. Moreover, such POPs are still released from some products that were manufactured prior to the introduction of associated regulations, and they circulate in the environment through treatment processes, leading to continuous human exposure (Černá et al., 2008; Panseri et al., 2019). In Korea, similar to in many other

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Abbreviations

BMI	Body mass index
CHMS	Canadian Health Measures Survey
HCB	hexachlorobenzene
KoNEHS	Korean National Environmental Health Survey
LOD	limit of detection
OCPs	organochlorine pesticides
PBDEs	polybrominated diphenyl ethers
PCBs	polychlorinated biphenyls
POPs	persistent organic pollutants
p,p′-DDE	*p,p′*-dichlorodiphenyldichloroethylene
p,p′-DDT	*p,p′*-dichlorodiphenyltrichloroethane
PCB52	2,2′,5,5′-tetrachlorobiphenyl
PCB118	2,3′,4,4′,5-pentachlorobiphenyl
PCB138	2,2′,3,4,4′,5′-hexachlorobiphenyl
PCB153	2,2′,4,4′,5,5′-hexachlorobipheny
PCB180	2,2′,3,4,4′,5,5′-heptachlorobiphenyl

countries, various POP components have been reported in the environment and in humans (Choo et al., 2020; Han et al., 2016; Lee et al., 2015; Lim et al., 2019).

Ingestion, mostly through food consumption, has been identified as a major route of exposure to POPs in the general population (Bräuner et al., 2011; Vaccher et al., 2020). Once absorbed, POPs accumulate in the adipose tissues of organisms, and persist in the body for decades (Arrebola et al., 2014; Yu et al., 2011). In addition, accumulated POPs can be transferred from mother to fetus through the blood or to breastfed infants through breast milk. However, in Korea, POP biomonitoring in the general population is generally limited to the populations with limited sample sizes (n < 500) or specific groups such as pregnant women or infants. For example, OCP and PCB levels were measured in maternal and cord blood serum from the Children's Health and Environmental Chemicals of Korea (CHECK) cohort (Choi et al., 2018). The CHECK cohort examined pregnant women (n = 148) recruited between February 2011 and December 2011 and their newborns. Several POPs were also reported in serum (n = 401) from participants who received health examinations in Korean Cancer Prevention Study-II (KCPS-II) (Moon et al., 2017).

The Stockholm Convention on POPs recommended commitments to the prevention of further harm to human health through the monitoring and reduction of POPs in the environment (UNEP, 2009). Consequently, many countries have conducted biomonitoring surveys of POPs in the general population, to identify reference levels and evaluate temporal trends of human exposure (Coakley et al., 2018; Porta et al., 2008; Sharkey et al., 2020). National biomonitoring surveys are essential for monitoring current exposure and evaluating the effectiveness of regulations governing the manufacture, import, and use of chemicals (Singh et al., 2019). In Korea, according to Article 14 of the Environmental Health Act, the Korean National Environmental Health Survey (KoNEHS), was initiated in 2009, (Choi et al., 2017; Park et al., 2016). KoNEHS aims to periodically estimate the representative values of environmental chemicals in the Korean population, at three-year intervals, and identify the major factors influencing their distribution. However, until Cycle 3, POPs were not included among the list of chemicals to be measured, leaving a big knowledge gap for the group of persistent environmental chemicals.

The aim of the present study was to provide the profile of POP exposure in a representative Korean adult population using an adult subpopulation that participated in the KoNEHS Cycle 3 (2015–2017). The results of this survey will help find POPs of concern among Korean adult population, and improve the design of KoNEHS to incorporate major POPs in future surveys.

2. Methods

2.1. Study population

This study was conducted using archived serum samples of adults who had participated in KoNEHS Cycle 3 (2015–2017). KoNEHS is an ongoing cross-sectional biomonitoring program that explores human exposure levels to major environmental contaminants in the general adult population in Korea, the factors associated with the exposure, and several key clinical indicators. Unlike the first two cycles which were focused on the adult population only, KoNEHS Cycle 3 extended its coverage to include children and adolescents. For adults, the same sampling strategy and field survey processes of KoNEHS Cycle 2 were applied. For this survey, fasting was not requested for the participating subjects. Detailed information on the KoNEHS research design can be found in previous studies (Choi et al., 2017; Park et al., 2016).

Representative subpopulations (n = 1295) were randomly selected among 3787 adult participants (19 years and older) of the KoNEHS Cycle 3, following classification into 8 groups by sex and age (19–39, 40–49, 50–59, 60 years and older), and consideration of the distribution of Korean adults in the 2015 census (Statistics Korea, 2016).

The participants were surveyed using face-to-face interviews, and data that could be linked to exposure to environmental substances, such as demographics, socioeconomic characteristics, transportation habits, indoor environments, and lifestyles, were collected. In addition, dietary habits were investigated using the food frequency questionnaire.

The present study was approved by the Institutional Review Board of the National Institute of Environmental Research (NIER), Korea (NIER-2015-BR-006-01).

2.2. Measurement of serum persistent organic pollutants (POPs)

Seven OCPs, 11 PCBs and 6 PBDEs were selected as target compounds and measured in the serum samples. These chemicals were chosen because of their high bioaccumulation and toxicity potentials. The measured OCPs included hexachlorobenzene (HCB), beta-HCH, gamma-HCH, *o,p′*-DDT, *p,p′*-DDT, *o,p′*-DDE and *p,p′*-DDE. PCBs included PCB52, PCB105, PCB118, PCB126, PCB138, PCB153, PCB157, PCB167, PCB169, PCB180 and 189. PBDEs included PBED28, PBDE47, PBDE99, PBDE100, PBDE153 and PBDE154.

The serum POPs concentrations were measured from serum that had been archived at −70 °C, using high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS, Agilent 6890/JEOL JMS-800D) (CDC, 2006; Moon et al., 2014). After adding an internal standard to the samples, they were stirred for 15–20 s and left to stand for 15 min. Subsequently, 1 mL ultrapure water was added and stirred. The solid phase extraction method was used to analyze 0.5 mL of the sample for POPs. An NH_2 cartridge that can easily distinguish C18 fatty acids was used for the efficient extraction of organic substances while excluding water-soluble substances. Silica gel (1 g) and Florisil (0.5 g) cartridges were used to remove other substances that could interfere with the extracted solvent thereafter, an internal standard was added through a syringe in a nitrogen-enriched atmosphere at ≤35 °C, and HRGC/HRMS analysis was performed.

Quality control (e.g., linearity and slope of the calibration curve, limit of detection [LOD], accuracy, and precision) was performed to ensure the reliability of the analysis and for verification of the analytical method. Quality and accuracy of the analytical method were assessed externally by participating in the German External Quality Assessment Scheme (G-EQUAS). LOD represents the minimum concentration of the substance that can be detected. It was determined by multiplying the standard deviation, which was obtained from seven experiments, with 3.143 (t-value) (α = 0.01; 99% significance level) after spiking the concentration to 2–5 times the expected LOD. The estimated LODs of the OCPs, PCBs, and PBDEs were 0.86–1.37, 0.41–0.76, and 0.35–0.92 ng/g lipid, respectively (Table 2).

Table 1
Demographic, socio-economic, and behavioral characteristics of the adult participants.

	n	%
Total	1295	100
Sex		
Male	637	49.19
Female	658	50.81
Age (years)		
19–29	187	14.44
30–39	275	21.24
40–49	266	20.54
50–59	252	19.46
60–69	206	15.91
≥70	109	8.42
Residence area		
Rural	195	15.06
Urban	1065	82.24
Coastal	35	2.70
Education level		
Middle school or lower	280	21.62
High school	412	31.82
College or higher	603	46.56
Monthly household income (US$/month)		
Low (<1,300)	153	11.81
Middle low (1,300–2,600)	210	16.22
Middle high (2,600–3,600)	276	21.31
High (≥3,600)	648	50.04
Not answered	8	0.62
Smoking status		
Non-smoker[a]	1035	79.92
Smoker	260	20.08
Body mass index (BMI)[b]		
Normal	496	38.30
Overweight	327	25.25
Obese	472	36.45

[a] Non-smoker includes former smokers.

[b] BMI (kg/m^2): Normal (<23.0), Overweight (23.0 ≤ BMI < 25), Obese (≥25).

The serum total lipid were analyzed by colorimetry using an UV spectrophotometer (Libra, Biochrom, UK). Quality control was ensured by analyzing the reference materials (D-Tek LLC, USA). The range of analytical measurement was 0.0003–5000 mg/dL. A lipid-adjusted concentration of POPs (ng/g lipid) was used for the analysis. The final lipid-adjusted concentrations were calculated by dividing the results with the total lipid concentrations.

2.3. Statistical analysis

The concentrations of POPs in the serum of subjects had a skewed distribution; therefore, they were log-transformed before statistical analysis. The results that were below the LOD were substituted with a value of LOD/$2^{1/2}$ (Croghan and Egeghy, 2016). The geometric mean and 95% confidence intervals of the lipid-adjusted concentrations of the POPs were calculated for descriptive statistics. When the values less than the LOD accounted for more than 40% of the total amount, we reported the proportions and the values greater than the LOD (Health Canada, 2015). Spearman correlation analysis was used for analyzing relationships among the POPs concentrations. In addition, the associations of POPs concentrations with each variable were analyzed using t-tests and Analysis of Variance. The following variables were included: sex (dichotomous), age (categorical), residence area (categorical), education level (categorical), smoking status (dichotomous), fish consumption (categorical), body mass index (categorical), and menopause status (dichotomous). Multiple regression analysis was performed to examine the factors associated with POP exposure. The relevant covariates were selected based on previous studies (Arrebola et al., 2018; Černá et al., 2008; Hardell et al., 2010). For the multiple regression analysis, covariates were included in the models, including age (19–29, 30–39, 40–49, 50–59, 60–69, or ≥ 70 years), sex (male or female), BMI (normal, overweight, or obese), and residence area (urban, rural, or coastal area). Factor analysis was performed to explore the association between the determined factors. Two factors with minimum eigenvalues greater than one were selected. Factor loadings greater than 0.4 were considered high. The statistical significance was determined at $p < 0.05$. All the statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, US).

3. Results

3.1. Characteristics of the participants

The general characteristics of the participants are presented in Table 1. The participating adults (n = 1295) were 637 males and 658 females with a mean age of 47 years. The majority of the proportion showed a normal BMI (<23, 38.3%) and resided in urban areas (82.2%).

3.2. Distributions of the concentrations of POPs

Among the 24 POPs (7 OCPs, 11 PCBs and 6 PBDEs) that were analyzed in the collected serum samples, those with detection rates of 60% or higher were three OCPs (HCB, *p,p′*-DDT and *p,p′*-DDE) and five PCBs (PCB52, PCB118, PCB138, PCB153 and PCB180). The most frequently detected POPs included *p,p′*-DDE (99.8%), followed by PCB180 (98.8%), PCB153 (98.8%), HCB (96.2%), PCB138 (95.2%), PCB118 (89.6%), *p,p′*-DDT (80.5%) and PCB52 (71.2%) (Table 2). None of the PBDEs were detected in over 60% of the samples; 2,2′,4,4′-tribromodiphenyl ether (PBDE47) had the highest detection rate (48.6%). Details of the serum concentrations are listed in Table S1.

3.3. Concentrations of organochlorine pesticides

The serum concentrations of several OCPs exhibited differences based on the demographic, socioeconomic, or behavioral characteristics of the participating adult population (Table 3). For HCB, which had a geometric mean of 67.08 ng/g lipid, males showed higher levels than females (73.39 vs. 61.49 ng/g lipid), and individuals aged 40–49 years had the highest concentrations (77.51 ng/g lipid). HCB levels tended to be higher among women before menopause (69.43 vs. 53.46 ng/g lipid). In the case of *p,p′*-DDT (geometric mean, 6.68 ng/g lipid), females had slightly but significantly higher levels (6.82 vs. 6.53 ng/g lipid). The *p,p′*-DDT levels tended to be higher among the older population, those living in coastal area, with lower levels of education, frequent fish consumers, and with relatively high BMI. In addition, women after menopause tended to exhibit higher *p,p′*-DDT concentrations. In the case of *p,p′*-DDE (geometric mean, 128.47 ng/g lipid), the trends based on sex, age, residence, education, fish consumption, BMI, and menopause were similar to those of *p,p′*-DDT.

3.4. Concentrations of polychlorinated biphenyls

Among the PCBs, PCB153 was present at the highest concentrations, with a geometric mean of 13.14 ng/g lipid, followed by PCB138 (8.84 ng/g lipid), and PCB180 (8.49 ng/g lipid) (Table 3). All PCBs concentrations were lower in females than in males, although statistical significance was not observed for certain PCBs like PCB118. The concentrations of all PCBs, excluding PCB52, were significantly higher among older adults, the residents in coastal areas, those with low education levels, frequent fish consumers, and the women after menopause ($p < 0.01$).

Table 2
Distributions of concentrations of measured persistent organic pollutants ($n = 1295$).

Analyte	LOD[a] (ng/g lipid)	Percentage below LOD[b]	GM[a] (ng/g lipid)	Percentile (ng/g lipid)				
				25th	50th	75th	95th	Max
OCP								
HCB	1.24	3.8	67.08	52.39	82.42	126.28	219.15	1026.22
beta-HCH	1.02	43.0	–	<LOD	4.76	9.45	22.03	79.08
gamma-HCH	1.37	91.5	–	<LOD	<LOD	<LOD	4.27	59.76
o,p′-DDT	0.86	99.5	–	<LOD	<LOD	<LOD	<LOD	445.06
p,p′-DDT	1.06	19.5	6.68	4.36	8.97	15.28	31.00	135.19
o,p′-DDE	1.01	100	–	–	–	–	–	–
p,p′-DDE	1.18	0.2	128.47	72.84	121.63	215.83	548.32	2565.63
PCB								
PCB52	0.51	28.8	1.57	<LOD	1.77	4.08	9.72	34.77
PCB105	0.41	46.0	–	<LOD	0.51	1.16	2.70	14.0
PCB118	0.72	10.4	2.66	1.50	2.75	4.94	10.96	44.21
PCB126	0.66	99.1	–	<LOD	<LOD	<LOD	<LOD	32.14
PCB138	0.52	4.8	8.84	5.87	10.13	17.12	35.01	151.73
PCB153	0.67	1.2	13.14	7.56	13.33	23.83	52.45	189.35
PCB157	0.67	91.0	–	<LOD	<LOD	<LOD	0.93	14.23
PCB167	0.62	76.1	–	<LOD	<LOD	<LOD	1.59	6.50
PCB169	0.41	97.5	–	<LOD	<LOD	<LOD	<LOD	16.30
PCB180	0.76	1.2	8.49	4.70	8.58	15.75	37.18	260.51
PCB189	0.47	88.1	–	<LOD	<LOD	<LOD	0.82	26.64
PBDE								
PBDE28	0.35	86.6	–	<LOD	<LOD	<LOD	1.87	13.08
PBDE47	0.59	51.4	–	<LOD	<LOD	1.71	4.80	120.68
PBDE99	0.61	85.9	–	<LOD	<LOD	<LOD	2.28	37.78
PBDE100	0.72	91.7	–	<LOD	<LOD	<LOD	1.07	12.01
PBDE153	0.92	63.6	–	<LOD	<LOD	2.28	7.16	75.31
PBDE154	0.74	95.1	–	<LOD	<LOD	<LOD	<LOD	6.85

[a] LOD: limit of detection; GM: geometric mean.

[b] If >40% of the samples were below the LOD, the percentile distribution is reported but mean was not calculated.

3.5. Factors associated with serum POP levels

The results of the multiple regression analysis, which was conducted with sex, age, residence area, education level, smoking status, and fish consumption frequency as independent variables revealed several demographic, socioeconomic, and behavioral factors influencing the serum POP levels among Korean adults (Table 4). Most substances, were present at lower concentrations in females than in males, tended to increase with an increase in age. In terms of residential area, the concentrations of *p,p′*-DDE, PCB153, and PCB180 were more positively correlated with living in urban or coastal areas than rural areas. The concentrations of all POPs, excluding HCB and PCB52, were also positively correlated with fish consumption more than once a week ($p < 0.05$, $p < 0.01$). The concentrations of *p,p′*-DDE, PCB52, PCB138, PCB153, and PCB180 were positively correlated with levels of education (college education level or above).

Furthermore, the correlation analysis revealed significant correlation between HCB and PCB52 (correlation coefficient = 0.515) (Table S2). Factor analysis also showed that HCB and PCB52 were grouped as a common factor (Factor 2, Table S3). In addition, *p,p′*-DDT, *p,p′*-DDE, PCB118, PCB138, PCB153, and PCB180 were grouped together (Factor 1), and had positive correlations with age, residential area, education level, and fish consumption (less than once per week); however, Factor 2 exhibited positive correlations with education level and fish consumption; however, the correlations were not significant.

4. Discussion

4.1. POP distribution among Korean adult population

This study showed that major POPs are widely present in the serum samples of a representative population of Korean adults, similar to in other countries (CDC, 2009; Health Canada, 2010). The occurrence patterns of most POPs, such as PCBs and PBDEs, among Korean adults, were generally similar to those reported in other national biomonitoring programs, except for HCB. The serum concentrations of HCB (67.08 ng/g lipid) observed among Korean adults were approximately seven-fold higher than those measured in Canada, and approximately twice as high as those in Spain (Table 5). HCB is an organochlorine compound that was used as a fungicide for many years, and shows a broad half-life that can be as high as to 22.9 years (anaerobic biodegradation in soil) (Barber et al., 2005). Even after inclusion in the Stockholm Convention on POPs (Miret et al., 2019), it is still released into the environment as a byproduct of several industrial processes (Starek-Świechowicz et al., 2017). Several studies have investigated various HCB exposure pathways (Barmpas et al., 2020; Bravo et al., 2017; Harmouche-Karaki et al., 2018; Saoudi et al., 2014). The reason for the high HCB levels in serum of Korean adults, however, is not clear, and is subject to the results of future investigations. In Korea, HCB emissions are reported to have increased from 2006 to 2009 owing to incineration processes in non-metallic and ferrous industries (Kim and Yoon, 2014).

Most PCBs assessed in this study were present at concentrations lower than those reported in similar biomonitoring studies in other countries (Table 5). In the present population, PCB153 was present at the highest concentrations among the measured PCBs. In the body, PCB153 and PCB180 have low removal rates because of their prolonged half-lifes in adipose tissue, and extremely slow metabolism (Phillips et al., 1989; We et al., 2010). In general, hexa- and hepta-chlorinated congeners are detected at high concentrations in human serum samples (Glynn et al., 2000), for example, PCB138, PCB153, and PCB180 (Health Canada, 2010; Haines et al., 2017; Porta et al., 2010; Singh et al., 2019; Wattigney et al., 2015).

Apparently, PCB52 concentrations are not significantly correlated

Table 3
Serum concentrations of POPs by demographic, socioeconomic, and behavioral characteristics (ng/g lipid).

	HCB		*p,p′* -DDT		*p,p′*-DDE		PCB52		PCB118		PCB138		PCB153		PCB180	
	GM	(95% CI)	GM	(95% CI)	GM	(95% CI)	GM	(95% CI)	GM	(95% CI)	GM	(95% CI)	GM	(95% CI)	GM	(95% CI)
Total	67.08	(63.07–71.35)	6.68	(6.24–7.14)	128.47	(122.77–134.43)	1.57	(1.47–1.67)	2.66	(2.54–2.80)	8.84	(8.36–9.35)	13.14	(12.51–13.80)	8.49	(8.08–8.92)
Sex																
Male	73.39	(66.46–81.04)*	6.53	(5.86–7.28)	129.43	(121.90–137.42)	2.03	(1.84–2.24)**	2.68	(2.49–2.89)	9.48	(8.63–10.41)*	15.52	(14.48–16.64)**	10.57	(9.81–11.40)**
Female	61.49	(57.10–66.21)	6.82	(6.29–7.39)	127.54	(119.15–136.53)	1.22	(1.13–1.32)	2.64	(2.48–2.82)	8.26	(7.76–8.80)	11.18	(10.46–11.95)	6.86	(6.45–7.30)
Age (years)																
19–29	72.55	(61.09–86.17)**	3.35	(2.80–4.00)**	63.13	(58.80–67.77)**	1.47	(1.24–1.75)**	1.47	(1.34–1.62)**	4.43	(3.84–5.11)**	5.70	(5.09–6.39)**	2.89	(2.64–3.18)**
30–39	60.91	(52.32–70.90)	3.24	(2.78–3.77)	77.16	(72.46–82.16)	1.71	(1.48–1.97)	1.60	(1.47–1.74)	5.74	(5.10–6.47)	8.26	(7.62–8.95)	5.08	(4.73–5.45)
40–49	77.51	(68.47–87.76)	8.96	(7.90–10.15)	114.75	(106.69–123.42)	2.03	(1.76–2.34)	2.04	(1.85–2.25)	7.03	(6.21–7.96)	11.80	(10.81–12.88)	8.43	(7.83–9.08)
50–59	71.00	(61.62–81.81)	8.41	(7.26–9.75)	174.75	(158.44–192.73)	1.57	(1.37–1.80)	4.14	(3.77–4.54)	12.73	(11.53–14.05)	18.75	(17.12–20.54)	12.46	(11.44–13.56)
60–69	52.46	(45.04–61.11)	12.78	(11.49–14.23)	252.62	(228.94–278.74)	1.08	(0.93–1.26)	5.16	(4.71–5.65)	17.33	(15.83–18.97)	25.67	(23.36–28.21)	17.23	(15.61–19.02)
≥70	73.36	(63.77–84.38)	11.35	(9.60–13.44)	283.49	(245.73–327.06)	1.47	(1.20–1.81)	5.24	(4.57–6.01)	18.10	(15.99–20.48)	28.56	(25.21–32.36)	21.62	(18.86–24.79)
Residence area																
Rural	69.24	(59.00–81.26)	7.46	(6.31–8.81)*	131.42	(114.62–150.69)**	1.44	(1.22–1.68)	2.96	(2.63–3.32)**	9.35	(8.25–10.60)**	13.61	(12.10–15.32)**	8.39	(7.39–8.52)**
Urban	66.31	(61.90–71.03)	6.43	(5.97–6.93)	126.09	(120.13–132.35)	1.59	(1.48–1.71)	2.58	(2.44–2.72)	8.56	(8.03–9.12)	12.83	(12.15–13.54)	8.38	(7.93–8.85)
Coastal	79.94	(64.10–99.70)	11.05	(6.69–18.25)	199.64	(156.44–254.76)	1.56	(1.03–2.37)	3.91	(2.79–5.47)	17.33	(12.97–23.15)	22.38	(16.60–30.19)	13.48	(9.39–19.35)
Education level																
≤ Middle school	60.71	(53.38–69.05)	11.36	(10.23–12.62)**	202.97	(185.16–222.50)**	1.21	(1.06–1.37)**	4.71	(4.35–5.10)**	13.96	(12.73–15.29)**	21.09	(19.37–22.95)**	13.99	(12.77–15.32)**
High school	65.12	(58.32–72.70)	7.42	(6.62–8.31)	140.21	(129.06–152.33)	1.60	(1.42–1.79)	2.73	(2.51–2.98)	9.28	(8.41–10.26)	14.12	(12.94–15.41)	9.65	(8.86–10.52)
≥ College	71.70	(65.45–78.55)	4.85	(4.37–5.39)	97.86	(92.32–103.72)	1.74	(1.59–1.91)	2.01	(1.88–2.15)	6.91	(6.35–7.52)	10.04	(9.36–10.76)	6.16	(5.75–6.61)
Smoking status																
Non-smoker	67.09	(62.90–71.57)	6.83	(6.35–7.35)	131.48	(124.78–138.55)*	1.47	(1.37–1.57)**	2.80	(2.65–2.95)**	8.83	(8.30–9.39)	13.03	(12.32–13.77)	8.39	(7.94–8.87)
Smoker	67.02	(56.57–79.40)	6.09	(5.16–7.18)	117.12	(107.36–127.78)	2.03	(1.75–2.35)	2.18	(1.95–2.44)	8.88	(7.78–10.13)	13.59	(12.25–15.07)	8.88	(7.93–9.93)
Fish consumption																
Rarely	65.13	(53.62–79.12)	5.13	(4.26–6.18)**	105.27	(91.80–120.71)**	1.34	(1.10–1.65)	2.22	(1.94–2.55)**	7.30	(6.16–8.64)**	10.93	(9.49–12.59)**	6.46	(5.52–7.56)**
< Once a week	66.19	(59.86–73.19)	5.50	(4.92–6.15)	110.82	(103.12–119.09)	1.59	(1.43–1.76)	2.30	(2.13–2.48)	7.87	(7.21–8.59)	11.26	(10.44–12.15)	7.32	(6.76–7.93)
≥ Once a week	68.20	(62.61–74.29)	8.18	(7.46–8.98)	150.01	(140.90–159.70)	1.60	(1.47–1.75)	3.10	(2.89–3.32)	10.07	(9.29–10.91)	15.36	(14.33–16.46)	10.08	(9.43–10.78)
BMI																
Normal	69.33	(62.77–76.58)	5.41	(4.84–6.04)**	115.11	(106.74–124.13)**	1.60	(1.45–1.78)	2.26	(2.10–2.44)**	8.07	(7.40–8.81)*	11.48	(10.60–12.43)**	7.86	(7.24–8.53)
Overweight	71.30	(63.04–80.64)	7.01	(6.14–8.01)	136.46	(124.97–149.01)	1.55	(1.37–1.76)	2.86	(2.60–3.14)	8.93	(7.94–10.04)	13.93	(12.58–15.42)	9.05	(8.23–9.95)
Obese	62.11	(56.06–68.82)	8.05	(7.24–8.96)	138.27	(128.47–148.83)	1.54	(1.38–1.71)	3.01	(2.77–3.27)	9.66	(8.80–10.60)	14.54	(13.47–15.69)	8.81	(8.10–9.57)
Menopause[a]																
No	69.34	(63.75–75.43)**	4.26	(3.79–4.78)**	81.09	(75.54–87.03)**	1.50	(1.35–1.68)**	1.67	(1.56–1.79)**	5.66	(5.24–6.10)**	7.05	(6.55–7.58)**	4.43	(4.15–4.74)**
Yes	53.46	(47.16–60.59)	11.79	(10.96–12.68)	216.16	(197.20–236.91)	0.95	(0.86–1.06)	4.52	(4.20–4.87)	12.84	(11.87–13.89)	19.14	(17.61–20.81)	11.41	(10.58–12.32)

*$p < 0.05$, **$p < 0.01$.
[a] Women only ($n = 658$).

Table 4
Factors determining serum concentrations of POPs based on multiple regression analysis.

	HCB		*p,p′* -DDT		*p,p′*-DDE		PCB52		PCB118		PCB138		PCB153		PCB180	
	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)
Sex																
Male	ref.															
Female	−0.20	(−0.32, −0.07)**	0.04	(−0.09, 0.16)	−0.05	(−0.12, 0.02)	−0.52	(−0.64, −0.39)**	−0.04	(−0.12, 0.04)	−0.18	(−0.28, −0.08)**	−0.37	(−0.45, −0.30)**	−0.51	(−0.57, −0.44)**
Age (years)																
19–29	ref.															
30–39	−0.15	(−0.36, 0.06)	−0.07	(−0.28, 0.13)	0.20	(0.08, 0.32)**	0.18	(−0.03, 0.39)	0.06	(−0.07, 0.20)	0.27	(0.10, 0.43)**	0.38	(0.25, 0.51)**	0.60	(0.49, 0.71)**
40–49	0.09	(−0.12, 0.30)	0.95	(0.74, 1.15)**	0.60	(0.48, 0.72)**	0.35	(0.14, 0.56)**	0.31	(0.18, 0.45)**	0.47	(0.30, 0.64)**	0.74	(0.61, 0.87)**	1.12	(1.00, 1.23)**
50–59	0.00	(−0.21, 0.22)	0.86	(0.65, 1.08)**	1.03	(0.91, 1.16)**	0.11	(−0.11, 0.32)	1.01	(0.87, 1.15)**	1.07	(0.90, 1.24)**	1.21	(1.08, 1.35)**	1.54	(1.42, 1.65)**
60–69	−0.28	(−0.50, −0.05)*	1.28	(1.05, 1.50)**	1.40	(1.27, 1.53)**	−0.21	(−0.44, 0.01)	1.23	(1.09, 1.38)**	1.40	(1.22, 1.58)**	1.56	(1.42, 1.70)**	1.91	(1.79, 2.03)**
≥70	0.02	(−0.25, 0.29)	1.18	(0.91, 1.44)**	1.52	(1.37, 1.68)**	0.03	(−0.24, 0.30)	1.26	(1.08, 1.43)**	1.42	(1.20, 1.63)**	1.63	(1.46, 1.79)**	2.08	(1.93, 2.22)**
Residence area																
Rural	ref.															
Urban	−0.08	(−0.25, 0.09)	0.05	(−0.12, 0.23)	0.16	(0.06, 0.26)**	0.02	(−0.15, 0.20)	0.06	(−0.06, 0.17)	0.09	(−0.05, 0.23)	0.12	(0.01, 0.23)*	0.20	(0.10, 0.29)**
Coastal	0.14	(−0.26, 0.55)	0.30	(−0.11, 0.70)	0.33	(0.09, 0.56)**	0.07	(−0.34, 0.48)	0.18	(−0.08, 0.45)	0.52	(0.20, 0.84)**	0.38	(0.13, 0.63)**	0.34	(0.12, 0.56)**
Education level																
≤ Middle school	ref.															
High school	0.05	(−0.14, 0.24)	0.06	(−0.14, 0.25)	0.19	(0.08, 0.30)**	0.19	(−0.01, 0.38)	−0.07	(−0.20, 0.06)	0.11	(−0.05, 0.26)	0.13	(0.01, 0.24)*	0.24	(0.14, 0.34)**
≥ College	0.15	(−0.07, 0.36)	−0.05	(−0.27, 0.16)	0.22	(0.09, 0.34)**	0.23	(−0.01, 0.44)*	−0.06	(−0.20, 0.08)	0.17	(0.00, 0.34)*	0.16	(0.03, 0.29)*	0.24	(0.12, 0.35)**
Smoking status																
Non-smoker	ref.															
Smoker	−0.13	(−0.30, 0.04)	0.06	(−0.11, 0.23)	0.04	(−0.06, 0.13)	0.05	(−0.12, 0.22)	−0.14	(−0.25, −0.03)*	0.10	(−0.04, 0.23)	0.04	(−0.06, 0.14)	0.02	(−0.07, 0.11)
Fish consumption																
Rarely	ref.															
< Once a week	0.01	(−0.20, 0.22)	0.07	(−0.14, 0.28)	0.06	(−0.06, 0.18)	0.13	(−0.08, 0.34)	0.04	(−0.10, 0.17)	0.08	(−0.09, 0.24)	0.02	(−0.11, 0.15)	0.12	(0.01, 0.23)*
≥ Once a week	0.04	(−0.17, 0.24)	0.34	(0.13, 0.54)**	0.21	(0.10, 0.33)**	0.13	(−0.07, 0.34)	0.20	(0.06, 0.33)**	0.17	(0.01, 0.33)*	0.15	(0.02, 0.27)*	0.23	(0.12, 0.33)**
BMI																
Normal	ref.															
Overweight	0.01	(−0.15, 0.17)	0.15	(−0.01, 0.31)	0.04	(−0.06, 0.13)	−0.07	(−0.23, 0.09)	0.11	(0.01, 0.22)*	−0.04	(−0.17, 0.08)	0.02	(−0.08, 0.12)	−0.07	(−0.16, 0.01)
Obese	-0.14	(-0.28, 0.01)	0.26	(0.11, 0.40)**	0.01	(-0.07, 0.10)	-0.11	(-0.26, 0.03)	0.13	(0.03, 0.22)**	-0.01	(-0.13, 0.11)	0.00	(-0.09, 0.09)	-0.18	(-0.26, -0.10)**
Menopause[a]																
No	ref.															
Yes	−0.31	(−0.58, −0.05)*	0.14	(−0.10, 0.38)	0.31	(0.12, 0.50)**	−0.36	(−0.63, −0.08)*	0.40	(0.22, 0.57)**	0.19	(0.00, 0.37)*	0.24	(0.06, 0.42)*	0.21	(0.05, 0.37)*

Adjusted for age, sex, BMI, residence area. [a]Women only ($n = 658$). *$p < 0.05$, **$p < 0.01$.

Table 5
Comparison of POPs concentrations among national-scale biomonitoring programs.

Chemical	Country[a]	Year of survey	Age (years)	*n*	GM[b] (ng/g lipid)	(95% CI[c]) (ng/g lipid)
HCB	Korea	2015–2017	≥20	1295	67.08	(63.07–71.35)
	Canada	2007–2009	20–79	1666	9.09	(8.02–10.30)
	US	2003–2004	≥20	1373	15.5	(14.7–16.2)
	Spain	2009–2010	18–65	712	28.50	(25.56–31.38)
	Belgium	2002–2006	50–65	1530	56.3	(54.6–58.0)
	Belgium	2012–2016	50–65	201	13.7	(12.1–15.5)
***p,p′*-DDT**	**Korea**	**2015–2017**	**≥20**	**1295**	**6.68**	**(6.24–7.14)**
	Canada[d]	2007–2009	20–79	1664	–	
	US[d]	2003–2004	≥20	1370	–	
***p,p′*-DDE**	**Korea**	**2015–2017**	**≥20**	**1295**	**128.47**	**(122.77–134.43)**
	Canada	2007–2009	20–79	1666	152.05	(127.03–182.00)
	US	2001–2002	≥20	1540	338	(303–376)
	US	2003–2004	≥20	1368	268	(217–332)
	Spain	2009–2010	18–65	934	158.8	(149.8–168.4)
	Belgium	2002–2006	50–65	1530	418	(394–444)
	Belgium	2012–2016	50–65	201	224	(199–253)
PCB52	**Korea**	**2015–2017**	**≥20**	**1295**	**1.57**	**(1.47–1.67)**
	Canada[d]	2007–2009	20–79	1661	–	
	US	2003–2004	≥20	1300	2.59	(2.36–2.84)
	Czech	2006	18–58	202	5	–
PCB118	**Korea**	**2015–2017**	**≥20**	**1295**	**2.66**	**(2.54–2.80)**
	Canada	2007–2009	20–79	1666	4.43	(3.78–5.20)
	Czech	2006	18–58	202	14	–
PCB138	**Korea**	**2015–2017**	**≥20**	**1295**	**8.84**	**(8.36–9.35)**
	Canada	2007–2009	20–79	1668	10.13	(8.92–11.51)
	US	2003–2004	≥20	1298	17.7[e]	(16.5–19.0)
	Spain	2009–2010	18–65	1880	31.89	–
	French	2006–2007	18–74	386	70.8	(64.4–77.7)
	Czech	2006	18–58	202	186	–
PCB153	**Korea**	**2015–2017**	**≥20**	**1295**	**13.14**	**(12.51–13.80)**
	Canada	2007–2009	20–79	1666	18.31	(15.83–21.16)
	US	2001–2002	≥20	1549	32.6	(29.5–36.1)
	US	2003–2004	≥20	1300	23.7	(22.3–25.1)
	Spain	2009–2010	18–65	1880	43.64	–
	French	2006–2007	18–74	386	113.3	(102.1–125.7)
	Czech	2006	18–58	202	423	–
PCB180	**Korea**	**2015–2017**	**≥20**	**1295**	**8.49**	**(8.08–8.92)**
	Canada	2007–2009	20–79	1666	15.21	(13.52–17.11)
	US	2001–2002	≥20	1547	23.0	(20.8–25.5)
	US	2003–2004	≥20	1298	19.0	(17.9–20.1)
	Spain	2009–2010	18–65	1880	55.97	–
	French	2006–2007	18–74	386	93.7	(83.1–105.5)
	Czech	2006	18–58	202	374	–

[a] Korea : Korean National Environmental Health Survey Cycle 3 (KoNEHS), This study; Canada : Canadian Health Measures Survey (CHMS); US : National Health and Nutrition Examination Survey (NHANES); French : *Etude National Nutrition Sante* (French National Nutrition and Health Survey, ENNS); Spain : BIOAMBIENT.ES (Spanish adult population); Belgium : Flemish Environmental and Health Survey (FLEHS); Czech : CZ-HBM project (Czech Human Biomonitoring).

[b] GM: geometric mean.

[c] CI: confidence interval.

[d] If > 40% of samples were below the LOD, the percentile distribution is reported but mean was not calculated.

[e] PCB135 + PCB158 (2009).

with most factors because it has the shortest half-life, resulting in lower accumulation in the body compared to other substances (Ritter et al., 2011; Rossi et al., 2010).

The serum concentrations of *p,p′*-DDE were similar to those reported in Canada and Spain, and lower than those observed in the US and Belgium (Table 5). The mean concentration of *p,p′*-DDE (128.47 ng/g lipid) was considerably higher than that of *p,p′*-DDT (6.68 ng/g lipid). DDT is metabolized over time to form *p,p′*-DDE, whose half-life is ≥ 8.6 years in serum (Wolff et al., 2000); therefore, it tends to persist longer than the parent compound (Arrebola et al., 2013; WHO, 1979).

PBDEs, which were added by the Stockholm Convention on POPs as new POPs in 2009, are expected to have high bio-persistence and toxicity. Information about their accumulation in the human body has attracted considerable attention from researchers (Kang et al., 2010; Kim et al., 2012; Moon et al., 2012). However, in the present study, the geometric means of the PBDEs could not be determined because the detection rates of all the PBDEs were lower than 60%. PBDE47 was present at a detection rate of 48.6%, which is the highest among the PBDEs, but it was difficult to estimate its accumulation level. Comparable results were reported by the Canadian Health Measures Survey

(CHMS) (Health Canada, 2010) and in pregnant women in the US (Zota et al., 2018). The CHMS survey, for instance, reported PBDE47 detection rate as the highest (75%), whereas the other PBDEs had lower detection rates, which is similar to the findings of the present study (Health Canada, 2010). Similar patterns have been observed in pregnant women in Korea (Choi et al., 2014).

4.2. Factors influencing serum POP levels

Depending on persistence and bio-accumulative potency, most POPs, excluding those with low molecular weight and lipophilicity (e.g., HCB and PCB52) showed serum concentration trends influenced by demographic factors such as age, sex, BMI, and menopause status, and other factors such as education, residence, smoking, and fish consumption (Tables 3 and 4).

4.3. Sex

Most POPs had higher concentrations in males than in females. The concentrations of POPs tended to be higher in menopausal women, except HCB and PCB52 (Tables 3 and 4). Furthermore, most POPs, except *p,p′*-DDT, PCB52, and PCB118, were slightly lower in women with breastfeeding history, but the differences were not statistically significant (Table S1). Excretion mechanisms specific to females, such as menstruation, and breastfeeding, may explain the sex difference (Hardell et al., 2010; Ibarluzea et al., 2011; Jovanović et al., 2019; Louis et al., 2011; Windham et al., 2005). The present observations are comparable to those that reported lower levels of POPs in women.

4.4. Age

In the present study, all substances, excluding HCB and PCB52, were significantly correlated with age, and PCB180 exhibited a particularly high positive correlation with age (Table 4). Age dependent increase in serum POPs levels has been documented in many studies. Among 444 subjects participating the Korean Cancer Prevention Study-II (2004–2011), the serum concentrations of *p,p′*-DDT, *p,p′*-DDE, PCB118, PCB138, PCB153, and PCB180 were positively correlated with age in both males and females ($p < 0.05$) (Moon et al., 2014). Similarly, according to the CHMS, the concentrations of PCBs and *p,p′*-DDE increased with an increase in age (Singh et al., 2019). In Korea, while the first regulation on POPs was implemented in the late 1960s, PCBs were first prohibited through the Electric Utility Act in 1979 (Kim and Yoon, 2014; Wattigney et al., 2015). Therefore, greater exposure that had taken place before 1970s among older Koreans could explain the relatively high serum concentrations (Moon et al., 2014; Kim and Yoon, 2014). Moreover, the age-related increase in POPs (PCBs and *p,p′*-DDE) in humans could be explained by an increase in half-life with age, and a reduction in the elimination rate with an increase in age (Bates et al., 2004; Černá et al., 2008; Hardell et al., 2010; Porta et al., 2012).

4.5. BMI

The concentrations of *p,p′*-DDT, *p,p′*-DDE, PCB118, PCB138, and PCB153 significantly increased with increasing BMI (Table 3). The observed relationship between serum POP levels and BMI is similar to observations reported from several human populations (Arrebola et al., 2014; Karmaus et al., 2009; Qin et al., 2010). The concentrations of POPs were higher in the group with the highest BMI (Hardell et al., 2010). The participants with a BMI of 24.5–35 had the highest concentrations of PCB153 (Wood et al., 2016). The only exception was PCB180 concentrations, which exhibited negative correlations with BMI in the present population (Table 4), which is also similar to the negative association reported previously between PCB180 and BMI (Dirinck et al., 2011). Although PCB serum concentrations could be lower in people with relatively high BMI because they are disproportionally accumulated in lipids (Agudo et al., 2009; Dirinck et al., 2011; Wolff et al., 2005), the reasons for such potentially negative relationships should be investigated further, since other factors such as age could also influence the serum POP levels (Collins et al., 2007; We et al., 2010).

4.6. Residence and fish consumption

Both residence in coastal areas and frequent fish consumption were significantly associated with higher POPs, which may be because of subjects living in coastal areas exhibiting relatively high fish and shellfish intake habits. Among the individuals living in coastal areas, none ate fish rarely, while 31.4% ate fish meals less than once a week, and 68.6% ate fish more than once a week (data not shown). Previous studies have reported that the primary sources of exposure to POPs are seafood, including fish, and dairy products (Lee et al., 2017; Schecter et al., 2010). PCB concentrations in serum as well as in breast milk and adipose tissue have been reported to be relatively high among individuals consuming fish (Qin et al., 2010).

4.7. Smoking

The serum concentrations of *p,p′*-DDE and PCB118 observed in the present study were higher in non-smokers than in smokers, which is similar to the observations reported from previous studies (Černá et al., 2008; Moon et al., 2017). The only exception was PCB52 which was higher among smokers. Other studies have reported that serum concentrations of PCBs are positively correlated with smoking (Deutch et al., 2003; Lackmann et al., 2000), while Apostoli et al. (2005) observed no significant correlation with smoking. Considering inconsistent observations among studies, smoking may not be a direct source of exposure to OCPs and PCBs, and could be associated with other sources of exposure to such compounds.

4.8. Strengths and limitations

This study employs a subset of the representative Korean adult population and provides, for the first time, the POP exposure profile of a representative Korean population based on KoNEHS Cycle 3 (2015–2017) data. Relatively high HCB serum levels were observed in the survey, which warrants further surveillance for the identification of risk groups and determinants of the sources of exposure. It should be noted that the POP concentrations available for some countries are based on the surveys conducted years before the present study, and may not allow direct comparison with those of the present study. In addition, since the present study was a cross-sectional study, it had limitations in terms of explaining the causal relationships between POPs in human serum and the factors influencing them. Therefore, additional analyses are required to determine the factors influencing the levels of exposure to POPs.

5. Conclusions

Korean adults are exposed to a wide range of POPs. Although most POPs were detected at levels similar to or lower than the levels reported in other national biomonitoring programs, HCB levels were several folds higher among Korean adults. Several demographic and behavioral factors were identified as influencing the serum POP levels among Korean adults. The present report will help facilitate the identification and prioritization of the chemicals of concern that warrant further environmental health management efforts. Major POPs will be added in the list of target chemicals in Cycle 5 (2021–2023) which will be measured among the general population. Such biomonitoring efforts would help identifying priority POPs among the general population of Korea and develop, exposure reduction policies.

Declaration

The results and conclusions in this report are those of the authors and do not necessarily represent the views of the Ministry of Environment and the National Institute of Environmental Research of Korea. The authors declare no competing financial interest.

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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.2021.113779.

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Healthcare provider satisfaction with environmental conditions in rural healthcare facilities of 14 low- and middle-income countries

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ABSTRACT

Healthcare provider (HCP) satisfaction is important for staff retention and effective health service delivery. Inadequate resources, understaffing, and ineffective organizational structure may reduce HCP satisfaction in low- and middle-income countries (LMICs). Some qualitative studies have described links between environmental conditions and job satisfaction in HCPs; however, few studies have explored this link using survey data. This study explores associations between HCP satisfaction and water, sanitation, and hygiene (WaSH) infrastructure, cleanliness, and infection prevention and control (IPC) practices in rural healthcare facilities (HCFs) in LMICs.

This study analyzes 2002 HCFs in rural areas of 14 LMICs. Generalized linear mixed-effects logistic regression models were used to analyze the association between HCP satisfaction, WaSH infrastructure, and cleanliness and IPC practices.

Most respondents reported that they were unsatisfied with water (65%), sanitation (68%), and hygiene infrastructure (54%) at their HCF. Insufficient supply and poor quality of WaSH resources were the most commonly reported reasons for provider dissatisfaction. Respondents were less likely to report dissatisfaction with cleanliness and IPC practices (36%). Dissatisfaction with cleanliness and IPC were most reported because patients and staff did not wash their hands at the correct times or with proper materials, or because the facility was not clean. Several characteristics of the WaSH environment were significantly associated with provider satisfaction at their HCFs, including acceptable water quality, readily available supply of water (on premises and improved), accessible supply of WaSH infrastructure to people with reduced mobility, accessible supplies of sanitation and hygiene materials, and sufficient training and budgeting for WaSH or IPC needs.

Our results suggest that the provision of on premises, improved water service accessible to people with reduced mobility, interventions that prioritize the acceptability of sanitation facilities within the local context, and the provision of hygienic materials are key interventions to improve HCP satisfaction. Dedicated funding and oversight should be established at the HCF level to ensure access to consumable hygiene and IPC products and maintenance of WASH infrastructure. Improvements to WaSH in HCF may improve HCP satisfaction and ultimately patient outcomes.

1. Introduction

Job satisfaction is "the attitude towards one's work and the related emotions, beliefs, and behavior," and "results from complex interactions between on-the-job experience, organizational environment, and motivation" (Peters et al., 2010). Healthcare providers (HCPs) who are more

Abbreviations: Healthcare provider, (HCP); healthcare facilities, (HCFs); water sanitation and hygiene, (WaSH); infection prevention and control, (IPC).

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satisfied with their job and work environment are more likely to demonstrate higher levels of effort towards quality improvement, provide lower-risk care for patients, and have less turnover (Alhassan et al., 2013; Mbaruku et al., 2014; Stewart et al., 2011).

The job of HCPs in rural areas of low- and middle-income countries (LMICs) can be especially demanding, and they often have little resources and institutional support (O'Neill and Sheffel, 2013). Increased pressure in the work environment affects job satisfaction, worker retention and health service delivery in rural settings (Mbaruku et al., 2014). Understaffing can lead to job dissatisfaction, in turn causing further staff shortages, labor unrest, and absenteeism (Gross et al., 2012; Mubyazi et al., 2012; Rowe et al., 2005). Dissatisfaction on the part of service providers can be passed on to patients in the form of impatient, distracted, or uncourteous behavior (Kruk et al., 2009). Improving the job satisfaction of HCPs in these settings is critical for improved patient and provider outcomes in healthcare facilities (HCFs).

The physical work environment – such as water, sanitation, and hygiene (WaSH) infrastructure, lighting, and infection control supplies – affects HCP job satisfaction. Peters et al. found that more than 90% of HCPs in India reported that "good physical conditions" were important in the "ideal job" and often ranked "good physical conditions" above "income" in importance (Peters et al., 2010). HCP dissatisfaction with staffing, poorer cleanliness and less orderliness of the workplace were associated with a greater risk for needlestick injury and pathogen exposure for HCPs (Lundstrom et al., 2002). Inadequate environmental conditions were linked to employee job frustration in HCFs in South Africa; rural HCPs had worse service delivery outcomes and expressed that transferring to another location with more resources would allow them to do their job more effectively (Tawana et al., 2019). In Ghana, HCPs in public and private facilities expressed that a major source of job dissatisfaction was due to the physical work environment of their clinics, and the low availability of resources and drugs (Alhassan et al., 2013). Insufficient resources were cited as a reason for job dissatisfaction for HCPs in government and public HCFs in Kenya, who were less likely to report adequate resources and safe water in the workplace (Ojakaa et al., 2014).

Understanding how the physical work environment impacts job satisfaction in HCPs can improve healthcare service delivery. Despite some qualitative evidence on job satisfaction in HCPs in rural, LMIC settings, few studies have explored this using survey data. We analyzed data from surveys conducted in 14 LMICs to explore the relationship between environmental conditions and HCP job satisfaction in HCFs, and to understand the factors influencing satisfaction with WaSH infrastructure and cleanliness and infection prevention and control (IPC) practices in rural HCFs in LMICs.

2. Materials and methods

Between July and December 2017, an evaluation of WaSH conditions in 14 low- and middle-income countries was conducted for the international non-governmental organization World Vision. As part of this evaluation, public clinics (such as health posts and health centers) in rural areas of Ethiopia, Ghana, Honduras, India, Kenya, Malawi, Mali, Mozambique, Niger, Rwanda, Tanzania, Uganda, Zambia, and Zimbabwe were assessed. Survey methods are described in detail by A. Z. Guo and Bartram (2019) but are summarized here briefly.

2.1. Study design and data collection

HCF sampling was nested within a population-based, cluster-randomized household survey design. Within the household survey sampling area in each country, 100 HCFs in areas where World Vision has programs and 100 HCFs in areas where World Vision does not have programs were randomly selected. Where fewer than 100 HCFs were identified in each of these categories, all HCFs in the sampling areas were surveyed.

Teams of trained enumerators travelled to each of the selected HCFs and attempted to interview a health provider at the facility – preferably the head nurse (42%), though a head doctor (13%) or a nurse who had worked at the facility for more than two years (23%) or other nurse (21%) could also respond if the head doctor was unavailable. Surveys were administered in the local language, and included both direct questions for respondents and enumerator observations of HCF characteristics, water service, sanitation and hygiene facilities, healthcare waste management and cleanliness practices. Definitions of all selected variables and a link to the full survey are available in Table 1. All responses were recorded in mWater (New York, NY, USA), a mobile survey tool which allowed for real-time quality checks by supervisors and researchers during the data collection period, including spot checking based on photos and checking for reasonable responses, duration of survey, and GPS location of surveys.

This study was approved by the UNC-Chapel Hill Institutional Review Board (IRB #17–0663) as well as agencies within each country (The Water Institute at UNC, 2019). Free and informed consent was obtained from all respondents in their own language before beginning the survey.

Full reports on data collection, survey methodologies and results, and surveys used are available at: http://waterinstitute.unc.edu/publication/world-vision-14-country-wash-evaluation/

2.2. Data processing and analysis

Data were cleaned and analyzed using R version 4.0.2 (R Core Team, 2020). Multivariable mixed-effects regression models were fitted using the lme4 package, version 1.1–23 (Bates, Mächler, Bolker and Walker, 2015). A general linear mixed model was used with a logit link function under a binomial family distribution for binary outcome variables. Satisfaction with water service, sanitation facilities, hygiene facilities, and cleanliness and IPC practices were coded as binary outcome variables (e.g., "satisfied with water" versus "not satisfied with water"). For each of these outcomes, approximately 20–40 binary, categorical, or ordered variables were selected *a priori* for testing based on plausible relationships with the outcome variables (Supplement 1). Random effects due to the country of survey were controlled for in each model and the variation in satisfaction attributable to country effects was assessed for each outcome according to the intraclass correlation coefficient (ICC). Responses of "do not know," "decline to state," and other non-responses were uncommon (<5% of responses) and imputed to the reference category.

Univariable logistic regressions were conducted to identify the variables with statistically significant relationships with each outcome variable ($p < 0.05$). For each outcome, all significantly associated variables were incorporated into a single corresponding multivariable mixed-effects logistic regression model as described above. Variables in these models were eliminated using a monitored stepwise procedure until all remaining variables had a corresponding p-value less than or equal to the significance-to-stay value (0.05) (Dietz et al., 2000; Suárez, Pérez, Rivera and Martínez, 2017). Pearson's correlation coefficient and the variance inflation factor were used to verify that none of the variables in the final models were highly correlated.

3. Results

Across the 14 studied countries, respondents at 2002 HCFs (over 98% of those contacted) consented to survey. The characteristics of facilities in the final sample are discussed in detail elsewhere (A. Z. Guo and Bartram, 2019). Respondents from India and Honduras reported consistently high satisfaction with environmental conditions compared to study countries in sub-Saharan Africa and in particular Malawi, Zambia, and Uganda, where respondents reported consistently low satisfaction with environmental conditions. A table displaying satisfaction with each category of environmental conditions (water, sanitation,

Table 1
Selected data on HCF characteristics, water service, sanitation and hygiene facilities, healthcare waste management and cleanliness practices, and administration and training associated with satisfaction with environmental conditions.

Grouping	Variable	Type	Definition
HCF characteristics	Electricity	Observed	Electricity available at the health facility on the day of visit
Water service	Source location	Reported	Location of the main water point (on premises, within 500 m, further than 500 m, other)
	Source availability	Reported	Water available from the main water point, on premises, at time of sampling
	Source type	Reported	Main water point is of an improved type according to JMP classifications at the time of analysis (WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation (JMP), 2019)
	Source condition	Reported	Main water point has not been out of service in the past two weeks
	Source continuity	Reported	Water is available from the main point 24 h per day
	Accessibility	Reported	Characteristics which would make water facilities difficult for people with limited mobility to use are present (e.g., path is difficult to navigate, steps at entrance, no handrails, etc.)
	Treatment	Reported	Health facility does "something" to make the water safer (e.g., boiling, chlorine, filtration, ceramic filter, other)
Sanitation and hygiene facilities	Facility number	Reported	Number of sanitation facilities
	Facility odor	Observed	Any sanitation facility has a "bad smell"
	Facility security	Observed	At least one sanitation facility has a door which can be locked from the inside
	Facility accessibility	Observed	Characteristics which would make sanitation facilities difficult for people with limited mobility to use are present (e.g., path is difficult to navigate, steps at entrance, no handrails, etc.)
	Open defecation	Reported	Open defecation occurs at the facility
	Hygiene near sanitation	Observed	Hand hygiene stations are available in or near (within 5 meters of) all sanitation facilities
	Soap and water	Observed	Soap and water available in all hygiene facilities
	Drying materials	Observed	Hygienic drying materials available in all hygiene facilities
	Staff hand rub	Reported	Medical staff carry alcohol-based hand rub or sanitizer while on duty
Healthcare waste management and cleanliness	Linen cleaning	Reported	Mattresses, pillows, and linens are cleaned between each patient with detergent
	Bins	Observed	Waste is segregated into at least three labeled bins at all points of care (sharps, infectious, and non-infectious general waste)
	Infectious waste	Reported	The mode of facility waste disposal is reported, then segregated into proper (e.g., "autoclaved") vs improper (e.g., "not treated and added to general waste") categories
	Surface cleaning	Reported	Floors, surfaces, and sanitation facilities are cleaned at least once a day with water and detergent or disinfectant
Administration and training	WaSH training	Reported	Water and sanitation training at the facility in the past 12 months
	WaSH budgeting	Reported	Annual budget for the facility which includes funding for WaSH and infection prevention/ control infrastructure, services, and personnel (and is sufficient to meet the needs of the facility)
	WaSH committee	Reported	Infection prevention and control, WaSH, or hygiene committee that employees belong to at the facility (and has met in the past 6 months)
	Oversight committee	Reported	Community-composed oversight committee at the facility (and has met in the past 6 months)
	IPC policy	Reported	Infection control policy, procedure, or document in place

and hygiene infrastructure and cleanliness and ICP practices) can be found in the supplementary materials (Supplement 2).

Most respondents reported that they were unsatisfied with WaSH infrastructure at their HCF. Overall, 68% of respondents were unsatisfied with the sanitation facilities, 65% were unsatisfied with the water service, and 54% were unsatisfied with the hygiene facilities. Survey respondent type was included in each model; head doctors were more likely to be dissatisfied with hygiene facilities (OR = 0.66, p = 0.028) than other respondents; nurses working more than 2 years at their respective facility were more likely to be satisfied with water services (OR = 1.40, p = 0.034) than other respondents; and head nurses as a survey respondent type were not significantly associated with any changes in satisfaction. Insufficient water quantity was the most frequently cited reason for dissatisfaction with WaSH infrastructure, followed by poor water quality (Fig. 1). Respondents were less likely to report that they were unsatisfied with cleanliness and IPC practices (36%). Dissatisfaction with cleanliness and IPC practices was most commonly reported because patients (40%) and other staff (23%) did not wash hands at the correct times, patients (33%) and other staff (28%) did not wash hands with proper materials, or because the facility was not cleaned well (34%). Reasons for HCP dissatisfaction with WaSH infrastructure and cleanliness and IPC practices are presented tabularly in the supplementary materials, alongside additional selected descriptive statistics from surveyed healthcare facilities (Supplement 3).

3.1. Satisfaction with water service

In a mixed-effects logistic regression model, satisfaction with water services at HCFs was significantly associated with continuous water service (OR = 2.81, p < 0.001); water available at the time of survey (OR = 2.21, p < 0.001); a water source on premises (OR = 2.01, p < 0.001); a water source that had not broken down in the last two weeks

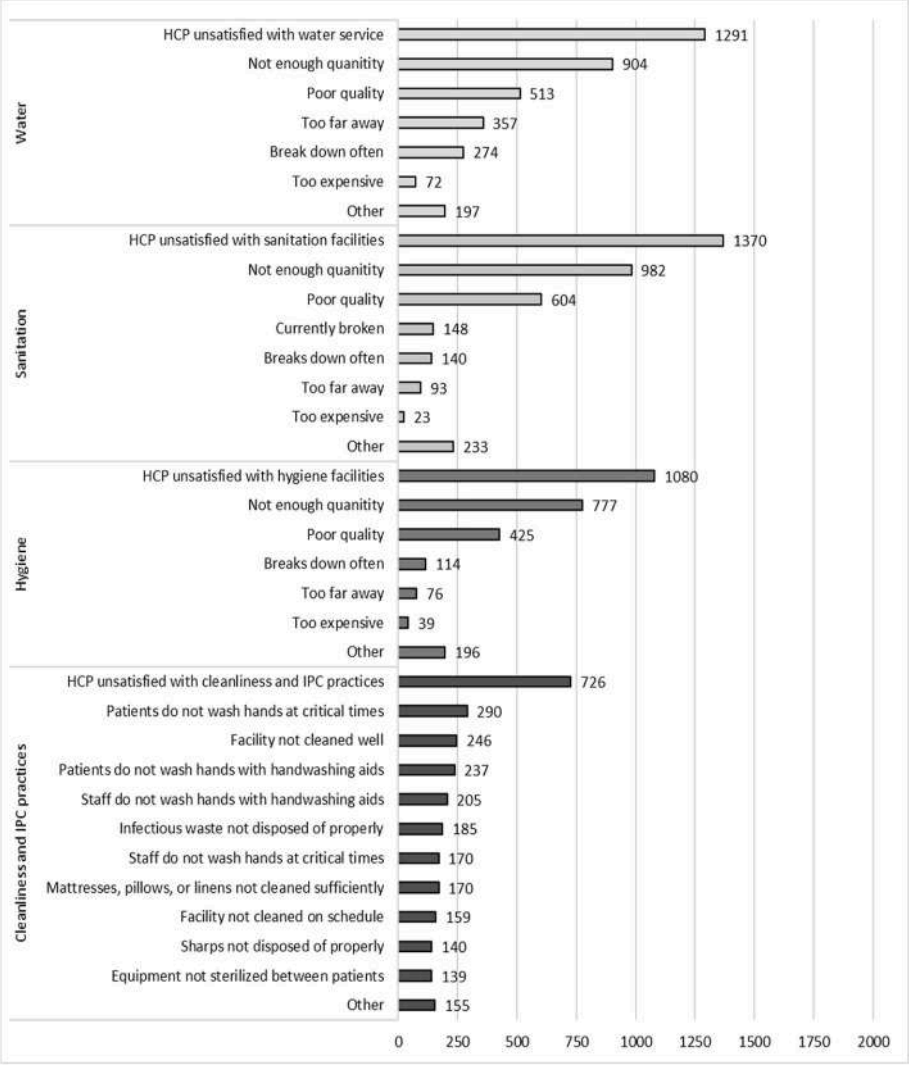

Fig. 1. Number of healthcare facilities dissatisfied with environmental conditions and the reported reasons for dissatisfaction (N = 2002).

(OR = 1.79, p < 0.001); and an improved main water source type (OR = 1.73, p = 0.007) (Table 2). Other than these direct water source characteristics, WaSH training for HCPs (OR = 1.41, p < 0.001) and working electricity (OR = 1.30, p = 0.030) were associated with increased satisfaction with water services. Satisfaction with water services was highest in Honduras (53%) and was lowest in Ethiopia (22%). Approximately 4% of variation in water satisfaction was attributable to differences by country (ICC = 0.039).

3.2. Satisfaction with sanitation facilities

Quantity, quality, and privacy of sanitation facilities were associated with satisfaction with sanitation services at HCFs (Table 3). Respondents were more likely to report that they were satisfied with sanitation facilities at HCFs where no open defecation was reported to occur (OR = 1.88, p < 0.001); at least one facility had a door that locked from the inside (OR = 1.62, p < 0.001); no sanitation facilities were reported to have a bad odor (OR = 1.42, p = 0.004); and had a greater number of hygiene facilities (OR = 1.08, p < 0.001). Respondents were more likely to be satisfied with sanitation where sanitation facilities (OR = 1.77, p < 0.001) and water points (OR = 1.40, p = 0.013) were accessible by people with reduced mobility. Other than direct characteristics of sanitation, hand hygiene stations with soap and water nearby (OR = 1.70, p < 0.001) and a sufficient budget for WaSH and IPC (OR = 1.75, p < 0.001) were associated with increased satisfaction with sanitation services. Satisfaction with sanitation facilities was highest in India (53%) and lowest in Zambia (15%). About 6% of variation in sanitation satisfaction was attributable to differences by country (ICC = 0.061).

3.3. Satisfaction with hygiene facilities

Satisfaction with hygiene facilities was associated with accessible water, the presence of hand hygiene stations and supplies, and institutional support (Table 4). An on premises water source (OR = 1.57, p = 0.003), a water source accessible for people with reduced mobility (OR = 1.57, p < 0.001) and a continuous water service (OR = 1.38, p = 0.012) were associated with increased hygiene satisfaction, as were alcohol-based hand rub carried by staff (OR = 1.66, p < 0.001); the presence of hygienic drying materials (OR = 1.63, p = 0.027) or hand hygiene stations with soap and water within 5 m of all sanitation facilities (OR = 1.39, p = 0.020); and hand hygiene stations with soap and water for at least one point of care (OR = 1.54, p < 0.001). A sufficient budget (OR = 1.73, p = 0.001) and an IPC or WaSH committee that had met in the past six months (OR = 1.33, p = 0.011) were associated with higher hygiene satisfaction. Satisfaction with hygiene facilities was highest in India (76%) and lowest in Ghana (29%). About 9% of variation in hygiene infrastructure satisfaction was attributable to differences by country (ICC = 0.088).

Table 2
Factors associated with statistically significant changes in reported satisfaction with water service within surveyed healthcare facilities (N = 2002).

Factor	Odds Ratio	95% CI	p-value
Survey respondent type			
Other nurse	Ref		
Head doctor	1.26	(0.87, 1.83)	0.219
Head nurse	1.27	(0.96, 1.67)	0.097
Nurse who worked at facility >2 years	1.40	(1.03, 1.90)	0.034
Location of main water source			
Further than 500 m	Ref		
Within 500 m	1.06	(0.70, 1.54)	0.779
On premises	2.01	(1.32, 2.79)	<0.001
Main water source is available during the survey			
No	ref		
Yes (reported, but not observed)	1.09	(0.65, 1.83)	0.741
Yes (reported and observed)	2.21	(1.48, 3.31)	<0.001
Electricity working on the day of the survey	1.30	(1.03, 1.64)	0.030
Facility provides WaSH training to healthcare providers	1.41	(1.14, 1.73)	<0.001
Main water source is an improved type	1.73	(1.16, 2.56)	0.007
Main water source has not broken down in the past 2 weeks	1.79	(1.30, 2.44)	<0.001
Main water source provides continuous (24-h) service	2.81	(2.03, 3.88)	<0.001

Table 3
Factors associated with statistically significant changes in reported satisfaction with sanitation facilities within surveyed healthcare facilities (N = 2002).

Factor	Odds Ratio	95% CI	p-value
Survey respondent type			
Other nurse	ref		
Head doctor	0.20	(0.47, 1.05)	0.085
Head nurse	0.71	(0.68, 1.22)	0.554
Nurse who worked at facility >2 years	1.05	(0.76, 1.45)	0.774
Facility has a budget that includes funding for WaSH/IPC needs			
No budget exists	ref		
Yes (insufficient)	0.87	(0.66, 1.15)	0.329
Yes (sufficient)	1.75	(1.26, 2.42)	<0.001
Number of sanitation facilities present	1.08	(1.04, 1.11)	<0.001
Water accessible for people with reduced mobility	1.40	(1.07, 1.83)	0.013
No sanitation facilities have a bad odor	1.42	(1.12, 1.80)	0.004
At least one sanitation facility has a door that locks from the inside	1.62	(1.26, 2.09)	<0.001
Hand hygiene stations with water and soap are available near all sanitation	1.70	(1.31, 2.20)	<0.001
At least one sanitation facility is accessible by people with reduced mobility	1.77	(1.30, 2.40)	<0.001
Open defecation does not occur at the facility	1.88	(1.34, 2.62)	<0.001

Table 4
Factors associated with statistically significant changes in reported satisfaction with hygiene facilities within surveyed healthcare facilities (N = 2002.

Factor	Odds Ratio	95% CI	p-value
Survey respondent type			
Other nurse	ref		
Head doctor	0.66	(0.46, 0.96)	0.028
Head nurse	0.90	(0.69, 1.18)	0.447
Nurse who worked at facility >2 years	0.95	(0.70, 1.27)	0.710
Location of main water source			
Further than 500 m	ref		
Within 500 m	1.09	(0.75, 2.43)	0.628
On premises	1.57	(1.17, 2.12)	0.003
None available	1.35	(0.77, 1.52)	0.322
Facility has a budget that includes funding for WaSH/IPC needs			
No budget exists	Ref		
Yes (insufficient)	0.98	(0.77, 1.26)	0.890
Yes (sufficient)	1.73	(1.24, 2.40)	0.001
Facility has an IPC/WaSH committee			
No	ref		
Yes, but they have not met in the past 6 months	1.00	(0.71, 1.41)	0.979
Yes, and they have met in the past 6 months	1.33	(1.07, 1.66)	0.011
Main water source provides continuous (24-h) service	1.38	(1.07, 1.79)	0.012
Hand hygiene stations with soap and water are available near all sanitation	1.39	(1.05, 1.83)	0.020
At least one point of care has a hand hygiene station with soap and water	1.54	(1.25, 1.91)	<0.001
Water accessible for people with reduced mobility	1.57	(1.22, 2.02)	<0.001
Hand hygiene stations with hygienic drying materials are available near all sanitation	1.63	(1.06, 2.51)	0.027
Medical staff carry alcohol-based hand rub while on duty	1.66	(1.33, 2.06)	<0.001

3.4. Satisfaction with cleanliness and IPC practices

Satisfaction with cleanliness and IPC practices in HCFs was associated with three determinant categories: availability of infrastructure and supplies, reported cleaning practices, and institutional support (Table 5). For infrastructure and supplies, presence of water accessible to people with reduced mobility (OR = 1.69, $p < 0.001$); a main water source within 500 m of the HCF (OR = 1.54, $p = 0.014$); water treatment (OR = 1.43, $p = 0.002$); working electricity (OR = 1.36, $p = 0.011$); and presence of bins for waste segregation at points of care (OR = 1.35, $p = 0.018$) were associated with increased satisfaction with cleanliness and IPC practices. Regarding reported cleaning practices, respondents were more likely to be satisfied with cleanliness and IPC practices if floors, surfaces, and sanitation facilities were cleaned with water, detergent, or disinfectant (OR = 1.54, $p = 0.002$); no open defecation was reported to occur at the facility (OR = 1.54, $p = 0.002$); mattresses, pillows, and linens were cleaned only intermittently (OR = 1.96, $p = 0.031$); and if infectious waste was disposed of using a safe method such as autoclaving or incineration (OR = 1.48, $p < 0.001$). Sufficient budgeting (OR = 2.14, $p < 0.001$); a community-composed oversight committee that had met in the past six months (OR = 1.54, $p < 0.001$); and an IPC policy, procedure, or document in place by the facility (OR = 1.62, $p < 0.001$) were all aspects of institutional support associated with increased satisfaction with cleanliness and IPC practices. Satisfaction with cleanliness and IPC practices was highest in India (87%) and lowest in Malawi (39%). Over

Table 5
Factors associated with statistically significant changes in reported satisfaction with cleanliness and infection prevention and control practices within surveyed healthcare facilities (N = 2002).

Factor	Odds Ratio	95% CI	p-value
Survey respondent type			
Other nurse	Ref		
Head doctor	0.81	(0.55, 1.18)	0.270
Head nurse	1.08	(0.81, 1.42)	0.610
Nurse who worked at facility >2 years	1.09	(0.80, 1.50)	0.572
Location of main water source			
Further than 500 m	ref		
Within 500 m, but not on premises	1.54	(1.09, 2.17)	0.014
On premises	1.23	(0.89, 1.68)	0.207
None available	1.18	(0.65, 2.17)	0.584
Facility does not clean mattresses, pillows, or linens with detergent			
No cleaning	Ref		
Linens, mattresses, or pillows are provided by the facility	1.25	(0.68, 2.36)	0.468
Cleaned, but not between every patient	1.96	(1.06, 3.70)	0.031
Cleaned, between every patient	1.03	(0.56, 1.89)	0.917
Facility has a budget that includes funding for WaSH/IPC needs			
No budget exists	Ref		
Yes (insufficient)	1.29	(0.99, 1.67)	0.060
Yes (sufficient)	2.14	(1.45, 3.15)	<0.001
Facility has a community-composed oversight committee			
No	ref		
Yes, but they have not met in the past 6 months	1.17	(0.82, 1.66)	0.379
Yes, and they have met in the past 6 months	1.54	(1.21, 1.95)	<0.001
Bins available for segregation of waste at all points of care	1.35	(1.05, 1.73)	0.018
Electricity working on the day of the survey	1.36	(1.07, 1.72)	0.011
Facility treats water to make it safer	1.43	(1.14, 1.82)	0.002
Infectious waste is disposed safely	1.48	(1.18, 1.84)	<0.001
Open defecation does not occur at the facility	1.54	(1.16, 2.04)	0.002
Facility has an IPC policy, procedure, or document	1.62	(1.28, 2.05)	<0.001
Water accessible for people with reduced mobility	1.69	(1.32, 2.17)	<0.001
Facility cleans floors, surfaces, and sanitation facilities with water, detergent, or disinfectant	1.85	(0.22, 2.78)	0.004

13% of variation in satisfaction with cleanliness and IPC was attributable to differences by country (ICC = 0.134).

4. Discussion

We assessed HCP satisfaction with WaSH infrastructure and IPC practices and cleanliness at 2002 rural HCFs across 14 LMICs. This is one of the first studies to quantitatively assess HCP satisfaction with water, sanitation and hygiene infrastructure and IPC practices in LMICs. HCP satisfaction directly affects patient outcomes (Alhassan et al., 2013; Mbaruku et al., 2014; Stewart et al., 2011) and may be especially important in rural and LMIC settings where a small staff with few financial resources determines the quality of healthcare available.

Our results demonstrate that most HCPs are unsatisfied with the water, sanitation, and hygiene infrastructure at their rural HCF, though they were somewhat less likely to report they were dissatisfied with cleanliness and IPC practices. Several indirect and direct characteristics of water, sanitation, and hygiene infrastructure were significantly associated with provider satisfaction at their HCFs, including high quality supply, readily available and accessible supply, and sufficient training and budgeting.

Most HCPs reported that they were unsatisfied with both the quantity and quality of water services at their HCFs. Previous assessments of water services in rural HCFs have shown that only 51% of HCFs in sub-Saharan Africa and 58% of those in Honduras have an improved water source type on premises (WHO/UNICEF, 2019) (data not available for India). Further, an assessment of HCFs in sub-Saharan Africa showed that 27% of rural HCFs lacked continuous water service, and that only 42% had access to a secondary source (A. Guo, Bowling, Bartram and Kayser, 2017).

Having a continuous (24-h) water service (OR = 2.81) was the variable most strongly associated with HCP satisfaction with water services. However, the location of the main water source was significantly associated with multiple aspects of HCP satisfaction, including: satisfaction with water service (on premises, OR = 2.01), hygiene infrastructure (on premises, OR = 1.57) and IPC practice and cleanliness (within 500 m but not on premises, OR = 1.54). The importance of water source location is supported by household water literature. Previous studies have shown that having water on premises increases quantity of household water use (Brown et al., 2013; Overbo et al., 2016), and improves water quality by reducing the risk of contamination during transport from the source and household storage (Overbo et al., 2016; Usman et al., 2018). Presence of a water source accessible for people with reduced mobility is also significantly associated with HCP satisfaction with sanitation facilities (OR = 1.4), hygiene facilities (OR = 1.57) and IPC practices and cleanliness (OR = 1.69).

As with water services, HCPs reported dissatisfaction with both the quality and quantity of sanitation facilities. Previous assessments of sanitation services in rural HCFs have shown that only 23% of HCFs in sub-Saharan Africa and 1% of those in Honduras have basic sanitation service, defined as an improved-type sanitation facility that is acceptable to users, accessible to people with limited mobility and sufficiently available (WHO/UNICEF, 2019) (data not available for India). Further, an assessment of rural HCFs in sub-Saharan Africa showed that 44% of sanitation facilities in HCFs faced issues with toilet privacy, cleanliness, or functionality (A. Guo et al., 2017).

Satisfaction with sanitation facilities was most strongly associated with whether open defecation occurs at the HCF (OR = 1.88). Open defecation was also significantly associated with satisfaction with IPC practices and cleanliness (OR = 1.54). People are motivated to practice open defecation by both active choice and compulsion (Bhatt et al., 2019), meaning that open defecation at HCFs could result from cultural norms (Thys et al., 2015), concerns about privacy (Bhatt et al., 2019), cleanliness, or other reasons (WHO/UNICEF, 2019). One previous study that found that 69% of people who used sanitation facilities at HCFs in Lesotho were dissatisfied with the toilet condition (WHO/UNICEF, 2019). One previous study found that 69% of people who used sanitation facilities at HCFs in Lesotho were dissatisfied with the toilet condition (WHO/UNICEF, 2019). Another study in sub-Saharan Africa found that, on average, 17% of toilets had privacy or cleanliness issues reported and that 20% of toilets were in need of repair (A. Guo et al., 2017). Any of these factors could affect decision-making about open defecation at the HCF for both HCPs and patients affecting both HCP satisfaction and related health outcomes. Accessibility of sanitation facilities for people with limited mobility was also strongly associated with HCP satisfaction with sanitation facilities (OR = 1.77).

HCP satisfaction with hygiene facilities was slightly better than satisfaction with water service or sanitation facilities. The factors most

strongly associated with HCP satisfaction with hygiene facilities were having a sufficient budget for WASH/IPC needs (OR = 1.73), medical staff that carry alcohol-based hand rub while on duty (OR = 1.66) and hand hygiene stations with hygienic drying materials available near all sanitation facilities (OR = 1.63). These results suggest that HCP satisfaction with hygiene is largely dependent on the availability of supplies, their amount/location, and a system which prioritizes funding for supplies, as opposed to the hygiene knowledge of HCPs. This finding is supported by previous literature which found associations between sufficient hygiene practices and hygiene materials such as soap, drying materials, and alcohol-based hand rub (Erasmus et al., 2010), but no association with theoretical knowledge, social influence, or moral obligation (De Wandel, Maes, Labeau, Vereecken and Blot, 2010; Naughton et al., 2015).

Respondents less often reported dissatisfaction with cleanliness and IPC practices than with WaSH infrastructure. However, dissatisfaction was still reported by 36% of respondents, and a number of factors – availability of infrastructure and supplies, reported cleaning practices, and institutional support – were associated with HCP satisfaction with cleanliness and IPC practices. Characteristics of water at the HCF, including proximal main water sources (OR = 1.54), water treatment (OR = 1.43), and accessibility of water to people with reduced mobility (OR = 1.69) were some of the most important infrastructure characteristics for satisfaction with cleanliness and ICP practices, similarly to HCP satisfaction with other environmental conditions studied here.

Cleaning practices, including the cleaning of floors, surfaces, and sanitation facilities with water, detergent, or disinfectant (OR = 1.54) and intermittent cleaning of mattresses, pillows, and linens (OR = 1.96) were also significantly associated with HCP job satisfaction. These specific factors do not yet have well-established links in the literature, however the importance of cleanliness to job satisfaction has been demonstrated (Lundstrom et al., 2002; Peters et al., 2010). Institutional support for cleanliness and IPC practices at the HCFs were closely associated with HCP satisfaction, including sufficient budgeting (OR = 2.14), a community-composed oversight committee that had met in the past six months (OR = 1.54), and an IPC procedure, or document in place by the HCF (OR = 1.62). This finding is supported by previous literature from Ghana indicating that oversight and organizational commitment are directly tied to job satisfaction in HCPs (Bonenberger et al., 2014), and analysis showing the importance of budgeting for cleanliness and IPC to maternity wards in HCFs of the same countries studied here (Cronk et al., 2021). This coincides with findings about oversight and dedicated budgets improving satisfaction with other environmental services like sanitation and hygiene infrastructure.

4.1. Limitations

This study was based on cross-sectional data, so our ability to infer causality is limited. Although sample size was comparatively large and variance assumptions were met, reducing survey information to binary or categorical variables reduced the amount of information available for models and thus the power of analyses. Spurious results due to logistic regression testing of large sets of variables was reduced by examining selected variables and results with subject-matter experts and alongside current literature. While surveys were conducted in private (away from patients, other staff, etc.) and respondents were informed that responses would be kept anonymous, respondents' answers may have been influenced by courtesy or social desirability bias due to face-to-face surveying. Only public clinics in rural areas of LMICs are studied here, so findings should not be interpreted for private, non-profit, or urban centers, or places with specialized care. We did not assess the impact of the presence of alcohol-based hand rub at the point of care (e.g., bedside) because this was not included in the survey, nor were other aspects of HCP satisfaction not available from the survey included in the analysis.

5. Conclusions

Our study suggests that governments and non-governmental organizations (NGOs) should facilitate substantial improvements to HCF WaSH infrastructure in order to improve both HCP satisfaction and patient outcomes in public, rural clinics of LMICs. With regards to water, our findings suggest that governments and NGOs working in this space should prioritize provision of on premises, improved-type water service accessible to people with reduced mobility at HCFs. This single intervention could improve the quantity of water available for all purposes, as well as increase HCP satisfaction with HCF water, hygiene, and cleanliness and IPC services. With regards to sanitation services, it is critical that future interventions prioritize the acceptability of sanitation facilities within the local context in order to prevent patients choosing to defecate in the open. Finally, our results suggest that the provision of hygienic materials may be more effective than the provision of hygiene education in improving handwashing compliance and HCP satisfaction, though provision of hygiene materials and education are often delivered together.

At the HCF level, we recommend that oversight committees include dedicated funding for WaSH and IPC needs. The sustainability of WASH infrastructure and services at HCFs depends on the ability of the service provider to ensure accessibility of the consumable hygiene and IPC products and the maintenance of WASH infrastructure. The inclusion of sufficient funding for WASH and IPC needs in the HCF budget affected HCP satisfaction with sanitation, hygiene and IPC practices and cleanliness.

Declaration of competing interest

Authors JBT and OO are both employed by World Vision, the sponsor for the original collection of data, but do not declare any influence from World Vision in their role in this manuscript. The other authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijheh.2021.113802.

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Association of silica dust exposure with mortality among never smokers: A 44-year cohort study

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ABSTRACT

The association of silica dust exposure with mortality among never smokers has not been well established. We aimed to evaluate the association of silica dust exposure with mortality among never smokers. We studied 17,130 workers employed for at least 1 year between January 1, 1960 and December 31, 1974, with follow-up until the end of 2013. Cumulative respirable silica dust exposure (CDE) was estimated by linking a job-exposure matrix to personal work history. We observed 3937 deaths during 589,357.26 person-years of follow-up. Significant positive exposure-response relationships were found between CDE and mortality from all cause (HR = 1.01, 95% CI = 1.01–1.02), respiratory tuberculosis (HR = 1.04, 95%CI = 1.02–1.06), CVDs (HR = 1.03, 95%CI = 1.02–1.04), and diseases of the respiratory system (HR = 1.06, 95%CI = 1.04–1.07). We found higher standardized mortality ratios for respiratory tuberculosis (2.62, 2.32–2.95), CVDs (1.43, 1.32–1.54), and pneumoconiosis (77.75, 68.21–88.25) among silica dust exposed workers. In addition, we estimated that 4.19%, 20.69%, 7.48% and 34.06% of deaths for all cause, respiratory tuberculosis, CVDs, and diseases of the respiratory system among Chinese workers were attributed to silica, after adjusting for other covariates. With regard to lung cancer, compared with unexposed group, the HRs and 95% CI were 0.94 (0.52–1.71), 1.86 (1.15–3.00), 1.65 (0.95–2.86) for low, medium, and high exposed workers, respectively. Long-term silica dust exposure is associated with increased mortality in the absence of cigarette smoking.

1. Introduction

Crystalline silica is one of the commonest minerals on earth, and silica dust has been reported to be one of the most serious occupational hazards in the workplace (Steenland and Ward, 2014). It is estimated that tens of millions of workers worldwide (Leung et al., 2012) and 23 million Chinese workers are exposed to silica dust. The adverse health effects of silica exposure are an increasing public health concern for decades. Long-term exposure to silica dust has been established to be associated with higher mortality of silicosis (Mundt et al., 2011), cardiovascular diseases (CVDs) (Liu et al., 2014), lung cancer (Liu et al., 2013) and other respiratory diseases (Chiazze et al., 2002).

In our previous study, we have explored the association between silica dust exposure and total and specific mortality among 74,040 workers (Chen et al., 2012). And we found positive association between silica dust exposure and mortality of respiratory diseases, lung cancer and CVDs. Meanwhile, studies revealed that smoking also play an important role in the occurrence and progress of these diseases (Duncan et al., 2019; Hirsch et al., 2017). As we know, cigarette smoking has been confirmed to be the main cause of lung cancer and respiratory diseases mortality. According to the report of centers for disease control and prevention in US, smoking causes about 90% of all lung cancer deaths and about 80% of all death from chronic obstructive pulmonary disease (COPD). Meanwhile, stroke and coronary heart disease caused

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by smoking are among the leading causes of death in the United States. In addition, the interaction between silica dust exposure and cigarette smoking on the risk of total and cause-specific mortality has also been reported previously (Brown, 2009; Wang et al., 2020). However, evidence on the association between silica dust exposure and diseases mortality among never smokers is limited.

Thus, we conducted a large cohort study of 17,130 never smokers from 20 metal mines and 9 pottery factories followed from January 1, 1960 to December 31, 2003. We aimed to assess the association of long-term exposure to silica dust with total and cause-specific mortality among never smokers and to determine population attribute risks (PAR) of mortality associated with the exposure among never smokers.

2. Material and methods

2.1. Study population

The silica cohort has been reported elsewhere (Chen et al., 1992, 2012). Briefly, the cohort was established in the late 1980s, it included 74,040 workers from 20 metal mines and 9 pottery factories worked for 1 year or more between January 1, 1960 and December 31, 1974. The cohort was retrospectively followed to 1960 and prospectively followed to the end of 2003. In the present study, we conducted analyses only among 17,130 never smokers.

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline. The study was approved by the Tongji Medical College Institutional Review Board (No. 0990–0279), written informed consent was obtained before interviews.

2.2. Silica exposure assessment

Silica exposure assessment was reported in detail in previous published papers (Dosemeci et al., 1993). In brief, quantitative assessment of silica exposure was conducted using a job-exposure matrix. In the matrix, the total dust concentration for each job title in one specific year in all workplaces of this job title and this specific year and such exposure matrix has been calculated for each mine or factory in the cohort since 1950. It was estimated using data from similar jobs or the same job at different years for missing data for years or jobs (less than 20%).

Work histories for each worker were obtained from personal employment records in mines/factory files. They include job titles and duration years of each worker's full employment. By linking the job exposure matrix, cumulative respirable silica dust exposure (CDE, mg/m^3-year) for each worker was estimated as follows:

$$CDE = \sum_{i=1}^{n} (Ci \times Ti) \quad (1)$$

where n is the total number of job titles; Ci is the 8 h time-weighted mean concentration of dust for the ith job title; and Ti is the working years for the ith job title.

We calculated CDE from the start date of silica-exposed work to the earliest one of the following: end of employment, lost to follow-up, died or the end of 2003.

2.3. Cause of death

All workers were tracked for their vital status by local hygienists during the follow-up period. Information on causes of death was collected from various ways: medical records in the hospital (60.5%); employment registers, accident records, or death certificates (35.2%); or oral reports from relatives (4.3%) (Chen et al., 2012). The 10th International Classification of Diseases (ICD-10) was used to code causes of death.

2.4. Statistical analysis

The basic characteristic of the participants was reported as mean (SD) for continuous variables and as number (percentages) for categorical variables. Cox proportional hazard models were used to calculate hazards ratios (HRs) and 95% confidence intervals (CIs) for CDE and the risk of selected causes of death, with adjustment for gender, year of hire (four categories: 1950 or earlier, 1951–1960, 1961–1970, 1971 or later), age at hire (continuous), and type of facility (four categories: tungsten mines, iron and copper mines, tin mines, and pottery factories). CDE was categorized into four groups based on the percentiles from the exposure distribution. The linear trend tests were conducted by including the median value for each level of dust as a continuous variable in the models. In addition, we evaluated the nonlinear relationship between CDE and total and cause-specific mortality by using restricted cubic splines with 4 knots at percentiles 5, 35, 65 and 95% of the distribution.

The population attributable risk percent (PAR%) was calculated as follows:

$$PAR\% = [P \times (RR\text{-}1)]/[P \times (RR\text{-}1)+1] \times 100\% \quad (2)$$

Where P is the percentage of silica-exposed workers among all industrial workers (16.3%) (China, 2009), and RR is the relative risk, which is estimated from HR in our study.

Standardized mortality ratios (SMR) were also used to reflect the death information. SMRs were calculated among 16,918 workers with excluding 212 deaths before 1970, as national death rate data in China were not available before 1970. The expected number of cause-specific deaths were calculated by multiplying the gender-, age-, periods-, and cause-specific person-years at risk with 5-year intervals for age and period by the corresponding mortality rates in China. All statistical analyses were performed using SAS 9.4 software (SAS Institute, Cary, NC), and a two-sided p-value < 0.05 was regarded as statistically significant.

3. Results

A total of 17,130 participants (mean [SD] age at hire, 23.99 [7.12] years; 10,992 [64.17%] men) were included in this study. The basic characteristics of participants based on CDE are summarized in Table 1. Finally, there were 1179 (6.88%) workers still working at the end of the follow up. A total of 8942 (52.20%) workers were exposed to silica dust. The average CDE and duration of silica dust exposure was 3.50 mg/m^3-y and 18.67 years, respectively. During a median follow-up of 34.40 years (589,357.26 persons-years), a total of 3937 deaths were reported. Mortality rate was 668.02 per 100,000 person-years for all participants, with 848.68 per 100,000 person-years for silica exposed workers and 460.65 per 100,000 person-years for no silica exposed workers.

The HRs and 95% CI for total and cause-specific mortality associated with CDE are revealed in Table 2. CVDs, malignant neoplasms, cerebrovascular diseases, and diseases of the respiratory system were the top 4 causes of death. Compared with unexposed workers, the mortality was significantly higher among silica-exposed workers from all cause (HR = 1.27, 95%CI = 1.18–1.36), respiratory tuberculosis (HR = 2.60, 95%CI = 1.95–3.46), CVDs (HR = 1.50, 95%CI = 1.28–1.74), and diseases of the respiratory system (HR = 4.17, 95%CI = 3.17–5.48). Among CVDs, it is more evident for pulmonary heart diseases (HR = 3.37, 95%CI = 2.53–4.50). Categorical CDE were significantly associated with higher risk of mortality from all cause, respiratory tuberculosis, CVDs (including pulmonary heart diseases), and diseases of the respiratory system (including pneumoconiosis). We also observed possible association of silica exposure with the mortality of lung cancer. Compared with unexposed group, we observed higher risks of lung cancer for medium (HR = 1.86, 95%CI = 1.15–3.00) and high exposed group (HR = 1.65, 95%CI = 0.95–2.86).

The nonlinear relationship between CDE and total and cause-specific

Table 1
Basic characteristics of participants based on CDE.

Characteristic	Total	Level of CDE[c]			
		Unexposed	Low	Medium	High
	(N = 17,130)	(N = 8188)	(N = 2980)	(N = 2981)	(N = 2981)
Status at the end of follow-up (n,%)					
Working	1179 (6.88)	825 (10.08)	159 (5.34)	167 (5.60)	28 (0.94)
Left	2831 (16.53)	2102 (25.67)	415 (13.93)	222 (7.45)	92 (3.09)
Retired	9183 (53.61)	3997 (48.82)	1867 (62.65)	1750 (58.71)	1569 (52.63)
Died	3937 (22.98)	1264 (15.44)	539 (18.09)	842 (28.25)	1292 (43.34)
Male (n,%)	10,992 (64.17)	3947 (48.20)	2441 (81.91)	2380 (79.84)	2224 (74.61)
Year of birth, mean ± SD	1938.52 ± 10.76	1940.57 ± 10.12	1942.29 ± 9.45	1937.03 ± 10.21	1930.63 ± 10.01
Year of hire, mean ± SD	1962.52 ± 7.28	1963.77 ± 6.94	1965.53 ± 6.53	1961.45 ± 6.86	1957.15 ± 6.29
Year of hire, (n, %)					
1915–1950	595 (3.47)	151 (1.84)	19 (0.64)	72 (2.42)	353 (11.84)
1951–1960	7904 (46.14)	3236 (39.52)	940 (31.54)	1684 (56.49)	2044 (68.57)
1961–1970	5527 (32.27)	3099 (37.85)	1198 (40.20)	765 (25.66)	465 (15.60)
1971–1975	3104 (18.12)	1702 (20.79)	823 (27.62)	460 (15.43)	119 (3.99)
Age at hire, mean ± SD	23.99 ± 7.12	23.20 ± 6.73	23.24 ± 5.98	24.41 ± 6.75	26.52 ± 8.74
Age at first exposure, mean ± SD[b]	23.25 ± 7.01	NA	24.83 ± 7.47	23.49 ± 6.46	21.43 ± 6.62
Year of first exposure, mean ± SD[b]	1959.90 ± 10.32	NA	1967.12 ± 8.08	1960.53 ± 7.86	1952.06 ± 8.86
Cumulative total exposure, mean ± SD, mg/m^3-y[b]	145.54 ± 171.83	NA	40.79 ± 38.76	80.48 ± 54.19	315.30 ± 200.28
CDE, mean ± SD, mg/m^3-y[b]	3.50 ± 4.11	NA	0.48 ± 0.25	1.95 ± 0.76	8.08 ± 4.20
Duration of silica dust exposure, mean ± SD[b]	18.67 ± 10.32	NA	13.85 ± 9.74	18.98 ± 9.44	23.18 ± 9.58
Pneumoconiosis cases (n,%)[a]	1442 (8.42)	NA	85 (2.85)	357 (11.98)	1000 (33.55)
Age at first diagnosis of pneumoconiosis, mean ± SD[a]	45.99 ± 10.29	NA	48.69 ± 8.23	47.62 ± 10.51	45.18 ± 10.27
Age at last exposure, mean ± SD[b]	43.01 ± 10.57	NA	39.51 ± 11.14	43.86 ± 10.12	45.65 ± 9.44

[a] Results was just among pneumoconiosis.
[b] Results was just among silica dust-exposed workers.
[c] Levels of CDE was tertiles of CDE of all workers exposed to silica dust: low, 0–0.967mg/m^3-y; medium, 0.968–3.693 mg/m^3-y; high, >3.693 mg/m^3-y.

Table 2
Estimated HRs for total and cause-specific mortality associated with CDE in the cohort.

Cause of Death (ICD-10 Codes)	Number of Death	HRs for Levels of CDE versus Unexposed			
		Low	Medium	High	P_{trend}
Malignant neoplasms (C00–C97)	701	1.04 (0.83–1.31)	1.14 (0.92–1.40)	0.98 (0.78–1.24)	0.72
Malignant neoplasm of nasopharynx (C11)	36	0.76 (0.27–2.19)	1.34 (0.59–3.03)	0.28 (0.07–1.08)	0.06
Malignant neoplasm of liver and intrahepatic bile ducts (C22)	202	1.05 (0.69–1.60)	1.02 (0.69–1.52)	1.03 (0.67–1.58)	0.93
Lung cancer (C33–C34)	129	0.94 (0.52–1.71)	1.86 (1.15–3.00)	1.65 (0.95–2.86)	0.11
Certain infectious and parasitic diseases (A00–B99, J65)	457	1.39 (0.93–2.09)	1.88 (1.37–2.57)	2.83 (2.14–3.73)	<0.001
Respiratory tuberculosis (A15–A16, J65)	398	1.42 (0.88–2.30)	2.22 (1.57–3.14)	3.19 (2.35–4.34)	<0.001
Cardiovascular diseases (I00–I52, I70–I99)	973	1.08 (0.84–1.39)	1.33 (1.09–1.61)	1.80 (1.51–2.13)	<0.001
Pulmonary heart diseases (I26–I27)	485	1.28 (0.76–2.13)	2.40 (1.70–3.40)	4.56 (3.36–6.18)	<0.001
Hypertensive heart disease (I11)	131	1.42 (0.81–2.48)	0.92 (0.56–1.51)	0.78 (0.50–1.23)	0.16
Ischemic heart disease (I20–I25)	179	1.12 (0.70–1.77)	1.44 (0.98–2.13)	0.75 (0.47–1.19)	0.10
Chronic rheumatic heart disease (I05–I09)	41	0.15 (0.02–1.18)	0.62 (0.26–1.49)	0.79 (0.36–1.71)	0.97
Cerebrovascular diseases (I60–I69)	643	1.06 (0.83–1.36)	1.06 (0.86–1.32)	0.91 (0.73–1.13)	0.28
Diseases of the respiratory system (J00–J99)	575	2.39 (1.63–3.49)	3.55 (2.58–4.88)	5.96 (4.40–8.06)	<0.001
Pneumoconiosis (J60–J65)*	293	1 (ref)	3.67 (1.86–7.26)	8.98 (4.46–18.08)	<0.001
Diseases of the digestive system (K00–K93)	198	1.14 (0.74–1.76)	1.12 (0.76–1.65)	0.77 (0.51–1.16)	0.12
External causes of morbidity and mortality (V01–Y98)	304	1.60 (1.20–2.15)	1.30 (0.93–1.80)	0.68 (0.44–1.06)	0.02
All diseases (A00–Y98)	3937	1.17 (1.05–1.30)	1.22 (1.11–1.34)	1.39 (1.27–1.52)	<0.001

Adjusted for gender, year at hire (five categories: 1950 or earlier, 1951–1960, 1961–1970, 1971 or later), age at hire (continuous), and type of facilities (four categories: tungsten mines, iron/copper mines, tin mines and pottery factories).

mortality was further evaluated by the spline curve in Fig. 1. The results confirmed that the mortality was significantly higher among silica-exposed workers from all cause, respiratory tuberculosis, pulmonary heart diseases, and diseases of the respiratory system (pneumoconiosis).

The PAR% of silica exposure was 4.19% for mortality from all causes, 20.69% from respiratory tuberculosis, 7.48% for mortality from CVDs (including 27.89% from pulmonary heart diseases), and 34.06% for mortality from diseases of the respiratory system, after adjusting for other covariates.

The SMRs for deaths from all and specific cause from 1970 to 2003 are reported in Table 3. Compared with the mortality from 1970 to 2003 in China, the mortality among silica-exposed workers significantly elevated for certain infectious and parasitic diseases (SMR = 4.19), respiratory tuberculosis (2.62), CVDs (1.43), and diseases of the respiratory system (1.40). Among CVDs, it is significantly higher for pulmonary heart diseases (1.92), hypertensive heart disease (2.46), and ischemic heart disease (1.26). In addition, significantly elevated mortality was also found for nasopharynx cancer (1.76) and liver cancer (1.28).

4. Discussion

In the present cohort study with a large sample and long-term follow-up, we found a significant expose-response relationship between long-term silica dust exposure and mortality among never smokers, including all cause, respiratory tuberculosis, non-malignant respiratory diseases, and CVDs. Meanwhile, the SMRs of the latter three diseases from 1970 to 2003 among silica exposed workers were still higher than

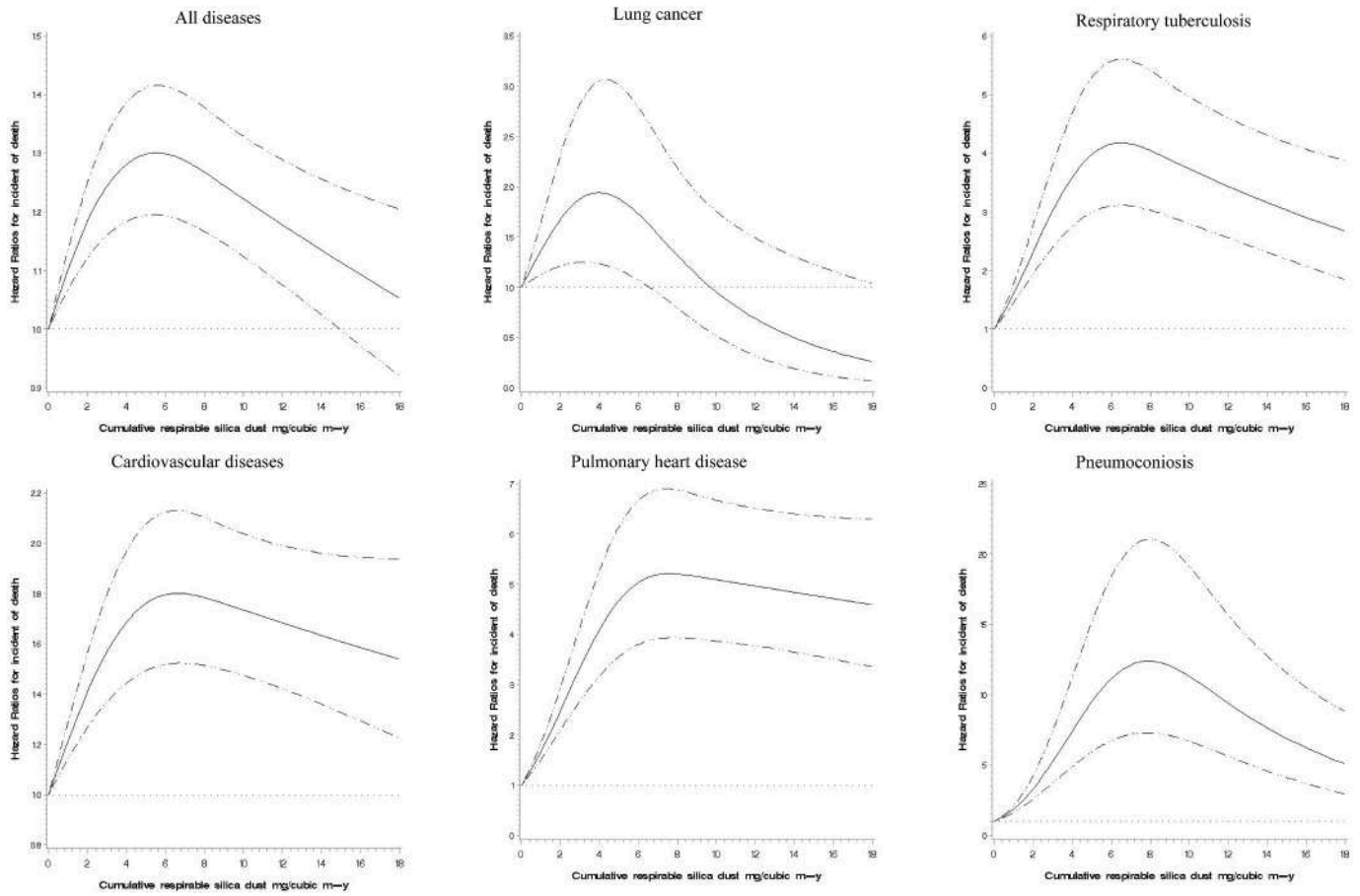

Fig. 1. Multivariable adjusted spline curves for association between cumulative respirable silica dust and mortality. Adjusted for sex, year of hire (four categories: 1950 or earlier, 1951–1960, 1961–1970, 1971 or later), age at hire (continuous), and type of facility (four categories: tungsten mines, iron and copper mines, tin mines, and pottery factories).

the general population.

The exposure-response relationships between CDE and mortality from all cause, respiratory tuberculosis, CVDs and diseases of the respiratory system have been reported in our previous study conducted among 74,040 workers (Chen et al., 2012). The present study reconfirmed the results among never smokers, which strongly indicated the relationship between silica dust exposure and mortality from these diseases. The findings may have important public health implications. Silica exposure is common in the workplace around the world and has been reported to be associated with several diseases, our study identified the significant independent attribution of silica exposure on all cause, respiratory tuberculosis, CVDs and diseases of the respiratory system in the absence of cigarette smoking.

The results for the association between silica exposure and above-mentioned diseases were consistent with other published cohort studies. Mannetje et al. verified the elevated silicosis mortality among silica-exposed workers in a pooled analysis of six cohorts (t Mannetje et al., 2002). Tse et al. confirmed the exposure-response relationship between silica dust exposure and mortality from non-malignant respiratory diseases (Tse et al., 2007). Few studies have reported the association between silica dust exposure and pulmonary heart diseases mortality. A retrospective cohort conducted among 6266 male workers found an increased standardized rate ratio (SRR) of 1.79 for pulmonary heart disease mortality (Dong et al., 1995), but the SRR was calculated by comparing the mortality rates with 11,470 male steel workers, the HRs were not reported either. Previous analysis of this cohort with 42, 572 workers revealed a positive exposure-response relationship between silica exposure and pulmonary heart diseases mortality (Liu et al., 2014). The exposure-response relationship could also be found in the current study, but the HRs were lower than those when cigarette smoking workers were excluded. Our present study conducted the exposure-response analyses with sufficient data on silica exposure, larger study population and longer follow-up time. In addition, we also found the exposure-response relationship between silica dust exposure and respiratory tuberculosis, which has been less studied before.

The association between silica dust exposure and lung cancer has been debated for several years (Keil et al., 2018). Most studies indicated the positive association between silica dust exposure and lung cancer, but others did not get the similar conclusion. A pooled analysis of 10 large silica-exposed cohorts with good-quality exposure data found a significant positive exposure-response relationship between silica exposure and lung cancer mortality (Steenland et al., 2001). In addition, a meta-analysis study revealed similar results, and showed studies with and without controlling for cigarette smoking yield similar relative risks (Lacasse et al., 2009). However, some experts disagreed with this conclusion, as the positive association between silica dust exposure and lung cancer could not be found in all industrial circumstances (Hessel et al., 2000). The main point for the debate is that it is difficult to rule out the risk caused by confounders, especially for tobacco smoking and occupational hazards other than silica. Sogl M et al. have confirmed a positive exposure-response relationship between silica dust and lung caner after adjusting for radon and arsenic with effect modifiers (Sogl et al., 2012); however, the individual information on the potential confounder smoking was not included in the analysis. Cigarette smoking is also a causative factor for lung cancer and the proportion of smokers is high among silica exposed workers (Zhang et al., 2009). Therefore,

Table 3
Estimated SMRs for death of workers in the cohort (N = 16,918), 1970–2003.

Cause of Death (ICD-10 Codes)	SMR (95%CI), 1970–2003				
	Total	Unexposed	Low	Medium	High
Malignant neoplasms (C00–C97)	0.72 (0.66–0.77)	0.68 (0.60–0.77)	0.85 (0.70–1.02)	0.83 (0.71–0.98)	0.62 (0.52–0.73)
Malignant neoplasm of nasopharynx (C11)	1.65 (1.14–2.31)	1.50 (0.80–2.56)	1.67 (0.54–3.79)	3.14 (1.62–5.44)	0.66 (0.13–1.85)
Malignant neoplasm of liver and intrahepatic bile ducts (C22)	1.14 (0.98–1.30)	0.94 (0.74–1.19)	1.40 (0.98–1.93)	1.39 (1.02–1.85)	1.11 (0.82–1.48)
Lung cancer (C33–C34)	0.59 (0.49–0.70)	0.51 (0.37–0.68)	0.54 (0.30–0.88)	0.84 (0.58–1.17)	0.55 (0.38–0.78)
Certain infectious and parasitic diseases (A00–B99, J65)	2.98 (2.69–3.29)	1.35 (1.06–1.69)	1.73 (1.18–2.45)	3.41 (2.72–4.22)	6.17 (5.34–7.09)
Respiratory tuberculosis (A15–A16, J65)	1.80 (1.61–2.00)	0.71 (0.53–0.92)	0.78 (0.48–1.19)	2.13 (1.67–2.67)	4.18 (3.59–4.84)
Cardiovascular diseases (I00–I52, I70–I99)	1.13 (1.06–1.20)	0.70 (0.62–0.80)	0.83 (0.66–1.03)	1.27 (1.10–1.46)	1.81 (1.64–1.99)
Pulmonary heart diseases (I26–I27)	1.28 (1.17–1.41)	0.36 (0.27–0.47)	0.48 (0.29–0.73)	1.33 (1.07–1.64)	2.98 (2.65–3.35)
Hypertensive heart disease (I11)	2.23 (1.86–2.65)	1.89 (1.38–2.52)	2.78 (1.68–4.32)	2.46 (1.62–3.57)	2.34 (1.65–3.20)
Ischemic heart disease (I20–I25)	1.18 (1.01–1.37)	1.07 (0.83–1.36)	1.48 (0.97–2.16)	1.85 (1.38–2.41)	0.77 (0.53–1.08)
Chronic rheumatic heart disease (I05–I09)	0.45 (0.32–0.62)	0.51 (0.31–0.79)	0.08 (0.01–0.39)	0.43 (0.17–0.87)	0.60 (0.31–1.05)
Cerebrovascular diseases (I60–I69)	0.86 (0.79–0.93)	0.85 (0.75–0.96)	1.00 (0.80–1.23)	0.96 (0.80–1.13)	0.76 (0.64–0.88)
Diseases of the respiratory system (J00–J99)	0.94 (0.86–1.03)	0.28 (0.21–0.35)	0.84 (0.63–1.10)	1.15 (0.95–1.38)	1.78 (1.58–2.00)
Pneumoconiosis (J60–J65)	45.40 (39.83–51.53)	–	15.64 (7.82–27.72)	53.91 (40.64–70.11)	128.10 (109.75–148.67)
Diseases of the digestive system (K00–K93)	0.64 (0.55–0.74)	0.52 (0.40–0.66)	0.85 (0.59–1.19)	0.91 (0.68–1.20)	0.53 (0.38–0.73)
External causes of morbidity and mortality (V01–Y98)	4.26 (3.75–4.82)	3.67 (2.96–4.50)	10.49 (8.24–13.16)	5.07 (3.81–6.61)	1.85 (1.24–2.65)
All diseases (A00–Y98)	0.83 (0.81–0.86)	0.65 (0.61–0.69)	0.88 (0.81–0.97)	0.94 (0.88–1.01)	1.01 (0.96–1.07)

SMRs were estimated based on Chinese national mortality rates (not available before 1970).

smoking is considered to be an important interfering factor in evaluating the carcinogenicity of silica dust. In the present study, our study confirmed that silica dust exposure was associated with lung cancer in the absence of cigarette smoking, though the higher risk of lung cancer in the high exposed group was not statistically significant. There are several possible reasons, including the healthy worker survivor effect, which refers to a depletion of the number of susceptible people in the population at high exposure levels and less reliable estimates at those levels. This phenomenon was also observed in studies of other occupational populations (Stayner et al., 2003). Our study could provide some important evidence for the association between silica dust exposure and lung cancer, as it was found in the absence of cigarette smoking in the present study.

Our study has several strengths. First, it was a long follow-up cohort study with large sample size. Second, the detail information of silica dust exposure during the lifetime was collected. The limitation should also be acknowledged. First, long-term exposure to silica dust was evaluated carefully, but measurement errors were inevitable. Silica concentrations before 1950 were estimated by using those in 1950, which may have led to the underestimation of exposure for those who worked before 1950 (4.0%). However, the results were almost the same when we excluded the workers whose silica exposure occurred before 1950 (data not shown). Second, the use of personal protective equipment was not considered in our study, but they were rarely used (<5% of the workers) or used improperly, indicating that the use of personal protective equipment had little effect on the results. Third, although a number of confounders were adjusted in our study, there were still other occupational risk factors which were not included, such as radon and polycyclic aromatic hydrocarbon (PAHs), which were also reported to paly an important role in respiratory diseases. However, less than 10% workplace of job titles have a level of radon exceeding the limit (0.3WL), and no statistical difference was found in the incidence of silicosis or lung cancer between the high radon exposure group (≥0.3WL) and low radon exposure group (<0.3WL) (P>0.05). In addition, the level of polycyclic aromatic hydrocarbon (PAHs) were also far less than the limit (0.1 mg/m^3), which may have little effect on health outcomes. Finally, smoking information for all participants were collected in 1995 and 2004 (Liu et al., 2013; Wang et al., 2020), respectively, and ever smokers (current and former smokers) were defined as those who had smoked cigarettes regularly for at least 1 year at any point in their lifetimes in this study, others were never smokers. Since cigarette smoking for the deceased were derived from their colleagues or next-of-kin, there may be information bias. In order to evaluate the accuracy of information, a data reliability analysis was examined for 1990 randomly selected subjects through face-to-face questionnaires, and the agreement on smoking status (never or ever) between next-of-kin and colleagues of decedents was 89.1%, and agreement on smoking status between self-report and next-of-kin (or colleagues) for living subjects was 93.6% (Liu et al., 2013).

5. Conclusions

Our study shows a significant exposure-response relationship between silica dust exposure and mortality from all cause, respiratory tuberculosis, non-malignant respiratory diseases, and CVDs among never smokers. In addition, elevated risk of lung cancer could also be found for silica exposed never smokers. Our findings could provide some information for the association between silica dust exposure and lung cancer.

Contributors

DW and WC designed research. DW conducted research, analyzed data, and wrote the paper. All authors contributed to the acquisition, analysis, or interpretation of the data, and revised the manuscript for important intellectual content. WC has primary responsibility for final content and is the study guarantor. All authors read and approved the final manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Declaration of competing interest

None declared.

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Associations of fine particulate matter and constituents with pediatric emergency room visits for respiratory diseases in Shanghai, China

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ABSTRACT

Background: Although ambient fine particulate matter ($PM_{2.5}$) has been associated with adverse respiratory outcomes in children, few studies have examined $PM_{2.5}$ constituents with respiratory diseases in children in China.

Objectives: To investigate the associations of short-term exposure to $PM_{2.5}$ and its constituents with pediatric emergency room visits (ERVs) for respiratory diseases in Shanghai, China.

Methods: We collected daily concentrations of $PM_{2.5}$ and its constituents in urban Shanghai from January 1, 2016, to December 31, 2018. Daily pediatric ERVs for four major respiratory diseases, including upper respiratory tract infection, bronchitis, pneumonia, and asthma, were obtained from 66 hospitals in Shanghai during the same period. Associations of exposure to daily $PM_{2.5}$ and constituents with respiratory ERVs were estimated using the over-dispersed generalized additive models.

Result: Short-term exposure to $PM_{2.5}$ and its constituents were associated with increased pediatric ERVs for respiratory diseases. Specifically, an interquartile range increase in the 3-day average $PM_{2.5}$ level (31 $\mu g/m^3$) was associated with 1.86% (95%CI: 0.52, 3.22), 1.53% (95%CI: 0.01, 3.08), 1.90% (95%CI: 0.30, 3.52), and 2.67% (95%CI: 0.70, 4.68) increase of upper respiratory tract infection, bronchitis, pneumonia, and asthma ERVs, respectively. As for $PM_{2.5}$ constituents, we found organic carbon, ammonium, nitrate, selenium, and zinc were associated with higher risk of respiratory ERVs in the single constituent and the constituent-$PM_{2.5}$ models.

Conclusion: Short-term exposure to $PM_{2.5}$ was associated with increased pediatric ERVs for respiratory diseases. Constituents related to anthropogenic combustion and traffic might be the dominant contributors of the observed associations.

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1. Introduction

The incidence of respiratory diseases, especially respiratory tract infection and asthma, has increased considerably in children in recent years and acute respiratory infection has become one of the major causes of hospitalization for young children around the world (Asher and Pearce, 2014; Shi et al., 2017). Previous studies suggested that hospital admission for pneumonia among children increased by 2.9 times from 2000 to 2015 globally with greater growth found in developing countries (Achilleos et al., 2017).

Several studies have suggested that ambient air pollution, especially fine particulate matter (particulate matter with an aerodynamic diameter $\leq$2.5 μm, $PM_{2.5}$) is a modifiable risk factor of respiratory diseases among children (Bouazza et al., 2018; Horne et al., 2018; Li et al., 2018; Liu et al., 2019; Nascimento et al., 2017). In China, increased pediatric visits for respiratory infection and asthma have been associated with $PM_{2.5}$ in previous studies, but the results were still inconsistent in magnitude (Liu et al., 2017; Zheng et al., 2015, 2017). One possible reason for such inconsistency is that as a mixture with complex constituents, ambient $PM_{2.5}$ may differ in respiratory toxicity as determined by chemical constituents from multiple sources (Liao et al., 2015). Although several studies have considered the associations of $PM_{2.5}$ chemical constituents and hospital visits for respiratory diseases, evidence for children were still limited compared to the general population (Kim et al., 2012; Ostro et al., 2009; Peng et al., 2009).

The aim of this time-series study is to examine the associations of short-term exposure to $PM_{2.5}$ and its constituents with EVRs for four common respiratory diseases, including upper respiratory tract infection, bronchitis, pneumonia, and asthma in children from Shanghai, China.

2. Materials and methods

2.1. Data collection

The study period was from January 1, 2016 to December 31, 2018. Daily pediatric ERVs during this period were extracted from electronic medical records of 66 hospitals in Shanghai, China (Fig. 1). We used the International Classification of Disease, Revision 10 (ICD10) to identify ERVs for upper respiratory tract infection (J06), bronchitis (J20/J21/J40), pneumonia (J12~J18), and asthma (J45~J46). Patients who were not residents of Shanghai were excluded. Details on data collection can be found in our previous publication (Liu et al., 2020).

Daily mass concentrations of $PM_{2.5}$ during the study period were calculated by averaging the daily means across ten fixed-site monitoring stations in the Shanghai National Air Quality Monitoring Network (Fig. 1). Daily concentrations of $PM_{2.5}$ constituents, including organic and elemental carbon, water soluble ions, and trace elements, were only available in the Shanghai Pudong Atmospheric Monitoring Supersite and thus were used to represent the $PM_{2.5}$ constituent levels in Shanghai (Fig. 1). Concentrations of organic (OC) and elemental carbon (EC) were measured by a semi-continuous OC/EC analyzer (model RT-4, Sunset Laboratory Inc.) with an upstream parallel-plate organic denuder and a $PM_{2.5}$ cyclone. Three major water-soluble inorganic ions, including nitrate (NO_3^-), sulfate (SO_4^{2-}) and ammonium (NH_4^+), were measured using the Monitor for AeRosols and Gases in ambient Air system (MARGA) (ADI, 2080; Applikon, Netherlands). Nine trace elements, including arsenic (As), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se), vanadium (V), and zinc (Zn), were measured by a nondestructive energy dispersive X-ray fluorescence spectrometry (Model XACT 625, Cooper Environmental, Services, LLC). Detailed descriptions of air pollution monitoring and relevant parameters can be found elsewhere (Niu et al., 2018).

Meteorological data, including daily mean temperature and relative humidity, were obtained from Shanghai Meteorological Bureau. Daily concentrations of other gaseous pollutants, including nitrogen dioxide (NO_2), sulfur dioxide (SO_2), carbon monoxide (CO), and ozone (O_3, 8-h maximum values) were also collected from the ten fixed-site monitoring stations for $PM_{2.5}$.

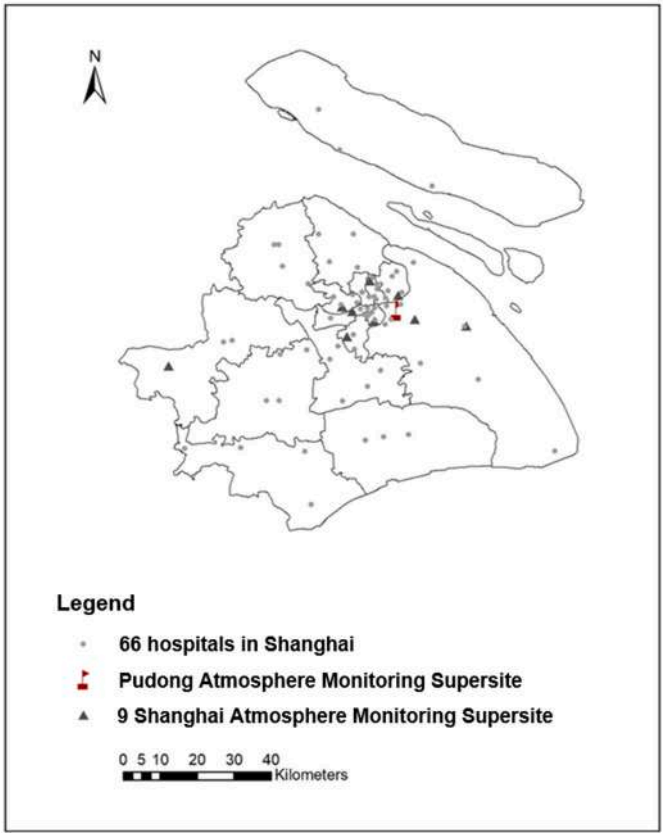

Fig. 1. The locations of 66 hospitals and atmospheric monitor stations in this study.

2.2. Statistical analysis

We used the generalized additive models (GAM) with a quasi-Poisson distribution to estimate the associations of daily $PM_{2.5}$ total mass and constituents with ERVs during the study period, respectively. Covariates considered in the models included: (1) a natural cubic spline with 7 degrees of freedom (df) per year for calendar days to control for long-term and seasonal trends; (2) natural cubic splines with 6 and 3 dfs for the current-day temperature and relative humidity; (3) a binary variable for public holidays; and (4) dummy variables of day of the week (DOW). To explore the potential lag effects of $PM_{2.5}$, we consider single-day exposures on the current (lag 0) and in 1, 2, and 3 days previously (lag 1–lag 3 days) and the cumulative average exposure for up to 3 days prior (i.e., lag 01, 02, and 03 days).

To further control for potential confounding by total $PM_{2.5}$ in the associations of constituents with respiratory ERVs, we then fitted constituents-$PM_{2.5}$ models by additionally adjusting for total $PM_{2.5}$ mass for each constituent, respectively. We also conducted sensitivity analyses to evaluate the robustness of our results. First, we replaced the current day temperature with moving averages from the current day to the previous 3, 7, and 14 days, respectively, to examine the possible lagged confounding of ambient temperature. Further, we considered a distributed lag nonlinear model (DLNM) for the 14-day average temperature to examine for residual confounding. Next, we used a natural spline of 10 df per year for calendar days to control for potential confounding by pollen and influenza seasons. Finally, we adjusted for daily SO_2, NO_2, CO, and O_3 in the models, separately, to control for confounding by co-exposure to these pollutants.

All analyses were conducted in R software (Version 3.3.5, R Foundation for Statistical Computing, Vienna, Austria) with the "mgcv" package. Results were presented as the percentage changes and 95% confidence intervals (CIs) in daily ERVs for each disease per interquartile range (IQR) increase of exposure to $PM_{2.5}$ and constituents. All statistical tests were two-sided and a $p < 0.05$ was considered statistically significant.

3. Results

3.1. Descriptive statistics

During the study period, we identified a total of 868,352 ERVs for upper respiratory tract infection (daily average = 792), 731,916 for bronchitis (daily average = 668), 126,802 for pneumonia (daily average = 116), and 108,817 for asthma (daily average = 99), respectively (Table 1). The daily ERVs for all four diseases distributed evenly by day of week (Supplementary Material Fig. 1). The daily average $PM_{2.5}$ was 40 μg/m^3 during the study period, which was higher than the World Health Organization Air Quality Guidelines (25 μg/m^3). Among the constituents, NO_3^- had the largest proportion (25.9%), followed by SO_4^{2-} (18.9%), NH_4^+ (16.4%), and OC (13.4%). The daily average temperature and relative humidity were 17.8 °C and 73%, respectively.

Spearman correlation coefficients among $PM_{2.5}$ constituents varied in both the magnitude and direction (Supplementary Material Table S1). Overall, there were moderate to high correlations (Spearman r = 0.47–0.90) with $PM_{2.5}$ for all constituents except for V, and the highest correlations were found between $PM_{2.5}$ and NH_4^+ (Spearman r = 0.90), OC and EC (Spearman r = 0.89), and NH_4^+ and NO_3^- (Spearman r = 0.89). Most of the $PM_{2.5}$ constituents had moderate and positive correlations with SO_2, NO_2, and CO, and weak and negative correlation with temperature and relative humidity.

3.2. Regression results

We observed positive associations of daily $PM_{2.5}$ levels and ERVs for all 4 respiratory diseases. The magnitude of the associations differed by lag days and by diseases and asthma had the strongest association with $PM_{2.5}$. As shown in Fig. 2, the strongest associations with $PM_{2.5}$ were found on lag02 days for upper respiratory tract infection, pneumonia, and asthma, and on lag01 for bronchitis. For example, an IQR increase in total $PM_{2.5}$ mass (31 μg/m^3) in lag 02 days was significantly associated with a 1.86% (95%CI: 0.52, 3.22), 1.53% (95%CI: 0.01, 3.08), 1.90% (95%CI: 0.30, 3.52), and 2.67% (95%CI: 0.70, 4.68) increase of ERVs in upper respiratory tract infection, bronchitis, pneumonia, and asthma, respectively. Generally, the model with exposures in lag 0–2 days had the strongest associations with the outcomes as well as the smallest quasi-Poisson Akaike Information Criterion (Supplementary Material Table S3), so we reported estimates in lag 0–2 days in the following analyses.

Fig. 3 showed the percentage change of daily ERVs for upper respiratory tract infection, bronchitis, pneumonia, and asthma with an IQR increase of $PM_{2.5}$ constituents in the single constituent models. Specifically, we found OC, SO_4^{2-}, NO_3^-, NH_4^+, Se, and Zn were associated with higher daily ERVs for all 4 respiratory diseases. Among all the constituents, NH_4^+ had the strongest associations with upper respiratory tract infection, bronchitis, and asthma, and Se had the strongest association with pneumonia. For instance, an IQR increase of NH_4^+ was association with 2.74% (95%CI: 1.55, 3.95), 2.40% (95%CI: 1.05, 3.77) and 1.86% (95%CI: 0.01, 3.75) increase of ERVs for upper respiratory tract infection, bronchitis, and asthma, respectively; and an IQR increase of Se was associated with 2.40% (95%CI: 0.79, 4.04) increase in pneumonia.

After adjusting for $PM_{2.5}$ total mass in constituent-$PM_{2.5}$ models, we found associations of SO_4^{2-} and NO_3^- with daily ERVs attenuated, while associations of OC, Se, and Zn were similar with the single constituent models (Fig. 4). Of note, estimates for NH_4^+ in the constituent-$PM_{2.5}$ models were higher than those in the single constituent models with an IQR increase of NH_4^+ was associated with 7.11% (95%CI: 4.21, 10.09),

Table 1
Daily emergency room visits for respiratory diseases, air pollution levels, and meteorological factors.[a].

variables	mean	SD	Minimum	P (25)	Median	P (75)	Maximum
Emergency Room visits							
Upper respiratory tract infection	792	284	260	609	754	916	2,390
Bronchitis	668	362	167	388	555	894	2,370
Pneumonia	116	58	35	77	101	141	824
Asthma	99	51	30	63	86	123	380
$PM_{2.5}$							
Total mass (μg/m^3)	40	26	6	21	33	51	184
OC (μg/m^3)	5	3	1	3	5	7	20
EC (μg/m^3)	2	1	0	1	2	3	10
SO_4^{2-} (μg/m^3)	8	4	0	4	6	10	37
NO_3^- (μg/m^3)	10	10	1	4	8	14	70
NH_4^+ (μg/m^3)	7	5	0	3	5	9	35
As (ng/m^3)	7	5	0	7	6	10	37
Cr (ng/m^3)	6	4	0	5	5	7	24
Cu (ng/m^3)	14	9	0	11	11	18	84
Mn (ng/m^3)	36	21	0	29	30	45	140
Ni (ng/m^3)	5	2	0	3	4	6	25
Pb (ng/m^3)	35	4	0	29	28	44	264
Se (ng/m^3)	3	2	0	3	3	5	4
V (ng/m^3)	7	5	0	8	5	10	30
Zn (ng/m^3)	155	96	0	141	126	201	712
Meteorological conditions							
Temperature (°C)	18	9	−6	10	19	25	35
Relative humidity (%)	73	12	29	64	74	82	100
Gaseous pollutants							
SO_2 (μg/m^3)	12	5	3	8	10	14	50
NO_2 (μg/m^3)	42	19	10	28	39	54	125
CO (mg/m^3)	0.7	0.2	0.4	0.6	0.7	0.8	1.9
O_3 (μg/m^3)	83	37	9	55	78	102	235

[a] Definition of abbreviations: SD = standard deviation, $PM_{2.5}$ = fine particulate matter, NO_2 = nitrogen dioxide, SO_2 = sulfur dioxides, CO = carbon monoxide, O_3 = ozone.

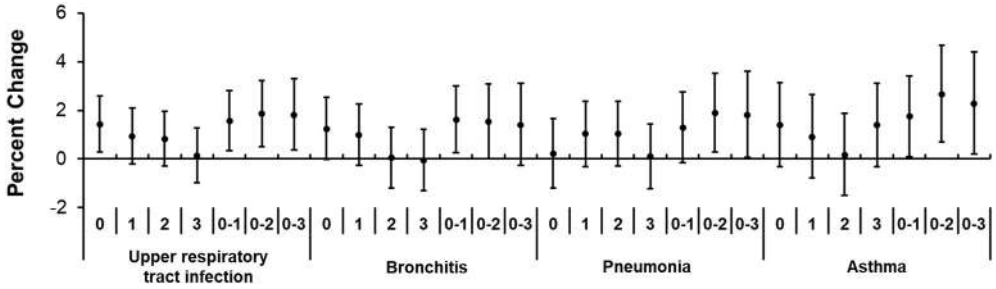

Fig. 2. Percentage changes (mean and 95% confidence intervals) in daily ERVs for 4 diseases per IQR (31 μg/m^3) increase in concentrations of $PM_{2.5}$ with different lag days.

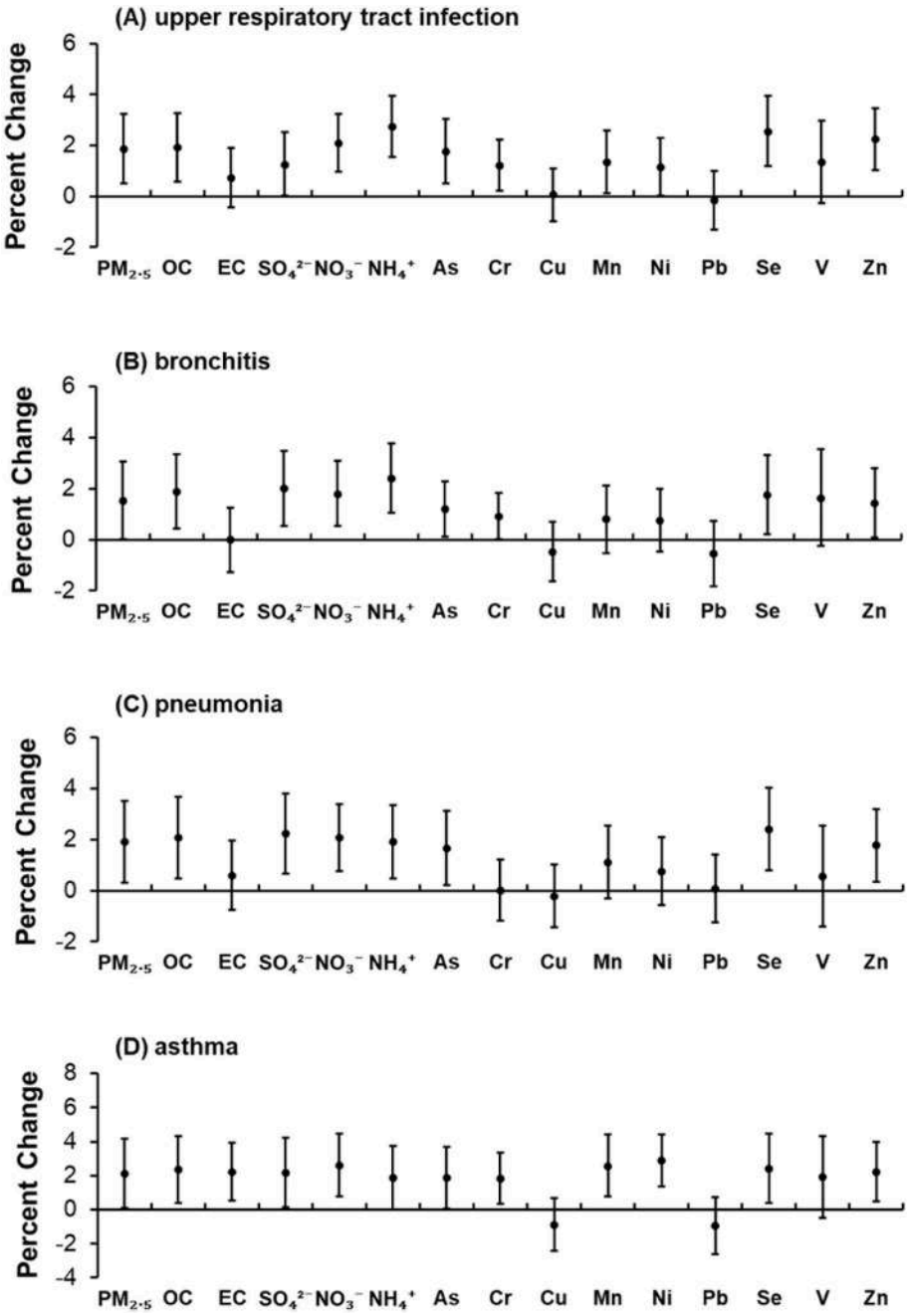

Fig. 3. Percentage changes (mean and 95% confidence intervals) in daily pediatric respiratory ERVs associated with an IQR increase of $PM_{2.5}$ and constituents (lag 0–2 days) in single-constituent models.

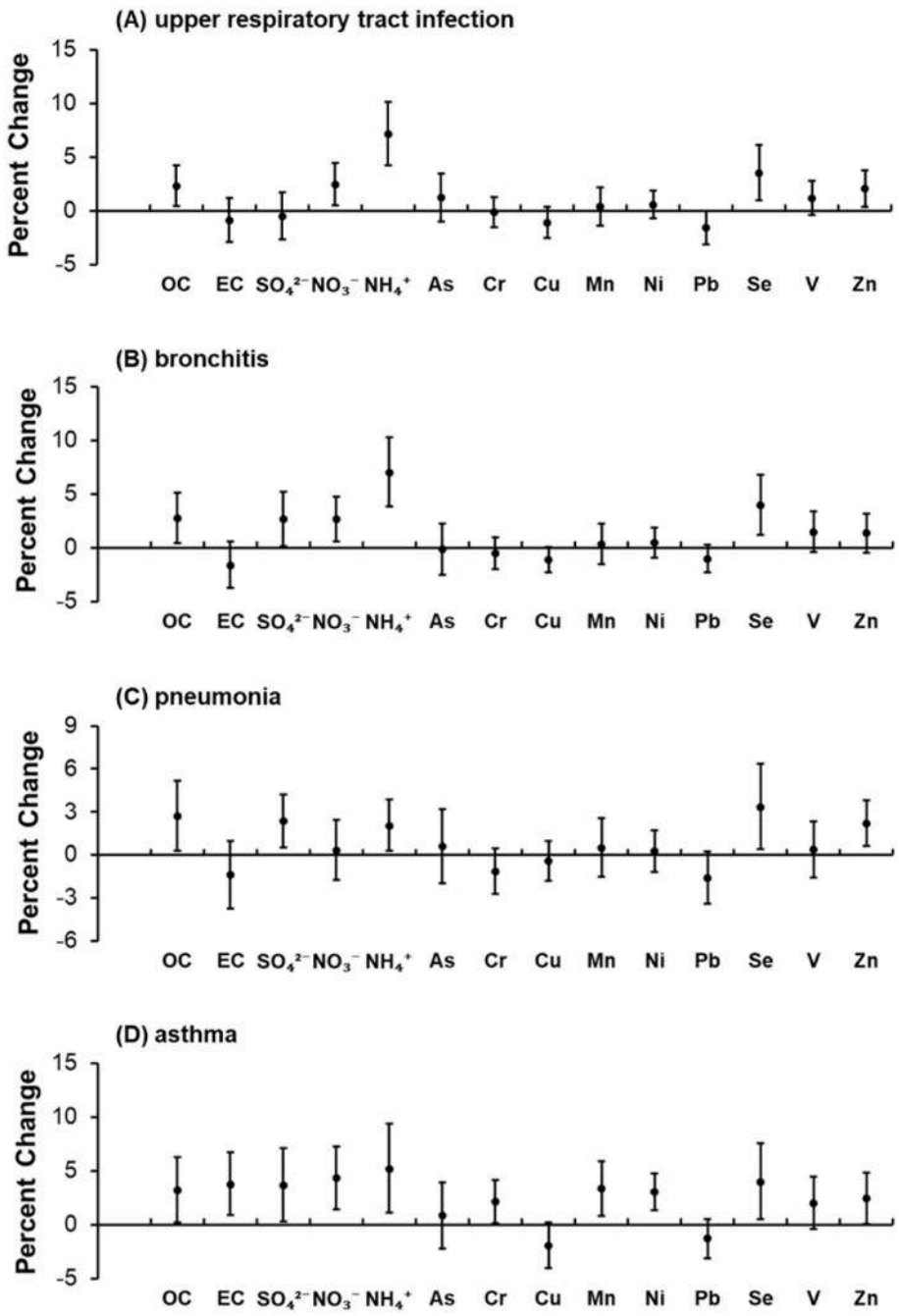

Fig. 4. Percentage changes (mean and 95% confidence intervals) in daily pediatric respiratory ERVs associated with an IQR increase of $PM_{2.5}$ constituents (lag 0–2 days) in constituent-$PM_{2.5}$ models.

7.00% (95%CI: 3.84, 10.25), 2.04% (95%CI: 0.26, 3.85) and 5.16% (95%CI: 1.15, 9.34) increase of ERVs for upper respiratory tract infection, bronchitis, pneumonia and asthma, respectively.

Estimates changed little for the associations of $PM_{2.5}$ constituents and respiratory ERVs when using longer lags or a DLNM model for temperature or using 10 df in the natural cubic spline for calendar days (Supplementary Material Table S2 and Figure S2 and S3). After adjusting for SO_2, NO_2, and CO in the models, the associations between $PM_{2.5}$ constituents and pediatric ERVs attenuated and the 95% CIs were wider (SSupplementary Material Figure S4~7), while the model estimates remained robust after adjusting for O_3.

4. Discussion

Results from this time-series study showed that ambient $PM_{2.5}$ and its chemical constituents such as OC, NH_4^+, Se, and Zn were associated with increased risk of pediatric emergency room visit for respiratory diseases in Shanghai, China. These associations were robust in sensitivity analyses. Our findings provided evidence to the associations of $PM_{2.5}$ constituents on children's respiratory diseases in China.

We found short-term exposure to $PM_{2.5}$ was associated with increased risk of pediatric respiratory ERVs. This result was consistent with previous evidences from China suggesting exposure to $PM_{2.5}$ would increase emergency room visits of respiratory diseases (Tian et al., 2017; Xu et al., 2016). Children are believed to be more vulnerable to ambient air pollution due to their higher breath rate, narrower airway,

undeveloped lung, and more time spent outdoors (Xing et al., 2020). However, so far, few studies have examined the associations between $PM_{2.5}$ level and pediatric ERVs for respiratory diseases in China (Liu et al., 2020). Several studies reported positive associations between $PM_{2.5}$ and children's respiratory hospital admissions in Asian countries but the magnitude of these associations differed due to the different outcomes of interest (e.g., hospitalization vs emergency visits), age, and lag periods (Hua et al., 2014; Lee et al., 2006). For instance, a time-series study in Vietnam suggested that a 39.4 μg/m^3 increase of 6-day average $PM_{2.5}$ was associated with 1.3% increase of hospital admission for bronchitis and asthma among children aged 1–5 (Nhung et al., 2018). Another study in Jinan, China showed children hospital admissions for upper respiratory infection increased by 1.57% per 10 μg/m^3 increase of $PM_{2.5}$ in lag 03 days (Liu et al., 2019). In addition, stronger associations of $PM_{2.5}$ with respiratory outcomes in children were reported by studies conducted in countries with lower $PM_{2.5}$ levels. For example, one study in US found that a 10 μg/m^3 increase of $PM_{2.5}$ exposure was associated with 15–32% increase in the odds of acute lower respiratory infection among children (Horne et al., 2018). Another study in Ontario, Canada showed each interquartile change (5.92 μg/m^3) in 3-day mean $PM_{2.5}$ was associated with a 7.2% increased risk of emergency room visits for asthma among children under 9 years old (Weichenthal et al., 2016). Possible explanations for such differences include variations of the exposure levels and chemical compositions of $PM_{2.5}$, and population characteristics by study location.

Among the carbonaceous constituents, we found OC was associated with ERVs for all the four major respiratory diseases and this association remained robust after adjusting for $PM_{2.5}$ total mass. Previous studies have linked OC with increased risk of adverse respiratory outcomes in different population (Kim et al., 2012; Peng et al., 2009; Wang and Lin, 2016). For example, a study in California suggested an IQR (4.5 μg/m^3) increase of OC was associated with an excess risk of 3.4% in respiratory hospital admission for children (Ostro et al., 2009). Another 18-year time-series study in Atlanta suggested an IQR (1.7 μg/m^3) increase of 3-day moving average concentration of OC predicted a 2% increase in emergency room visits for pneumonia and an 1.9% increase for upper respiratory infection among children aged 0–4 (Darrow et al., 2014). However, most current studies were conducted in developed countries, while evidence from developing countries such as China was scarce.

We also observed NH_4^+, SO_4^{2-}, and NO_3^-, which were the predominant compositions of $PM_{2.5}$, were associated with increased pediatric ERVs. In addition, the estimate of NH_4^+ was inflated after adjusting for total $PM_{2.5}$, which may suggest multi-collinearity in the model. Although the adverse health effects of these soluble ions in $PM_{2.5}$ have been documented in the literature, evidence in children were still sparse and inconsistent (Ferreira et al., 2016; Hwang et al., 2017; Ostro et al., 2009). A time-series study suggested that an IQR (3 μg/m^3) increase of sulfate was associated with a 1.3% increase in emergency room visits for upper respiratory infection among children aged 0–4 (Darrow et al., 2014). Another two studies from Taiwan found that NH_4^+, NO_3^-, and SO_4^{2-} were associated with increased respiratory mortality or asthma ERVs in the general population (Hwang et al., 2017; Wang et al., 2019).

Our results suggested trace element compositions, especially Se and Zn, were associated with increased pediatric ERVs for respiratory diseases. Among the limited epidemiological evidence on the trace element constituents of $PM_{2.5}$ and respiratory disease, Zn has been suggested to be associated with increased asthma risk in children. For example, Gehring et al. showed that exposure to PM constituents, in particular Fe, Cu, and Zn, may increase the risk of asthma and allergy in children (Gehring et al., 2015). Hirshon et al. found associations between Zn in particles and increased asthma morbidity among children (Hirshon et al., 2008). However, furhter evidence is warranted to confirm these findings.

There were several studies concerned with the potential biological mechanisms linking $PM_{2.5}$ and respiratory adverse events, among which oxidative stress was regarded as a crucial pathophysiological mechanism of $PM_{2.5}$-induced respiratory disease (Valavanidis et al., 2013; Zhang et al., 2016). Reactive oxygen species (ROS) could directly combine with particles or generate via cellular redox reaction through stimulation from specific particle components (Dellinger et al., 2001). For example, previous studies have hypothesized that the magnitude of ROS generation could be driven by transition metals (e.g., iron, copper, manganese, vanadium, nickel, chromium) and organic compounds (Cho et al., 2005; Gurgueira et al., 2002). $PM_{2.5}$ components can also impair respiratory system by inducing inflammatory response (Schaumann et al., 2004). NO_3^- has been reported to have the strongest toxic effect on the respiratory system in young mice among the three major $PM_{2.5}$ water-soluble inorganic components (NH_4^+, NO_3^- and SO_4^{2-}) (Zhang et al., 2021b). The supposed molecular mechanisms included inflammatory response, dysregulating lung gene expression, immune signaling, lysosome and circadian rhythms (Zhang et al., 2021a).

Our findings of $PM_{2.5}$ chemical constituents indicated that the observed adverse respiratory effects of $PM_{2.5}$ were contributed by particles from fuel combustion and industrial emission. The major sources of organic carbon include motor vehicles, heavy fuel oil burning, and industry in Shanghai. Soluble ions are mainly contributed by coal burning, industrial emission, and secondary sources and Se and Zn are mainly from coal burning and industrial emission (Zhou, 2020). Therefore, regulation on these emission sources should be given a higher priority for the purpose of health protection.

Our study has several limitations. First, we relied on $PM_{2.5}$ constituents measured in a single monitoring station to represent the overall exposure level in Shanghai. Therefore, exposure measurement errors are possible as this single station cannot fully capture the geographic variations of $PM_{2.5}$ constituents in an area of more than 6,000 km^2. However, such exposure measurement errors were likely non-differential and thus may bias the results towards the null (Zeger et al., 2000). Second, this study was conducted in one of the largest cities of China. Therefore, our results may not be applicable to children in other developing countries or even to the general children population in China. Moreover, we only considered ERVs in the analysis, while outpatient clinic visits and hospital admissions were not included. Children who ended up with hospital admissions usually had more severe condition and only account for a small proportion of the patients. Meanwhile, outpatient visits were sometimes scheduled and thus might introduce misclassification in the analysis. Therefore, ERVs can be more appropriate when investigating the short-term effects of air pollution on health (Winquist et al., 2012). Third, although we adjusted for potential confounders such as meteorological conditions, seasonality, and co-exposure to other gaseous pollutants, residual confounding by factors such as influenza and pollens was possible. Finally, the high correlations between $PM_{2.5}$ constituents and total $PM_{2.5}$ may limit our ability to estimate the associations of these constituents with the outcome that is independent of total $PM_{2.5}$. Therefore, our results should be interpreted with caution.

5. Conclusion

In this time-series analysis, we found short-term exposure to $PM_{2.5}$ and its constituents, mainly OC, NH_4^+, Se and Zn, were consistently associated with increased pediatric ERVs of respiratory diseases in Shanghai, China. Our results suggested that constituents related to anthropogenic combustion and traffic might dominate the adverse respiratory effects of $PM_{2.5}$ among children. This study added to the limited evidence on $PM_{2.5}$ and its constituents with children's respiratory health in developing countries.

Acknowledgement

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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.2021.113805.

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Attributes of drinking water, sanitation, and hygiene associated with microbiological water quality of stored drinking water in rural schools in Mozambique and Uganda

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ABSTRACT

Contaminated drinking water causes morbidity and mortality worldwide, especially in low- and middle-income countries. Drinking water quality has been studied extensively in household settings, but little research is available on drinking water quality in schools. School settings are of particular importance, because children are more susceptible than adults to a variety of diseases from contaminated drinking water. Many school water, sanitation and hygiene (WaSH) interventions have been studied for their efficacy to reduce diarrheal disease incidence, but few have evaluated drinking water quality, which reflects an important exposure pathway between WaSH services and health outcomes. Using school surveys developed from internationally established WaSH indicators and field microbiological water quality tests, we studied 374 rural schools in Mozambique and Uganda to understand the association between specific WaSH services and drinking water microbiological contamination, specifically testing most probable number (MPN) of *Escherichia coli*, an indicator of fecal contamination, per 100 mL. In Mozambique and Uganda, 71% and 83% respectively of rural schools had low risk drinking water quality (<1 *E. coli*/100 mL); thirteen percent and seven percent had very high-risk water quality (≥100 *E. coli*/100 mL). When accounting for all WaSH services studied, schools that used an improved-type water source had 0.22 times less *E. coli* in stored drinking water in Mozambique (95% CI: 0.07, 0.65) and 0.12 times less *E. coli* in Uganda (95% CI: 0.02, 0.80). In Mozambique, use of a water source within 30 minutes for travel and collection and the presence of water and soap/ash for handwashing were also significantly associated with less *E. coli* in drinking water. The findings of this study provide public health practitioners with implementable WaSH services to improve school drinking water quality, which has implications for the health, learning environment, and cognitive development of school children in rural Mozambique and Uganda.

1. Introduction

Contaminated drinking water continues to cause substantial morbidity and mortality worldwide (Clasen et al., 2007; Hunter et al., 2010; Wolf et al., 2018a). As of 2019, drinking water sources of an estimated two billion people were contaminated with feces and over 800,000 people die annually from diarrhea caused by poor water, sanitation, and hygiene, including nearly 300,000 children (World Health Organization, 2019). Community settings, like schools and health facilities, have become spotlights for water, sanitation, and hygiene (WaSH) programming in low-income countries (LICs), to reduce disease exposure in commonly frequented areas outside the home. WaSH services that protect users from pathogen exposure are sparse in these settings (Guo et al., 2017; Morgan et al., 2017) and poorly financed (Alexander et al., 2016; McGinnis et al., 2017), and monitoring for quality of services in countries is infrequent and inadequate (United Nations Children's Fund and World Health Organization, 2018b; World Health Organization and United Nations Children's Fund, 2019).

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Evidence from household studies for effective WaSH strategies informs interventions in school and other extra-household settings. Meta-analyses of household WaSH interventions show household water treatment, safe storage, and sanitation interventions reduce diarrheal risk (Hunter, 2009; Wolf et al., 2014) and are associated with lower odds of intestinal protozoa infections (Speich et al., 2016). In cross-sectional studies, the type of water source and residual chlorine treatment are consistent predictors of *Escherichia coli* (*E. coli*) contamination (Gupta et al., 2007; Jeandron et al., 2019; Trevett et al., 2004), but various water storage and usage practices show no association in Honduras (Trevett et al., 2004).

Schools offer a particular opportunity for improving health related to WaSH and water quality, as children spend a substantial portion of their daytime hours in school. While household water quality has improved, school-aged children are exposed to waterborne disease through poor school drinking water quality. Further, children are more susceptible than adults to diarrheal disease and other waterborne illnesses (Jasper et al., 2012). Water treatment with hygiene or sanitation in schools decreases respiratory illness (Patel et al., 2012) and parasitic infections (Erismann et al., 2017; Freeman et al., 2013), and increases health-related knowledge and behaviors (Chard and Freeman, 2018; Hetherington et al., 2017), enrollment, and gender parity (Garn et al., 2013). School water treatment and handwashing interventions also reduce absenteeism, especially for girls (Trinies et al., 2016). School hand hygiene interventions alone also reduce absences due to a variety of respiratory and enteral infections (Talaat et al., 2011). Lastly, schools are a learning environment and have the potential to be places where safe WaSH practices are learned by students (Anthonj et al., 2021), and these teachings can then be shared with their families and communities (Bresee et al., 2016; Dreibelbis et al., 2014).

Many researchers have tested the effect of school WaSH interventions on diarrheal disease incidence or missed school days (McMichael, 2019), but few have evaluated the quality of drinking water (microbiological or chemical) as an intermediary in this causal relationship. As improvement of drinking water quality is a mechanism for how school WaSH interventions improve health of school-aged children, we study microbiological stored drinking water quality in 374 rural schools in Mozambique and Uganda and analyze the association between school WaSH services and microbiological water quality.

2. Methods

2.1. Sampling, study population, data collection tools

The sampling methodology, survey instrument, and study population have been previously described (Morgan et al., 2017). Briefly, we conducted a stratified random sample of schools in specific rural regions of ten sub-Saharan African countries; here we describe results from the schools in Mozambique and Uganda that had stored drinking water and collected a water sample from it. These two countries were selected from the original study as the random sample of schools with water quality samples from stored drinking water was sufficiently large in each. Data collection consisted of two components: a standardized survey instrument to evaluate access, quantity, quality, continuity, and reliability of WaSH services (previously described (Morgan et al., 2017)); and microbiological water quality testing of stored drinking water. GPS coordinates of schools were also collected.

2.2. Ethics

Free and informed participant consent was obtained from all school officials surveyed. The Institutional Review Board of the University of North Carolina at Chapel Hill approved this study protocol on June 3, 2014 (IRB Reference ID: 14–0763). This study was approved by the corresponding national governing bodies: the Uganda National Council for Science & Technology (UNCST) and the Directorate of Water in the Ministry of Public Works and Housing in Mozambique.

2.3. WaSH factors analyzed

We analyzed descriptive statistics of WaSH services of the 374 rural schools in Mozambique and Uganda, including water source type, access (distance to source), storage, removal; sanitation facility type and condition; and hand hygiene access. Estimates were weighted based on stratified random sampling in selected rural regions, to account for different probabilities of selection of schools. We tested the association of these WaSH factors in schools on school microbiological water quality using estimated *E. coli.* We describe the variables and regression model here.

We used the WHO/UNICEF Joint Monitoring Programme categorizations of “improved” and “unimproved” types of water source and sanitation (World Health Organization, 2012). Improved-type drinking water sources decrease the risk of fecal contamination compared to unimproved-type drinking water sources, but do not guarantee microbial water safety (Bain et al., 2014; Shaheed et al., 2014). Improved-type water sources include piped water, boreholes, protected dug wells or springs, rainwater, and packaged water. Improved-type sanitation limits human contact with excrement, and include flush sewer systems, septic tanks, ventilated pit latrines, composting toilets, and pit latrines with slabs. We analyzed water storage by observing the use of a safe container (covered container, with a narrow opening, or with a wide opening and water treatment) and safe removal methods (pouring, spigot, tap, or long ladle for extracting water) (Centers for Disease Control, 2014). We analyzed hand hygiene facilities by assessing the presence of water and soap/ash for handwashing, a widely used indicator for hand-hygiene access (United Nations Children’s Fund and World Health Organization, 2018a). We also analyzed the presence of materials for hand-drying.

The conditions of improved-type sanitation in schools were observed by the presence of the following aspects of each sanitation facility: doors, doors that can be closed, doors with locks, holes in the structure, stability of latrine slab, caving walls of the structure, latrine pits that were too large, latrine pits that were caving in, used paper on slabs, and flies swarming.

2.4. Water quality testing

As this study was conducted within a larger multi-country, multi-site evaluation, we used *E. coli* as the microbial indicator as the organism is an indicator of fecal contamination, it does not grow naturally in the environment (Edberg et al., 2000), and field-based and laboratory testing are available, inexpensive, and are simple to conduct in rural areas of LICs. In each school, a 100 mL water sample was collected from the stored drinking water consumed by students, used by the school, in the same way members of the school extract water for drinking. We analyzed water quality using Aquagenx (Chapel Hill, NC) Compartment Bags to determine the most probable number (MPN) of *E. coli* (Stauber et al., 2014) according to the manufacturer’s instructions (Aquagenx, 2013).

2.5. Regression model

WaSH indicators measured in the survey were treated as ordinal predictor variables, and MPN of *E. coli* in 100 mL of stored drinking water as a discrete outcome variable. Predictor variables were selected for the model based on potential for contamination of stored drinking water and were indicator variables for the following WaSH services: improved-type water source; treatment of stored water; safe container for stored drinking water; safe removal method of stored drinking water; water source within 30 minutes roundtrip; improved-type sanitation; water and soap/ash present for handwashing. The association between each WaSH factor and water quality was tested in bivariate (unadjusted)

analysis using a negative binomial regression model (El-Shaarawi et al., 1981), because our outcome, concentration of *E. coli*, was discrete and overdispersed around zero. Unadjusted covariates that were significant at $p < 0.05$ or considered necessary to control for (e.g. treatment of water) were included in a multivariate (adjusted) model. Tests for collinearity of these factors were conducted before inclusion in an adjusted model, and interaction terms between predictors were evaluated. Model results are reported as incidence rate ratios.

Frequencies were calculated using PROC SURVEYMEANS and PROC SURVEYFREQ in SAS 9.4 (SAS Institute, Cary, NC, USA). The negative binomial regression model was computed using *nbreg* with the *irr* option in Stata 14 (StataCorp, College Station, TX, USA). Figures for descriptive statistics were generated using R 3.6.0. Maps were produced using the "sf" package in R 3.6.0. Schools with missing GPS points were mapped to their respective districts.

3. Results

3.1. School demographics

We studied 374 rural schools, 124 in Mozambique and 250 in Uganda, serving 206,487 total students (Table 1). Districts sampled were geographically disparate rural areas (Fig. 1), and sampled schools were predominantly primary schools. The median number of students enrolled was 374 in Mozambique and 510 in Uganda (Table 1). In Mozambique, the median numbers of boy and girl students were 191 (IQR: 35, 366) and 172 (IQR: 66, 322), respectively. In Uganda, the median numbers of boy and girl students were 238 (IQR: 180, 372) and 263 (IQR: 190, 375), respectively.

4. Descriptive statistics

4.1. Water quality

Seventy-one percent of rural schools in Mozambique and 83% in Uganda had <1 *E. coli* MPN/100 mL (Fig. 2A), the lowest health risk in the latest WHO classification (World Health Organization, 2017). Thirteen percent of rural schools in Mozambique and seven percent in Uganda had ≥100 *E. coli* MPN/100 mL, WHO's highest health risk category. Fewer schools in each country fell into the intermediate risk categories. Boreholes, an improved-type water source, were the most common water source used by rural schools in both countries (Fig. 2B). Stored drinking water of the highest health risk level (>100 *E. coli* MPN/100 mL) was found in rural schools with improved-type water sources in both countries (piped and purchased sources in Mozambique, boreholes and protected springs in both countries, and rainwater in Uganda).

4.2. Water source type and storage

Of schools with water sources, 89% percent of rural schools in Mozambique and 95% in Uganda had an improved-type water source; in 92% and 85%, respectively, the water source was within 30 minutes of the school, including collection time (Fig. 3A). Forty-eight percent of rural schools in Mozambique and 78% in Uganda had safe storage containers, and 19% and 62%, respectively, had means for safe removal of drinking water. Three percent of schools in Mozambique reported no storage of drinking water because they had on-plot water sources.

Ten percent and 17% of schools reported treatment of drinking water in Mozambique and Uganda, respectively. Treatment methods included boiling and chlorine in both countries, with one school in Uganda reporting filtration.

4.3. Sanitation type and quality

Sanitation facilities were predominantly of an improved type, though the conditions of sanitation facilities varied. Sixty-two percent in Mozambique and 91% in Uganda had improved-type sanitation facilities. Of schools with improved-type sanitation, the most frequent problem was a lack of doors: only 46% of rural schools in Mozambique and 58% in Uganda had doors on all latrines. (Fig. 3B).

4.4. Hand hygiene

The availability of handwashing facilities was notably absent: only 2% of rural schools in Mozambique and 14% in Uganda had water and soap or ash for handwashing present on the day of the survey. Only 2% of rural schools in each country had water, soap or ash, and drying materials for handwashing present.

4.5. Regression model

Several WaSH factors in rural Mozambique schools had unadjusted estimates that significantly correlated with *E. coli* MPN/100 mL in stored drinking water (Table 2). Schools with an improved-type water source had 0.29 (95% CI: 0.13, 0.64) times the incidence rate of *E. coli* in stored drinking water compared with schools with unimproved-type water sources. Schools with water sources within 30 minutes for collection had 0.28 (95% CI: 0.12, 0.68) times the incidence rate of *E. coli* as schools with more distant water sources. Schools with safe storage containers had 3.71 (95% CI: 1.38, 9.92) times the incidence rate of *E. coli* compared with schools without safe containers, and schools with means for safe removal of stored water (e.g. with a tap or ladle) had 2.47 (95% CI: 1.10, 5.54) times the incidence rate of *E. coli* as schools that did not. Schools with water and soap/ash for handwashing on the day of the survey had 0.04 (0.01, 0.19) times the incidence rate of *E. coli* than with schools without these materials for handwashing. Schools that had hygienic materials for hand-drying, in addition to water and soap/ash, on the day of the survey had 0.08 (0.06, 0.13) times the incidence rate of *E. coli* as schools without all three handwashing materials. In testing for collinearity, none of these variables had correlation coefficients above 0.8, and were all included in an adjusted model.

In an adjusted model with the selected predictors of water quality in Mozambique, an improved-type water source (IRR: 0.22, 95% CI: 0.07, 0.65), water sources within 30 minutes (IRR: 0.25, 95% CI: 0.08, 0.81) and water and soap/ash for handwashing present (IRR: 0.12, 95% CI: 0.02, 0.73) remained significant, each associated with less *E. coli*. A similar adjusted model is observed when the handwashing indicator includes materials for drying. An improved-type water source and water sources within 30 minutes have similar incidence rate ratios, and water, soap/ash, and drying materials are associated with 0.27 times the incidence rate of *E. coli* (95% CI: 0.10, 0.76).

Predictors of water quality in schools in Uganda showed a different picture. Schools with piped water sources or other improved-type water

Table 1
Demographics of rural schools studied.

Country	Districts sampled	Schools with water quality samples (n)	Median total students (IQR)	Median male students (IQR)	Median female students (IQR)	Median number of teachers (IQR)
Mozambique	13	124	374 (133, 698)	191 (35, 366)	172 (66, 322)	6 (3, 15)
Uganda	10	250	510 (372, 757)	238 (180, 372)	263 (190, 375)	10 (7, 13)

IQR: Interquartile range.

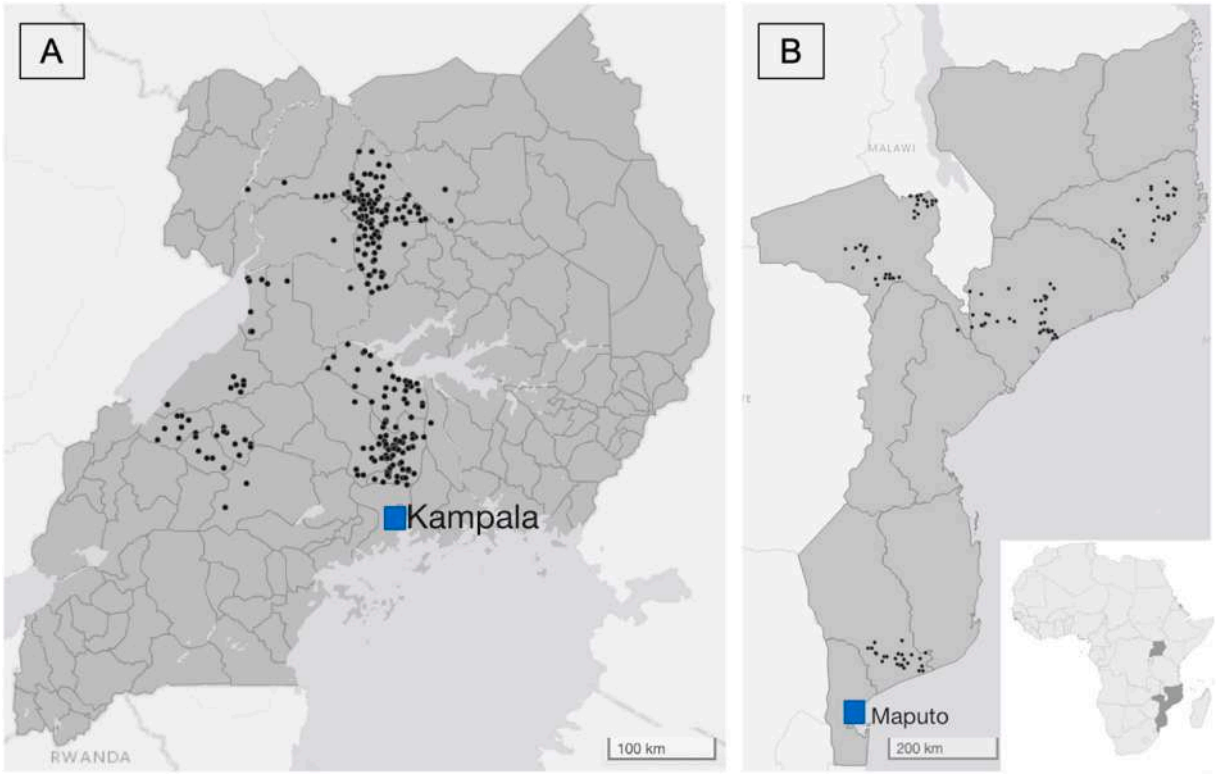

Fig. 1. Geographic locations of surveyed schools in Uganda (A, n = 250) and Mozambique (B, n = 124).

sources had 0.09 (95% CI: 0.02, 0.55) times the incidence rate of *E. coli* as schools with unimproved-type water sources. Schools that treated water had 2.36 (95% CI: 4.22, 11.22) times the incidence rate of *E. coli* as schools that didn't treat water. Lastly, schools with improved sanitation had 0.28 (95% CI: 0.10, 0.74) times the incidence rate of *E. coli* as schools with unimproved or no sanitation.

No collinearity was observed, and these three predictors were included in an adjusted model for Uganda. In the adjusted model, only an improved-type water source remained a significant predictor of water quality (IRR: 0.12, 95% CI: 0.02, 0.83).

In both countries, neither the method of water treatment (boiling or chlorination) nor specific conditions of latrines on visual inspection were associated with amount of *E. coli* contamination.

5. Discussion

We provide one of very few studies of rural school drinking water quality in LIC settings and that assesses the WaSH factors associated with safer microbiological drinking water quality in rural schools. Our large study (n = 374 rural schools) represents a sample from broad geographic areas in both countries studied, providing comparable and generalizable findings. Drinking water quality in both countries was good overall: 71% of schools in Mozambique and 83% in Uganda had <1 *E. coli* MPN/100 mL, with most schools drawing drinking water from boreholes. These water quality estimates are similar to previously published data from these countries (Agensi et al., 2019; Holcomb et al., 2020). In both Mozambique and Uganda, an improved-type water source was associated with less *E. coli* in unadjusted and adjusted models, with a piped water source in Ugandan schools additionally associated with less *E. coli*. This finding is not surprising, based on water quality evidence from household monitoring (Kirby et al., 2016; Shields et al., 2015). In Mozambique, additional significant predictors included a water source within 30 minutes and handwashing materials present on the survey day (water and soap/ash). Proximity to water source affects health in multiple ways. First, longstanding evidence from households suggests closer water sources leads to increased water quantity and better hygiene, regardless of water quality (Stelmach and Clasen, 2015). Second, and more specific to waterborne diarrheal disease, schools with closer water sources likely refill storage containers more often, which presents fewer potential opportunities for contamination of water during transport or longer storage periods. This is consistent with previous research in households that show an increase in disease incidence as distance from a water sources increases (Wang and Hunter, 2010).

In unadjusted models, rural schools in Mozambique with a safe storage container had 3.52 times the *E. coli* incidence rate in stored drinking water than schools that did not use a safe storage container; schools with means to remove drinking water safely had 2.47 times the *E. coli* incidence rate compared with schools that did not. The adjusted model for Mozambique controlled for these safe storage components, as well as water source type, source within 30 minutes for collection, treatment of stored water, and hand hygiene. In the adjusted model, use of a safe storage container and safe removal of water no longer significantly increased incidence rate of *E. coli.* Further, schools with handwashing facilities (water and soap/ash present for handwashing) had 0.12 times the *E. coli* incidence rate compared with schools without hand hygiene, while controlling for other significant WaSH predictors. This evidence suggests that water and soap/ash for handwashing reduces incidence rate of *E. coli* in school drinking water in Mozambique, regardless of safety of water storage. Handwashing with soap and water reduces the fecal contamination of hands of students and teachers accessing stored water, which can lead to less *E. coli* contamination of stored drinking water. Although studies of handwashing in households have established this relationship (Wolf et al., 2018b), our findings are the first to show that in schools, handwashing materials are associated with significantly less *E. coli* in stored water, regardless of stored water practices.

In unadjusted models of rural schools in Uganda, treatment of water and improved-type sanitation were significant predictors of *E. coli,* in addition to water source type. Reported water treatment was associated with increased incidence rate of *E. coli* in drinking water, contrary to

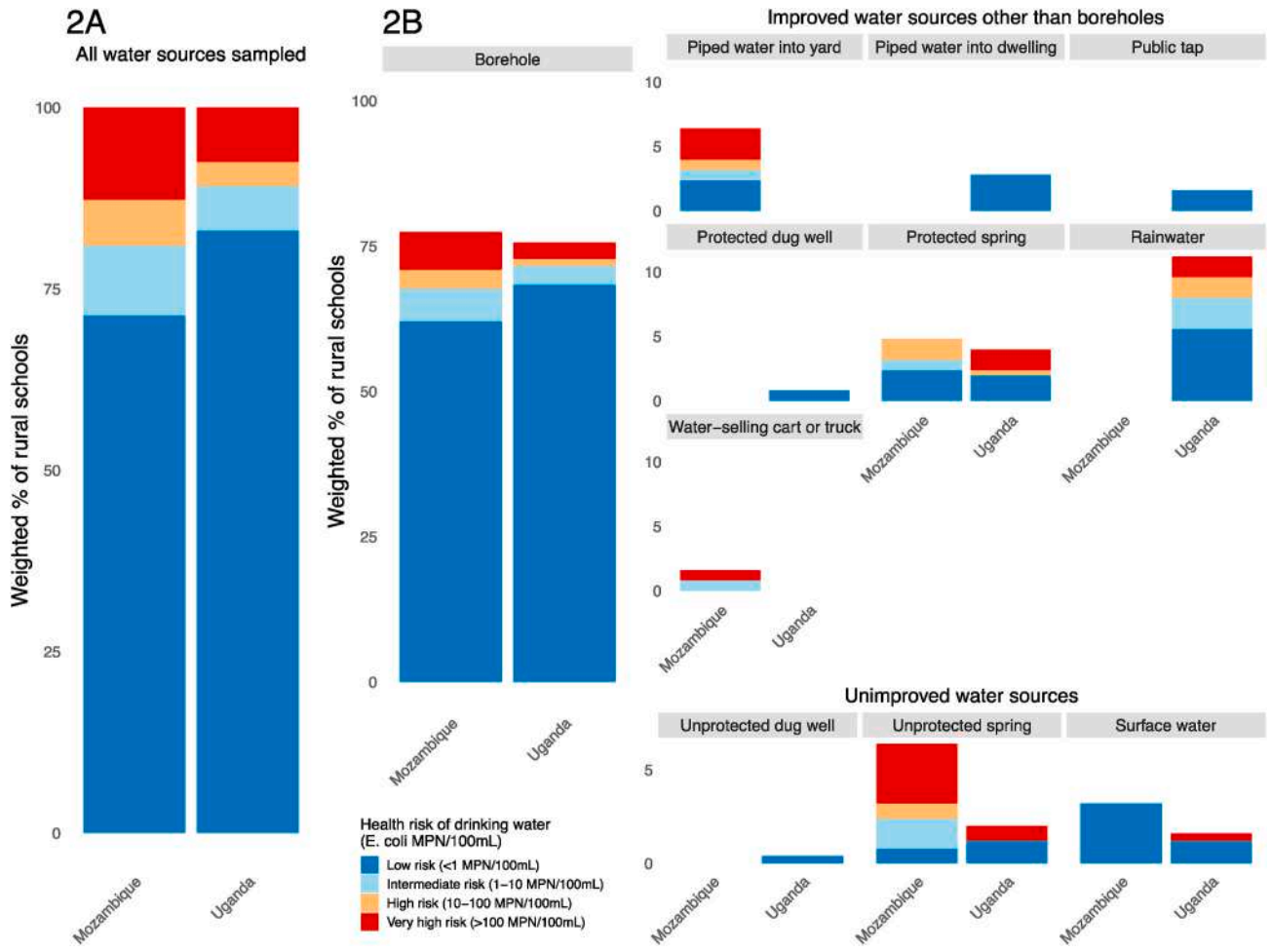

Fig. 2. A: Weighted percent of rural schools with drinking water in each WHO water quality risk level based on *E. coli* MPN/100 mL. B: Weighted percent of rural schools in each WHO water quality risk level by school water source type. Boreholes were the most common drinking water source type in schools. Other drinking water sources were less frequent; as such, the y-axis is shown on a smaller scale.

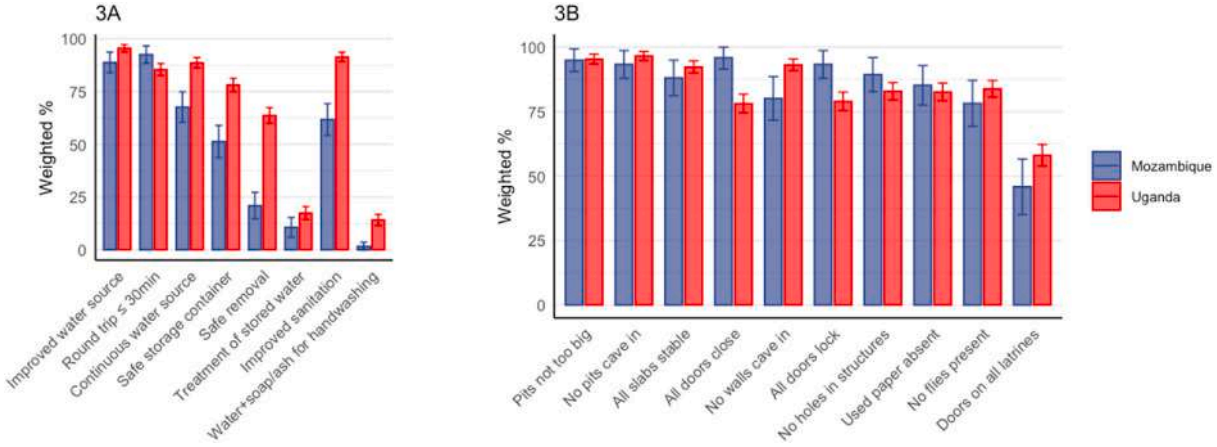

Fig. 3. A: Weighted percent of rural schools with each water, sanitation, and hygiene service studied in cross-sectional surveys, by country. B: Weighted percent of rural schools with improved-type sanitation facilities with all sanitation facilities meeting the conditions studied in cross-sectional surveys, by country. Error bars indicate 95% confidence intervals of population-level weighted percentages.

expectation. In the adjusted model, when controlling for use of a piped water source or other improved-type water source, improved-type sanitation, and treatment of water, water treatment was not associated with increased *E. coli;* only a piped water source or other improved-type remained significant. These findings suggest that of the WaSH services studied in Uganda, water source type most significantly predicts *E. coli* incidence rate in drinking water.

In both countries, specific conditions of latrines were not significantly associated with amount of *E. coli* contamination, a finding that has been similarly observed with sanitary inspections and water quality

Table 2
Incidence rate ratios (IRR) for *E. coli* most probably number in stored drinking water by water, sanitation, and hygiene service.

	Mozambique			Uganda		
	N	Unadjusted IRR (95% CI)	Adjusted IRR (95% CI)	N	Unadjusted IRR (95% CI)	Adjusted IRR (95% CI)
Improved-type water source	124	0.29 (0.13, 0.64)	0.22 (0.07, 0.65)	250	0.09 (0.02, 0.55)	0.12 (0.02, 0.80)
Water treatment	118	1.07 (0.30, 3.84)	0.39 (0.11, 1.33)	**250**	**2.36 (4.22, 11.22)**	1.75 (0.68, 4.52)
Continuous water source	118	5.53 (0.80, 38.15)		250	2.24 (0.33, 15.21)	
Safe Container	**124**	**3.52 (1.32, 9.40)**	3.58 (0.84, 15.19)	250	2.13 (0.71, 6.43)	
Safe Removal	**124**	**2.47 (1.10, 5.54)**	1.01 (0.29, 3.55)	249	1.98 (0.81, 4.86)	
Round trip < 30 min (self-reported)	**124**	**0.28 (0.12, 0.68)**	**0.25 (0.08, 0.76)**	250	0.61 (0.23, 1.65)	
Improved sanitation	123	0.94 (0.48, 1.87)		**250**	**0.28 (0.10, 0.74)**	0.44 (0.15, 1.29)
Feces absent on all latrines	123	3.26 (0.87, 12.22)		250	1.57 (0.58, 4.22)	
Water + soap/ash for handwashing observed	**122**	**0.04 (0.01, 0.19)**	**0.12 (0.02, 0.73)**	250	1.09 (0.36, 3.35)	
Water + soap/ash + drying materials observed for handwashing	**122**	**0.08 (0.06, 0.13)**	**0.27 (0.10, 0.76)**	250	2.30 (0.38, 13.93)	

§In Uganda, the variable for main water source included a category for piped sources. In Mozambique, this variable was binary, with only two options, improved- and unimproved-type water sources.
Bolded cells indicate null value (1.00) is not included in 95% confidence interval of estimate.
CI: confidence interval.

of water handpumps (Kelly et al., 2021).

5.1. Limitations

First, we did not sample microbial contaminants at the various points of study (water sources, door handles and other surfaces of sanitation facilities, hygiene facilities), nor at timepoints between storage and use, so we cannot isolate exact times or places of contamination. This is worth noting given the JMP "improved" and "unimproved" type classification does not involve sanitary inspection of water sources or sanitation facilities. Evidence from households suggests such contamination of drinking water after collection is common (Levy et al., 2008). We also did not sample water at different points in time throughout the year, which is important because seasonality can affect water quality. Second, while most indicators used were observations, we rely on self-reported time to water source due to insufficient GPS collection at the water source, which is less accurate than Euclidean distance measured with GPS (Ho et al., 2014). Finally, as this study was part of a larger multi-site, multi-country evaluation, we were unable to test other microbiological or physiochemical parameters associated with poor health outcomes through additional laboratory evaluation, but would recommend it in future studies.

This study concerns WaSH services associated with water quality in rural schools, in order to inform future school WaSH interventions that can be most successful in improving water quality, and thus, reducing disease incidence and absenteeism. Past studies that found school WaSH interventions had no effect on reducing disease incidence or absenteeism evaluated whole WaSH programs, not individual WaSH services, or relied on self-reported disease (Chard et al., 2019; Garn et al., 2017), while implementation of specific interventions, such as handwashing with soap and additional toilets in a recent study of schools in Nepal, has been shown to reduce intestinal parasitic infections (Shrestha et al., 2020). To avoid inaccuracies of subjective disease recall in the study of school WaSH interventions, future studies could focus on objective measures of disease, such as enteric pathogen antibodies (Chard et al., 2018) or on water quality (such as *E. coli* in stored water as a proxy for disease risk).

These results support the findings of water quality studies in other household and extra-household settings that show sanitation facilities and hand hygiene influence water quality, in addition to water source type and water treatment (Guo and Bartram, 2019; Holcomb et al., 2020). These findings have already been used by the funder to improve WaSH conditions and services in schools in respective settings. As we highlight WaSH services associated with school drinking water quality, our findings complement a recent study evaluating school system factors and water quality (Cronk et al., 2020), which found fewer schools in Mozambique and Uganda to have the lowest WHO risk water quality (61% and 56%, respectively, compared with our findings of 71% and 83%), despite sampling occurring after the present study. Our findings, as well as those of Cronk et al. (2020), are important for policymakers, school administrators, and public health practitioners in Mozambique and Uganda, as they provide specific WaSH services with potential to improve water quality and subsequently the health, learning environment, and cognitive development of their young people.

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Characterizing exposures to flame retardants, dioxins, and furans among firefighters responding to controlled residential fires

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ABSTRACT

Firefighters may encounter items containing flame retardants (FRs), including organophosphate flame retardants (OPFRs) and polybrominated diphenyl ethers (PBDEs), during structure fires. This study utilized biological monitoring to characterize FR exposures in 36 firefighters assigned to interior, exterior, and overhaul job assignments, before and after responding to controlled residential fire scenarios. Firefighters provided four urine samples (pre-fire and 3-h, 6-h, and 12-h post-fire) and two serum samples (pre-fire and approximately 23-h post-fire). Urine samples were analyzed for OPFR metabolites, while serum samples were analyzed for PBDEs, brominated and chlorinated furans, and chlorinated dioxins. Urinary concentrations of diphenyl phosphate (DPhP), a metabolite of triphenyl phosphate (TPhP), bis(1,3-dichloro-2-propyl) phosphate (BDCPP), a metabolite of tris(1,3-dichloro-2-propyl) phosphate (TDCPP), and bis(2-chloroethyl) phosphate (BCEtP), a metabolite of tris (2-chloroethyl) phosphate (TCEP), increased from pre-fire to 3-hr and 6-hr post-fire collection, but only the DPhP increase was statistically significant at a 0.05 level. The 3-hr and 6-hr post-fire concentrations of DPhP and BDCPP, as well as the pre-fire concentration of BDCPP, were statistically significantly higher than general population levels. BDCPP pre-fire concentrations were statistically significantly higher in firefighters who previously participated in a scenario (within the past 12 days) than those who were responding to their first scenario as part of the study. Similarly, firefighters previously assigned to interior job assignments had higher pre-fire concentrations of BDCPP than those previously assigned to exterior job assignments. Pre-fire serum concentrations of 2,3,4,7,8-pentachlorodibenzofuran (23478-PeCDF), a known human carcinogen, were also statistically significantly above the general population levels. Of the PBDEs quantified, only decabromodiphenyl ether (BDE-209) pre- and post-fire serum concentrations were statistically significantly higher than the general population. These results suggest firefighters absorbed certain FRs while responding to fire scenarios.

1. Introduction

Firefighters' exposures to flame retardants (FRs) including polybrominated diphenyl ethers (PBDEs), non-PBDE brominated flame retardants (NPBFRs), organophosphate flame retardants (OPFRs), and brominated and chlorinated dioxins and furans have increasingly become a topic of concern. PBDEs have been in use since the 1970s, are environmentally persistent, and can remain structurally unchanged on surfaces for long periods of time (e.g., years) (Alexander and Baxter, 2016; Easter et al., 2016). The increased interest in firefighters' exposures to FRs can largely be attributed to their presence in modern home furnishings (e.g., upholstered furniture, carpet padding, electronics),

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accumulation in humans, and association with adverse health effects (Herbstman et al., 2010; Linares et al., 2015).

Studies that have indicated an elevated risk of cancer for firefighters (Daniels et al., 2014; Jalilian et al., 2019; Lee et al., 2020; Pinkerton et al., 2020), the International Agency for Research on Cancer (IARC) designation of firefighting as a Group 2B possible human carcinogen (International Agency for Research on Cancer (IARC), 2010), and the complex mixture of combustion byproducts (e.g., polycyclic aromatic hydrocarbons (PAHs), formaldehyde, benzene, FRs) firefighters can be exposed to on the fireground have further raised concerns. IARC has not classified the potential carcinogenicity of PBDEs in humans to date. However, the National Toxicology Program (NTP) found evidence of PBDE carcinogenicity in rodent studies (National Toxicology Program , 2016). Other compounds firefighters are exposed to include dioxins, 2,3,7,8-tetrachlorodibenzo-para-dioxin (2378-TeCDD) and 2,3,4,7,8-pentachlorodibenzofuran (23478-PeCDF), which have been classified by IARC as Group 1 known human carcinogens, and a variety of other combustion byproducts that are known, probable, or possible human carcinogens (International Agency for Research on Cancer (IARC), 2010).

Over the past 10 years, the usage of penta-, octa-, and deca-PBDEs has been restricted globally by the Stockholm Convention (United Nations Environment, 2017). The use of organophosphate flame retardants (OPFRs) in furniture and other household items has increased as a result of PBDE's usage restriction following the classification of this compound class as a persistent organic pollutant (POPs) (Dishaw et al., 2011; National Institute of Environmental Health Sciences (NIEHS), 2018). The potential toxic effects of OPFRs are not fully understood. However, two OPFRs, tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and tris (2-chloroethyl) phosphate (TCEP), are listed in California Prop 65 as potentially carcinogenic (Environmental Protection Agency (EPA)U.S.E.P.A. and Cooke, 2017). Tris(1-chloro-2-propyl) phosphate (TCPP) has been found to be toxic to human cells at high concentrations (An et al., 2016), while triphenyl phosphate (TPhP or TPP) has been found to negatively affect development in zebrafish, mice, and rats (Du et al., 2016; Patisaul et al., 2013; Wang et al., 2018).

Studies have found a variety of FRs, dioxins, and furans on firefighter personal protective equipment (PPE) (Alexander and Baxter, 2016; Easter et al., 2016; Fent et al., 2020b; Mayer et al., 2019) and in air samples taken from a residential room-and-contents fire environment (Fent et al., 2020b). In addition, dust collected from fire stations has been found to contain higher FR levels (e.g., BDE-209 and TDCPP) than other occupational settings (Shen et al., 2015). A more recent study in Canada found fire station dust has high levels of BDE-209 (Gill et al., 2020). These studies suggest that firefighters have the potential to be exposed to these compounds while at the scene of a fire and may also bring the contamination back to their stations.

Biomonitoring and exposure assessment studies have also detected FRs in specimens collected from firefighters. Specifically, a study conducted by Shaw et al. reported elevated concentrations of PBDEs in firefighters' serum compared to the general population (Shaw et al., 2013). Park et al. (2015) reported similar findings, including relatively high serum levels of decabromodiphenyl ether (BDE-209) (Park et al., 2015). Another study reported higher levels of organophosphate flame retardants (OPFRs) metabolites in a sampling of firefighters' urine compared with the general population (Jayatilaka et al., 2017). In part because of these studies, a recent systematic review on occupational exposure to FRs listed firefighters as a workforce warranting further investigation (Gravel et al., 2019).

Exposure to combustion byproducts such as polycyclic aromatic hydrocarbons (PAHs) is also thought to be dependent on the job assignment for firefighters. Previous studies have reported that firefighters assigned to interior response activities (e.g., fire suppression or search and rescue) had higher biological levels of PAH metabolites compared to other job assignments (e.g., outside ventilation, incident command, pump operations, overhaul) on the fireground (Fent et al., 2020a). It is reasonable to assume that FR exposure may follow a similar pattern.

The purpose of this study was to characterize the biological levels of OPFR metabolites (in urine), and PBDEs, brominated and chlorinated furans, and chlorinated dioxins (in serum) in firefighters responding to controlled residential fire scenarios with modern home furnishings (containing FRs). This study design also allowed us to compare how exposures vary over time for firefighters assigned to different job assignments.

2. Methods

2.1. Study design

The study design is described in detail elsewhere (Fent et al., 2020b; Horn et al., 2018). Briefly, over a period of 2 weeks in the summer of 2015, 12 fires were ignited in a 111 m^2 wood-frame residential structure with gypsum board wall/ceiling linings and typical residential furnishings, containing a variety of FRs, including OPFRs, NPBFRs, and PBDEs (as reported in Fent et al., 2020b). The two bedrooms where the fires were ignited were furnished with a double bed (covered with a new foam mattress topper, comforter, and pillow), stuffed chair, side table, lamp, dresser, and flat screen television. The floors were covered with re-bonded polyurethane foam padding and new polyester carpet. Floor coverings in the fire rooms and nearby hallway were replaced after each fire. A fire was ignited and allowed to grow until the rooms approached flash-over conditions and became ventilation limited (typically 4–5 min) and then the firefighters were dispatched by apparatus from a nearby staging area and arrived on scene within 1 min. After each fire, the drywall and furniture were replaced. Study results reported here were collected from firefighters prior to and after three of the 12 fires.

A crew of twelve firefighters was paired up by job assignment to carry out a coordinated fireground response to a controlled residential fire, which was repeated the next day using a different fire suppression tactic. Approximately one to two weeks later, the returning firefighters were reassigned to new positions and repeated this experiment. This was done on a total of three crews (12 firefighters per crew, 4 burns per crew). Five firefighters dropped out of the study and were unable to return a week later and were replaced with new participants (resulting in a total of 41 participants). However, urine and serum specimens analyzed for FRs, dioxins and furans were only collected from one of the four fires for 36 firefighters. Crew A previously responded to a fire scenario as part of this study seven days prior to the fire where specimens were collected; Crew B responded to a fire scenario twelve days prior to the fire where specimens were collected; and Crew C provided specimens on the first fire they responded to as part of this study. The variability for each crew's recent fire exposure as part of this study allowed us to compare how time since last exposure impacted FR, dioxin, and furan urinary and serum concentrations. More information on the timing of the fire scenarios relative to the specimen collections is provided in Fig. 1. All firefighters participating in the fire scenarios wore a full PPE ensemble that included a protective hood, gloves, turnout gear, and self-contained breath apparatus (SCBA). Each firefighter was provided brand new turnout jackets, hoods, and gloves prior to the first scenario. Relevant demographic information for participating firefighters is provided in Table 1. Tobacco use was an exclusion criteria for this study.

Firefighters were assigned to one of three groups for each scenario. Firefighters assigned to interior response either pulled a primary hoseline and suppressed all active fire or entered the structure and searched for and rescued two simulated occupants (75 kg mannequins). Firefighters assigned to exterior response created openings in the windows and roof to ventilate the structure and/or completed typical exterior operations on the fireground (incident command (IC), pump operation). Importantly, these firefighters never entered the structure. Firefighters assigned to overhaul were outside the structure during active fire, either holding a secondary line or as a rapid intervention team (RIT). After the

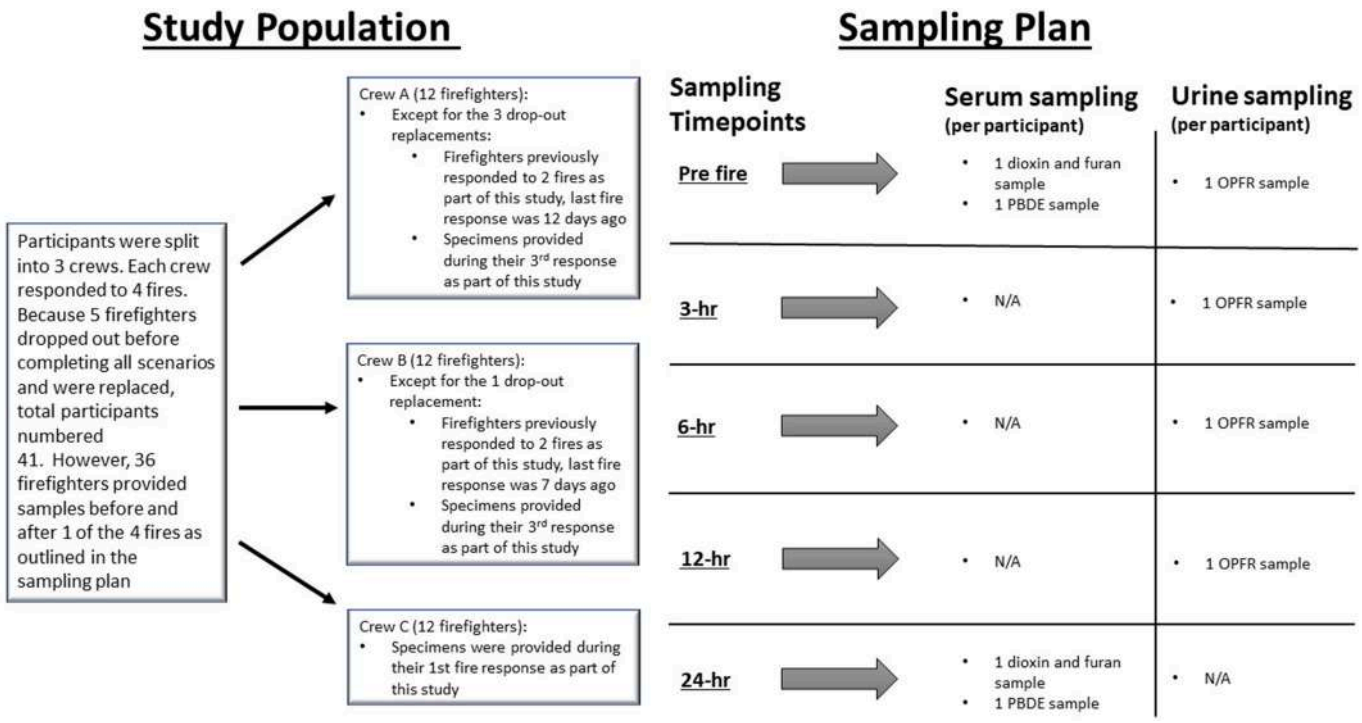

Fig. 1. Study population and sampling strategy for controlled residential fire responses with furnishings containing flame retardants.

Table 1
Characteristics of study participants.

Characteristic	Frequency
Sex	
Male (%)	32 (89)
Female (%)	4 (11)
Age	
Median (Range)	36 (21–52)
BMI	
Median (Range)	26.9 (20.5–34.2)
Home State	
Illinois (%)	22 (61)
Georgia (%)	4 (11)
Indiana (%)	4 (11)
South Dakota (%)	3 (8.3)
Wisconsin (%)	2 (5.5)
Ohio (%)	1 (2.8)

fire was suppressed by the interior firefighters, overhaul firefighters entered the structure to search for and suppress any smoldering items in the fire rooms, walls, and ceilings.

Immediately after completion of the assigned task, the firefighters walked to an open bay (approximately 40 m from the structure) where PPE was removed, turnout jackets hung in individual lockers and fire-fighting gloves placed on a shelf. Firefighters used skin cleansing wipes immediately post-fire and showered within an hour after the scenario. After doffing their gear, firefighters entered an adjacent bay where they provided biological samples. Firefighters provided a spot urine sample prior to the scenario (pre-fire) and 3 subsequent spot urine samples after the scenario (3-h, 6-h, 12-h post-fire). Firefighters also provided one serum sample prior to the fire (pre-fire serum) and one serum sample approximately 23 h after the scenario (post-fire serum).

2.2. Urine sampling

Prior to urine collection, participants were instructed to thoroughly rinse hands with water only and air dry their hands, avoiding the use of paper towels. Participants were also instructed to avoid touching the internal surface of the urine cup or the lid to avoid contaminating the sample. Participants were asked to provide a minimum 60 mL of urine for each void. Urine was put on ice and within 4 h, aliquoted into multiple tubes for analyses including 5 mL and 2 mL polypropylene vials for FR and creatinine quantification, respectively and then frozen at −20 °C. The samples were then shipped to the lab on dry ice and stored frozen until analysis.

2.3. Blood sampling

Blood was collected in multiple collecting tubes including two red top 10 mL glass blood collection tubes, and the samples were placed in a rack to clot for 2 h at room temperature. Blood samples were then centrifuged for 15 min at 1000-1300×*g*. Investigators pipetted serum from each participant's red-top tubes into separate 10 mL amber glass jars, one for PBDEs and serum lipids and one for dioxins and furans, and then froze the samples at −20 °C The samples were then shipped to the lab on dry ice and stored frozen until analysis.

2.4. Sample analyses

Urine samples (N = 144) were analyzed for eight OPFR metabolites and one NPBFR metabolite at the Centers for Disease Control and Prevention (CDC) as described by Jayatilaka et al. (2017) (Table 2). The OPFR metabolites measured were: diphenyl phosphate (DPhP), bis(1,3-dichloro-2-propyl) phosphate (BDCPP), bis(1-chloro-2-propyl) phosphate (BCPP), bis(2-chloroethyl) phosphate (BCEtP), di-p-cresylphosphate (DpCP), di-o-cresylphosphate (DoCP), dibutyl phosphate (DBuP), and dibenzyl-phosphate (DBzP); the NPBFR was 2,3,4,5-tetrabromobenzoic acid (TBBA). Specific gravity was measured in the field with a handheld refractometer (Atago, Uricon-Ne Product numbers 2722. Reading range 1.000–1.050 UG). Creatinine was measured at CDC using an enzymatic method with a Roche/Hitachi Cobas® c501 chemical analyzer (Roche Diagnostics, Inc., Indianapolis, IN). After enzymatic hydrolysis of 400-μL urine samples and off-line

Table 2
Flame retardant, dioxin, and furan biomarkers quantified in urine and serum.

Type of sample	Parent Chemical	Biomarker
Organophosphate Flame Retardants (OPFRs)		
Urinary	Triphenyl phosphate (TPP or TPhP), Isopropylphenyl diphenyl phosphate t-Butylphenyl diphenyl phosphate 2-Ethylhexyl diphenyl phosphate	Diphenyl phosphate (DPhP)
	Tris(1,3-dichloro-2-propyl) phosphate (TDCPP)	Bis(1,3-dichloro-2-propyl) phosphate (BDCPP)
	Tri-p-cresyl phosphate (TpCP)	Di-p-cresyl phosphate (DpCP)
	Tris(1-chloro-2-propyl) phosphate (TCPP or TClPP)	Bis(1-chloro-2-propyl) phosphate (BCPP)
	Tributyl phosphate (TBP or TBuP)	Dibutyl phosphate (DBP or DBuP)
	Tribenzyl phosphate (TBzP)	Dibenzyl phosphate (DBzP)
	Tris(2-chloroethyl) phosphate (TCEP)	Bis(2-chloroethyl) phosphate (BCEtP)
	Tri-o-cresyl phosphate (ToCP)	Di-o-cresyl phosphate (DoCP)
	Non-PBDE-brominated flame retardants (NPBFRs)	
	2-Ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB)	2,3,4,5-Tetrabromobenzoic acid (TBBA)
Polybromianted Diphenyl Ethers (PBDEs)		
Serum	2,2′,4-tribromodiphenyl ether (BDE-17)	BDE-17
	2,4,4′-tribromodiphenyl ether (BDE-28)	BDE-28
	2,2′,4,4′-tetrabromodiphenyl ether (BDE-47)	BDE-47
	2,3′,4,4′-tetrabromodiphenyl ether (BDE-66)	BDE-66
	2,2′,3,4,4′-pentabromodiphenyl ether (BDE-85)	BDE-85
	2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99)	BDE-99
	2,2′,4,4′,6-pentabromodiphenyl ether (BDE-100)	BDE-100
	2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153	BDE-153
	2,2′,4,4′,5,6′-hexabromodiphenyl ether (BDE-154)	BDE-154
	2,2′,3,4,4′,5′,6-heptabromodiphenyl ether (BDE-183)	BDE-183
	2,2′,3,3′,4,4′,5,5′,6-nonabromodiphenyl ether (BDE-206)	BDE-206
	decabromodiphenyl ether (BDE-209)	BDE-209
	Brominated furans	
	2,3,7,8-tetrabromodibenzofuran (2378-TeBDF)	2378-TeBDF
	2,3,4,7,8-pentabromodibenzofuran (23478-PeBDF)	23478-PeBDF
	1,2,3,4,7,8-hexabromodibenzofuran (123478-HxBDF)	123478-HxBDF
	Chlorinated dioxins	
	2,3,7,8-Tetrachlorodibenzodioxin (2378-TeCDD)	2378-TeCDD
	1,2,3,7,8-Pentachlorodibenzodioxin (12378-PeCDD)	12378-PeCDD
	1,2,3,4,7,8-Hexachlorodibenzodioxin (123478-HxCDD)	123478-HxCDD
	1,2,3,6,7,8-Hexachlorodibenzodioxin (123678-HxCDD)	123678-HxCDD
	1,2,3,7,8,9-Hexachlorodibenzodioxin (123789-HxCDD)	123789-HxCDD
	1234678-HpCDD	1234678-HpCDD
	Octachlorodibenzodioxin (OcCDD)	OcCDD
	Chlorinated furans	
	2,3,7,8-Tetrachlorodibenzofuran (2378-TeCDF)	2378-TeCDF
	1,2,3,7,8-Pentachlorodibenzofuran (12378-PeCDF)	12378-PeCDF
	(2,3,4,7,8-Pentachlorodibenzofuran) 23478-PeCDF	23478-PeCDF
	1,2,3,4,7,8-Hexachlorodibenzofuran (123478-HxCDF)	123478-HxCDF
	1,2,3,6,7,8-Hexachlorodibenzofuran (123678-HxCDF)	123678-HxCDF
	123789-HxCDF	123789-HxCDF
	2,3,4,6,7,8-Hexachlorodibenzofuran (234678-HxCDF)	234678-HxCDF
	1,2,3,4,6,7,8-Heptachlorodibenzofuran (1234678-HpCDF)	1234678-HpCDF
	1,2,3,4,7,8,9-Heptachlorodibenzofuran (1234789-HpCDF)	1234789-HpCDF
	Octachlorodibenzofuran (OcCDF)	OcCDF

solid phase extraction, target OPFR and NPBFR metabolites were separated via reversed phase high-performance liquid chromatography, and detected by isotope dilution-electrospray ionization tandem mass spectrometry.

Serum samples collected from firefighters were analyzed at CDC for a panel of PBDEs, brominated and chlorinated dioxins and furans performed by gas chromatography isotope dilution high resolution mass spectrometry (GC-IDHRMS) employing a DFS (Thermo DFS, Bremen, Germany) instrument, as previously detailed (Jones et al., 2012).

2.5. Data analysis

Descriptive statistics were displayed as frequency (%), mean ± standard deviation (SD), median, and range for firefighter characteristics. Number of samples, number of samples with concentrations below the limit of detection (LOD), geometric mean (GM), and geometric standard deviation (GSD) were provided for urine and serum concentrations by job assignment and by exposure time. LOD divided by square root of two was assigned to non-detectable concentrations (Hornung and Reed, 1990). Urinary concentrations were adjusted for creatinine (Boeniger et al., 1993).

A Welch's *t*-test or unequal variances *t*-test was used to determine concentration differences for all analytes between the U.S. general population aged 18 years and older and firefighters by job assignment and exposure time. The comparisons were also applied to each sex. A paired *t*-test was utilized to examine whether the change in serum concentrations from pre to post-fire was significantly different from zero. Concentrations for urinary and blood samples were log transformed because corresponding distributions were skewed to the right. For urinary samples, a mixed model with individual firefighter as a random effect was utilized to account for the statistical correlation among exposure time from the same firefighter. The model incorporated the use of maximum likelihood estimation method to reduce bias resulting from the data with non-detectable or left-censored concentrations (Jin et al., 2011). Univariable analyses of longitudinal urinary data were carried out using the log-transformed concentration as the dependent variable. Covariates treated as fixed effects, including exposure times (pre-fire, 3-h post, 6-h post, and 12-h post) and job assignments (exterior, interior, and overhaul), were evaluated. With respect to urine samples, an analysis of covariance (ANCOVA) was used to examine whether the means of a dependent variable, post urine concentration, were equal across job assignments, while statistically controlling for the effect of pre urine concentration. Statistical tests were two-sided at the 0.05 significance level. All analyses were performed in SAS version 9.4 (SAS Institute, Cary, NC).

3. Results

3.1. OPFR urinary results

Urinary concentrations of FRs measured among the majority of firefighters responding in three job assignment classifications during four urine collection times are summarized in Table 3. DPhP, BDCPP, and BCEtP were detected more frequently (detection rate > 60%) than the other metabolites measured in this study. Overall, GM concentrations of DPhP and BDCPP at multiple collection time points were higher than concentrations found in the general population. Specifically, 3-h and 6-h post-fire DPhP GM concentrations for all three job assignments (ranging from 1.38 μg/g creatinine to 1.75 μg/g creatinine) were statistically significantly greater than the GM of the general population (0.80 μg/g creatinine). Additionally, GM concentrations of BDCPP in the three job assignments during the four collection times ranged from 1.86 μg/g creatinine to 3.32 μg/g creatinine and were statistically significantly greater than the GM of general population (0.79 μg/g creatinine). We also stratified by sex and compared DPhP, BDCPP, and BCEtP concentrations with the general population in Supplemental Materials (Table S1). Results for the other urinary biomarkers detected less frequently (<60%) are provided in Supplemental Materials (Table S2).

Results of univariable analyses of repeated measures data with natural logarithm of urinary concentrations as the dependent variable are presented in Table 4. For DPhP and BDCPP, maximum urinary concentrations occurred 3-h post-firefighting, but this increase relative to the pre-fire concentrations was only statistically significant for DPhP (p-value is < 0.001). The mean urinary concentrations of DPhP and BDCPP decreased with each subsequent collection, however the 12-h post-fire DPhP concentrations were still higher than the pre-fire levels (p-value is < 0.05). For BCEtP, maximum urinary concentrations occurred 6-h post-firefighting (p-value is < 0.05 compared to the pre-fire concentrations), but then decreased to levels below the pre-fire concentrations (p-value is < 0.001) 12-h post-fire. There were no statistically significant differences in DPhP, BDCPP, and BCEtP for 3- and 6-h urinary mean concentrations among the three job assignments, adjusting for pre-fire concentrations. However, firefighters assigned to overhaul had statistically significantly higher 6-h BDCPP concentrations compared to those assigned to interior response in this analysis despite the requirement that firefighters wore SCBA during overhaul response.

Univariable results using pre-fire urinary concentrations as the dependent variable are provided in Table 5. Pre-fire BDCPP urinary concentrations were statistically significantly higher for firefighters who previously worked a scenario 7 days ago compared to those who were responding to their first scenario as part of this study (p-value is < 0.05). When comparing firefighters who last participated in a fire scenario 7 days and 10 or more days ago, firefighters who participated 10 days or more ago had statistically significantly lower BDCPP concentrations by comparison (p-value is < 0.05). When examining the job assignment for the previous scenario, firefighters who were previously assigned to interior response had statistically significantly higher pre-fire BDCPP concentrations than firefighters previously assigned to exterior response (p-value is 0.030).

Table 3
Firefighter urine biomarker concentrations[A] (μg/g creatinine) by job assignment compared to the general population (GP).

		Pre-fire Concentration			3-Hour Post-fire Concentration			6-Hour Post-fire Concentration			12-Hour Post-fire Concentration		
Biomarker	Job Assignment	N (N < LOD[B])	GM (GSD)	P-value (vs. GP)	N (N < LOD[B])	GM (GSD)	P-value (vs. GP)	N (N < LOD[B])	GM (GSD)	P-value (vs. GP)	N (N < LOD[B])	GM (GSD)	P-value (vs. GP)
DPhP	**All Firefighters**	36 (3)	0.97 (1.98)	0.103	36 (3)	1.67 (1.94)	**<0.001[E]**	36 (0)	1.58 (1.96)	**<0.001[E]**	36 (1)	1.20 (2.13)	**0.003[E]**
	Exterior	12 (2)	0.95 (2.37)	0.489	12 (1)	1.55 (2.05)	**0.009[E]**	12 (0)	1.38 (2.15)	**0.032[E]**	12 (0)	1.22 (2.18)	0.088
	Interior	12 (1)	1.04 (1.92)	0.196	12 (1)	1.72 (2.11)	**0.005[E]**	12 (0)	1.66 (2.31)	**0.012[E]**	12 (0)	1.28 (2.53)	0.105
	Overhaul	12 (0)	0.92 (1.74)	0.403	12 (1)	1.75 (1.75)	**<0.001[E]**	12 (0)	1.72 (1.43)	**<0.001[E]**	12 (1)	1.10 (1.78)	0.080
	General Population[C]	1901 (187)	0.80 (2.59)	Reference	**	**	Reference	**	**	Reference	**	**	Reference
BDCPP	**All Firefighters**	36 (0)	2.38 (2.12)	**<0.001[E]**	36 (0)	2.70 (1.97)	**<0.001[E]**	36 (0)	2.57 (2.01)	**<0.001[E]**	36 (0)	2.13 (1.99)	**0 < .001[E]**
	Exterior	12 (0)	2.73 (2.22)	**<0.001[E]**	12 (0)	3.32 (2.11)	**<0.001[E]**	12 (0)	2.63 (2.07)	**<0.001[E]**	12 (0)	2.23 (1.97)	**0 < .001[E]**
	Interior	12 (0)	2.09 (2.10)	**0.003[E]**	12 (0)	2.25 (1.83)	**<0.001[E]**	12 (0)	2.07 (1.98)	**<0.001[E]**	12 (0)	1.86 (2.06)	**0.002[E]**
	Overhaul	12 (0)	2.38 (2.13)	**<0.001[E]**	12 (0)	2.64 (1.95)	**<0.001[E]**	12 (0)	3.11 (1.96)	**<0.001[E]**	12 (0)	2.33 (2.01)	**0 < .001[E]**
	General Population[C]	1886 (174)	0.79 (2.83)	Reference	**	**	Reference	**	**	Reference	**	**	Reference
BCEtP	**All Firefighters**	36 (6)	0.28 (3.01)	**0.048[D]**	36 (8)	0.34 (2.09)	0.117	36 (1)	0.36 (1.83)	0.170	36 (5)	0.20 (2.03)	**<0.001[D]**
	Exterior	12 (2)	0.47 (2.43)	0.630	12 (2)	0.38 (1.94)	0.701	12 (1)	0.37 (1.75)	0.538	12 (2)	0.23 (1.81)	**0.005[D]**
	Interior	12 (3)	0.24 (2.92)	0.119	12 (4)	0.33 (2.10)	0.302	12 (0)	0.33 (1.78)	0.195	12 (1)	0.17 (2.51)	**0.006[D]**
	Overhaul	12 (1)	0.20 (3.37)	0.058	12 (2)	0.31 (2.34)	0.256	12 (0)	0.37 (2.02)	0.641	12 (2)	0.21 (1.82)	**0.002[D]**
	General Population[C]	1897 (240)	0.41 (3.10)	Reference	**	**	Reference	**	**[Ga]	Reference	**	**	Reference

A. Metabolites with less than 60% detection rate are summarized in Supplemental Materials (Table S2).
B. Limit of detection (LOD) for each analyte in μg/L: DPhP = 0.16, BDCPP = 0.11, BCEtP = 0.08.
C. Ospina, M., Jayatilaka, N., Wong, L.-Y., Restrepo, P., Calafat AM., 2018 Exposure to organophosphate flame retardant chemicals in the U.S. general population: Data from the 2013–2014 National Health and Nutrition Examination Survey. *Environmental International*. 110, 32–41. Participants aged 18 and older are included.
D. Results were significantly lower than the general population.
E. Results were significantly higher than the general population.
** GM and GSD of general population were listed in the pre-fire columns.

Table 4
Univariable analysis using urine metabolite concentrationsA (μg/g creatinine) as the dependent variable.

Outcome	Logarithm of DPhP Concentration			Logarithm of BDCPP Concentration			Logarithm of BCEtP Concentration		
Covariate	Estimate (SE)	Factor	P-value	Estimate (SE)	Factor	P-value	Estimate (SE)	Factor	P-value
Exposure Time									
Pre-Fire	Reference			Reference			Reference		
3-Hour Post	0.54 (0.10)	1.72	**<0.001**	0.13 (0.08)	1.13	0.141	0.15 (0.12)	1.16	0.243
6-Hour Post	0.52 (0.10)	1.68	**<0.001**	0.08 (0.08)	1.08	0.374	0.31 (0.12)	1.37	**0.013**
12-Hour Post	0.23 (0.10)	1.26	**0.022**	−0.11 (0.08)	0.89	0.191	−0.34 (0.12)	0.71	**0.009**
3-Hour Post	Reference			Reference			Reference		
6-Hour Post	−0.03 (0.10)	0.97	0.792	−0.05 (0.08)	0.95	0.548	0.17 (0.12)	1.18	0.176
12-Hour Post	−0.31 (0.10)	0.73	**0.003**	−0.24 (0.08)	0.79	**0.007**	−0.48 (0.12)	0.62	**<0.001**
6-Hour Post	Reference			Reference			Reference		
12-Hour Post	−0.28 (0.10)	0.75	**0.006**	−0.19 (0.08)	0.83	**0.032**	−0.65 (0.12)	0.52	**<0.001**

Outcome	**Logarithm of 3-Hour Post DPhP Concentration**			**Logarithm of 3-Hour Post BDCPP Concentration**			**Logarithm of 3-Hour Post BCEtP Concentration**		
Covariate[B]	**Estimate (SE)**	**Factor**	**P-value**	**Estimate (SE)**	**Factor**	**P-value**	**Estimate (SE)**	**Factor**	**P-value**
Job Assignment									
Exterior	Reference			Reference			Reference		
Interior	0.05 (0.21)	1.05	0.812	−0.22 (0.20)	0.80	0.284	0.23 (0.18)	1.26	0.213
Overhaul	0.15 (0.21)	1.16	0.491	−0.14 (0.20)	0.87	0.480	0.29 (0.18)	1.34	0.119
Interior	Reference			Reference			Reference		
Overhaul	0.10 (0.21)	1.10	0.652	0.08 (0.20)	1.08	0.703	0.06 (0.17)	1.07	0.705

Outcome	**Logarithm of 6-Hour Post DPhP Concentration**			**Logarithm of 6-Hour Post BDCPP Concentration**			**Logarithm of 6-Hour Post BCEtP Concentration**		
Covariate[B]	**Estimate (SE)**	**Factor**	**P-value**	**Estimate (SE)**	**Factor**	**P-value**	**Estimate (SE)**	**Factor**	**P-value**
Job Assignment									
Exterior	Reference			Reference			Reference		
Interior	0.13 (0.21)	1.14	0.536	−0.03 (0.15)	0.97	0.820	0.13 (0.20)	1.13	0.524
Overhaul	0.25 (0.21)	1.28	0.237	0.27 (0.15)	1.31	0.077	0.35 (0.20)	1.41	0.095
Interior	Reference			Reference			Reference		
Overhaul	0.12 (0.21)	1.13	0.567	0.31 (0.15)	1.36	**0.048**	0.22 (0.19)	1.25	0.258

A. No univariable analysis was conducted for metabolites with less than 60% detection rates (BCPP, DBuP, DpCP, TBBA, DoCP, and DBzP).
B. Logarithm of pre-fire concentration was adjusted for in the model.

3.2. PBDE and brominated and chlorinated dioxin and furan serum results

The levels of the PBDEs which were detected most frequently (>60%) in serum samples are summarized in Table 6. Six compounds (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, and BDE-209) were detected in more than 60% of the samples. Several of these compounds were below the levels reported in the general population, and no analytes significantly increased from pre- to post-fire. Concentrations for these six compounds were also stratified by sex and compared to the general population in Supplemental Materials (Table S3) The remaining PBDEs are summarized in Supplemental Materials (Table S4).

Although the change from pre- to post-fire was not statistically significant, BDE-209 was detected more frequently and had statistically significantly greater GM concentrations (2.91 and 3.01 ng/g lipid for pre- and post-fire serum samples) than the general population (1.89 ng/g lipid; p-values < 0.001). Pre- and post-fire serum GM concentrations of BDE-209 in the overhaul group (3.82 and 3.53 ng/g lipid, respectively) were also statistically significantly greater than the general population (p-values < 0.001), while firefighters assigned to exterior and interior response had higher post-fire serum GM concentrations (2.69 and 2.86 ng/g lipid, correspondingly) compared to the general population (respective p-values <0.05). Pre-fire serum BDE-209 concentrations were also used as the dependent variable to see how previous job assignment or days since last assignment impacted exposures, but results were similar and not statistically significant (data not shown).

Firefighters also provided serum samples that were pooled by job assignment groupings and analyzed for brominated and chlorinated furans and chlorinated dioxins, summarized in Supplemental Materials (Table S5). Compared to the brominated furans, chlorinated dioxins and furans were detected more frequently in the serum. Firefighters were found to have statistically significantly higher pre-fire GM serum concentrations of 23478-PeCDF, and pre- and post-fire GM serum concentrations of 1,2,3,4,7,8-Hexachlorodibenzofuran (123478-HxCDF), 1,2,3,6,7,8-Hexachlorodibenzofuran (123678-HxCDF), and 2,3,4,6,7,8-Hexachlorodibenzofuran (234678-HxCDF) than the general population. Job assignment did not appear to have a strong effect on the serum concentrations. The few statistically significant findings by job assignment appeared to be related to the precision in the measurements (GSD) rather than the magnitude of the differences. Additionally, there were no statistically significant increases in serum concentrations from pre to post-fire.

4. Discussion

This study was designed to simulate a fire environment where firefighters responded to realistic scenarios and were assigned to common job assignments including interior, exterior and overhaul response. The fire environment included common home furnishings containing FRs. Specifically, this study characterized firefighters' exposure to FRs during common job assignments through urinary and serum samples.

We measured statistically significantly higher concentrations of BDCPP and DPhP in firefighters' urine post-fire compared to the general population. Interestingly, firefighters' pre-fire BDCPP concentrations were also statistically significantly higher than the general population, which was not true for DPhP or BCEtP. Additionally, we found DPhP concentrations in samples taken post-fire (3-h, 6-h, 12-h) were statistically significantly higher than pre-fire samples. The fact that BDCPP and DPhP are the most abundant OPFR urinary metabolites measured in this study is consistent with our previous environmental monitoring results (Fent et al., 2020b). Median air concentrations of TPhP (the parent compound of DPhP) were 3000-fold higher than any other OPFRs

Table 5
Univariable analysis using pre-fire urine metabolite concentrations[A] (μg/g creatinine) as the dependent variable.

Outcome	Logarithm of Pre DPhP Concentration			Logarithm of Pre BDCPP Concentration		
Covariate	Estimate (SE)	Factor	P-value	Estimate (SE)	Factor	P-value
Days Since Last Fire Scenario (Categorical)						
NA (N = 16)	Reference			Reference		
7 Days (N = 11)	−0.31 (0.27)	0.73	0.259	0.58 (0.28)	1.78	**0.045**
10 (N = 1) and 12 (N = 8)	−0.15 (0.29)	0.86	0.610	−0.20 (0.29)	0.82	0.508
7 Days	Reference			Reference		
10 and 12 Days	0.16 (0.31)	1.18	0.604	−0.77 (0.32)	0.46	**0.021**
Pre-Fire Group						
NA	Reference			Reference		
Exterior	−0.17 (0.34)	0.85	0.633	−0.31 (0.37)	0.73	0.409
Interior	0.04 (0.29)	1.04	0.899	0.62 (0.31)	1.86	0.055
Overhaul	−0.60 (0.30)	0.55	0.055	0.16 (0.33)	1.17	0.628
Exterior	Reference			Reference		
Interior	0.20 (0.38)	1.22	0.599	0.93 (0.41)	2.54	**0.030**
Overhaul	−0.44 (0.39)	0.65	0.273	0.47 (0.42)	1.60	0.275
Interior	Reference			Reference		
Overhaul	−0.64 (0.35)	0.53	0.074	−0.46 (0.37)	0.63	0.224

A. No univariable analysis was conducted for metabolites with less than 60% detection rates (BCPP, DBuP, DpCP, TBBA, DoCP, and DBzP).

analyzed in this study (408 μg/m^3) and TPhP was detected most frequently during overhaul as well. Surface wipe samples were also taken from turnout jackets worn by firefighters responding to these scenarios, and TDCPP (the parent compound of BDCPP) and TPhP were two of the most abundant compounds measured (Fent et al., 2020b). TPhP was also detected in bulk samples taken from headboard padding and chair cushions that were burned in the scenarios, while TDCPP was only detected in carpet padding (Table S6; Fent et al., 2020b). A previous publication found similar urinary results, reporting elevated concentrations of DPhP and BDCPP in firefighters' urine collected at the same training academy (Jayatilaka et al., 2017) where samples were collected for this study.

BCEtP pre-fire concentrations were lower than the general population, but the 6-h post-fire concentrations were statistically significantly increased from the pre-fire concentrations (though not statistically significantly higher than general population levels). Of note, we did not detect TCEP (the parent compound of BCEtP) in air or on turnout gear, although it was found in the bulk sample of carpet liner included in the scenarios (Table S6; Fent et al., 2020b). Nevertheless, the increase in urinary concentrations of BDCPP, DPhP, and BCEtP after firefighting suggest biological uptake of the parent compounds.

We stratified DPhP, BDCPP, and BCEtP urinary concentrations by sex and compared to the general population. Males in this study were more likely than their female counterparts to have concentrations above the male general population, but this is likely due in large part to the small sample size for females (n = 4). We also compared urinary concentrations by job assignment. Firefighters assigned to overhaul had statistically significantly higher 6-h BDCPP concentrations compared to interior firefighters. However, those who were previously assigned to interior response (a week or more prior) had statistically significantly higher pre-fire BDCPP urinary concentrations compared to those previously assigned to exterior or overhaul. Additionally, firefighters who last participated in a scenario 7 days prior had statistically significantly higher pre-fire urinary concentrations of BDCPP compared to those who were participating in their first scenario as part of this study. It is likely that the exposure from the previous scenario contributed to firefighters' elevated pre-fire BDCPP concentrations, particularly for those who were previously assigned to interior response. It is also possible the firefighters were exposed to FRs through their occupation. For example (Shaw et al., 2013), measured higher levels of BDCPP in California firefighters compared to the general population. Unfortunately, we did not survey firefighters in this study to determine whether they had responded to emergency fires in the period before specimen collections. A recent publication estimated BDCPP has an elimination half-life of 54 days (Wang et al., 2020) based on concentrations in human plasma and urine, much longer than previously thought (Carignan et al., 2013). Hence, we cannot rule out that work-related exposures from months ago or non-occupational exposures (e.g., diet or contaminated dust in the home) could contribute to the concentrations measured here.

DPhP urinary concentrations were more likely to increase post-fire (3-h, 6-h, 12-h) from pre-fire levels compared to all other analytes (including BDCPP) measured in this study. While TPhP appears to have slower permeation through the skin than many of the other OPFRs (absorption flux in ng cm^{-2} h^{-1}; TCEP = 10, TDCPP = 0.10, TPhP = 0.093) (Frederiksen et al., 2018), it was measured in air during the fires and after suppression at median concentrations that were several orders of magnitude higher than the other OPFRs (Fent et al., 2020b). DPhP post-fire concentrations were marginally higher for firefighters assigned to interior or overhaul compared to those assigned to exterior response. DPhP has a much shorter estimated half-life of 9.5 days (Wang et al., 2020) than BDCPP, which may explain why the firefighters' pre-fire urinary concentrations were near general population levels regardless of the previous job assignment or how long it had been since they participated in a fire scenario. Though differences are not statistically significant, DPhP concentrations were lower for those previously assigned to overhaul compared to those assigned to interior response. Previous studies have found interior response activities like fire suppression and search and rescue led to higher exposures than exterior response activities or overhaul (Fent et al., 2020a, 2020b). Other studies have also explored TPhP exposure in other industries. Estill et al. (2021) found nail salon technicians had DPhP urinary concentrations lower than the current study, but still higher than the general population, while an older study found aircraft technicians had DPhP concentrations similar to those reported here (Schindler et al., 2014).

BDE-209 was the only PBDE that appeared to be higher than general population levels. However, there was not a statistically significant change in serum concentrations of BDE-209 from pre- to post-fire for all firefighters or for firefighters stratified by job assignment. Thus, although BDE-209 was the most abundant PBDE measured in air (both during overhaul and the fire period) and deposited on turnout jackets and hoods used in this study, there is no evidence of significant uptake of BDE-209 over a 23-h period after firefighting as part of this study. Interestingly, firefighters assigned to overhaul had pre-fire serum concentrations that were higher than the general population, suggesting that they may have been exposed before starting the scenario.

However, when we evaluated the effect of previous job assignment and time since last fire scenario on pre-fire BDE-209 serum concentrations, no statistically significant effects were found. There may be a low-level source of chronic BDE-209 exposure among the firefighters in this study that contributed to the serum levels we measured. Alexander and Baxter (2016) found that BDE-209 was one of the most abundant PBDE contaminants on used gear, while Shen et al. (2015) found high levels of BDE-209 in dust samples taken from firehouses relative to samples taken from other occupational settings. Previous studies have also found BDE-209 serum levels for firefighters that were statistically significantly higher than the general population (Park et al., 2015; Shaw et al., 2013).

Table 6
Firefighter PBDE serum concentrations[A] (ng/g lipid) by job assignment compared to the general population (GP).

		Pre-fire Serum Concentration			Post-fire Serum Concentration			
Analyte	Job assignment	N (No. < LOD[B])	GM (ng/g lipid) (GSD)	P-value (vs GP)	N (No. < LOD[B])	GM (ng/g lipid) (GSD)	P-value (vs GP)	P-value (Pre vs Post)
BDE-28	**All firefighters**	36 (4)	0.53 (2.25)	**0.029[D]**	36 (2)	0.54 (2.15)	**0.027[D]**	0.922
	Exterior	12 (2)	0.43 (1.88)	**0.016[D]**	12 (0)	0.43 (1.81)	**0.011[D]**	0.498
	Interior	12 (2)	0.47 (2.06)	0.065	12 (2)	0.47 (1.87)	**0.039[D]**	0.226
	Overhaul	12 (0)	0.74 (2.69)	0.928	12 (0)	0.77 (2.59)	0.823	0.984
	General Population[C]	1637 (178)	0.72 (1.78)	Reference	**	**	Reference	
BDE-47	**All firefighters**	36 (0)	8.49 (2.59)	**0.008[D]**	36 (0)	8.37 (2.57)	**0.006[D]**	0.869
	Exterior	12 (0)	5.94 (1.88)	**0.001[D]**	12 (0)	5.73 (1.86)	**<0.001[D]**	0.172
	Interior	12 (0)	7.58 (2.28)	**0.038[D]**	12 (0)	7.60 (2.23)	**0.034[D]**	0.447
	Overhaul	12 (0)	13.59 (3.29)	0.955	12 (0)	13.47 (3.25)	0.974	0.921
	General Population[C]	1637 (0)	13.32 (1.89)	Reference	**	**	Reference	
BDE-99	**All firefighters**	36 (0)	1.58 (2.80)	**0.007[D]**	36 (0)	1.49 (2.76)	**0.003[D]**	0.816
	Exterior	12 (0)	1.08 (2.01)	**0.001[D]**	12 (0)	0.95 (2.01)	**<0.001[D]**	0.081
	Interior	12 (0)	1.32 (2.62)	**0.035[D]**	12 (0)	1.31 (2.46)	**0.024[D]**	0.135
	Overhaul	12 (0)	2.76 (3.30)	0.852	12 (0)	2.68 (3.23)	0.918	0.899
	General Population[C]	1637 (0)	2.59 (2.12)	Reference	**	**	Reference	
BDE-100	**All firefighters**	36 (1)	1.58 (2.52)	**<0.001[D]**	36 (0)	1.67 (2.28)	**<0.001[D]**	0.992
	Exterior	12 (0)	1.24 (1.56)	**<0.001[D]**	12 (0)	1.19 (1.52)	**<0.001[D]**	0.091
	Interior	12 (0)	1.60 (2.08)	**0.017[D]**	12 (0)	1.54 (2.10)	**0.014[D]**	0.204
	Overhaul	12 (1)	1.99 (3.91)	0.361	12 (0)	2.52 (2.87)	0.657	0.949
	General Population[C]	1637 (0)	2.90 (1.88)	Reference	**	**	Reference	
BDE-153	**All firefighters**	36 (0)	5.66 (2.42)	**<.001[D]**	36 (0)	5.53 (2.44)	**<0.001[D]**	0.907
	Exterior	12 (0)	4.61 (2.22)	**0.008[D]**	12 (0)	4.45 (2.23)	**0.006[D]**	0.347
	Interior	12 (0)	4.37 (2.05)	**0.003[D]**	12 (0)	4.33 (2.09)	**0.003[D]**	0.962
	Overhaul	12 (0)	9.00 (2.68)	0.769	12 (0)	8.80 (2.72)	0.715	0.790
	General Population[C]	1637 (0)	9.81 (1.93)	Reference	**	**	Reference	
BDE-209	**All firefighters**	36 (2)	2.91 (1.79)	**<0.001[E]**	36 (0)	3.01 (1.57)	**<0.001[E]**	0.687
	Exterior	12 (1)	2.35 (1.71)	0.191	12 (0)	2.69 (1.56)	**0.020[E]**	0.359
	Interior	12 (1)	2.75 (1.87)	0.062	12 (0)	2.86 (1.61)	**0.012[E]**	0.720
	Overhaul	12 (0)	3.82 (1.66)	**<0.001[E]**	12 (0)	3.53 (1.53)	**<0.001[E]**	0.257
	General Population[C]	1637 (27)	1.89 (1.64)	Reference	**	**	Reference	

A. PBDEs with less than 60% detection rate are summarized in Supplemental Materials (S4).
B. LOD: limit of detection. Observations below the LOD were substituted using LOD/square root of 2.
C. The data are from the National Health and Nutrition Examination Survey (NHANES) (2020). 2015–2016 data documentation, codebook, and frequencies. Brominated Flame Retardants (BFRs) - Pooled Samples (BFRPOL_I). Available at https://wwwn.cdc.gov/Nchs/Nhanes/2015-2016/BFRPOL_I.htm. Accessed 12 November 2020.
D. Results were significantly lower than the general population.
E. Results were significantly higher than the general population.
** GM and GSD of general population were listed in the pre serum columns.

Of note, BDE-209 has a half-life of 15 days, while tri- to hexaBDEs have half-lives in the range of one to four years (Sjödin et al., 2020; Thuresson et al., 2006). Hence, serum concentrations of BDE-209 represent relatively recent exposures (i.e., within the last month) while lower brominated congeners serum concentrations represent years of accumulated exposure possibly masking any exposures occurring in the last fire scenario.

While BDE-209 concentrations were above the general population, the other BDEs detected most frequently in this study were statistically significantly lower than the general population. To our knowledge, this is the first study reporting lower BDE levels for firefighters compared to the general population, indicating firefighters' exposure to this class of FRs may be decreasing following their usage restriction.

None of the serum concentrations of dioxins or furans increased from pre- to post-fire. In general, chlorinated furans were more likely to be above general population levels than chlorinated dioxins even before the fires (general population data were not available for brominated furans). Specifically, 23478-PeCDF pre-fire concentrations were statistically significantly above the general population. 23478-PeCDF is a Group 1 known human carcinogen, according to IARC (International Agency for Research on Cancer (IARC), 2010), and thus exposure to this compound should be reduced as much as possible. It should be noted that levels in wipe samples of the firefighters' gloves were below the LOD for 23478-PeCDF (Fent et al., 2020b). However, the analysis of chlorinated furans in wipe samples was qualitative in nature, so caution should be exercised when interpreting these findings.

The types and makeup of furnishings and additive FRs in those furnishings will vary greatly from one structure to another. Hence, while we attempted to create a representative residential fire that could be replicated across all three participant crews, these fires certainly do not represent potential exposures across all structure fires. The FRs that dominated in the environmental and biological samples collected in this study could be more or less prevalent in different structure fires. For example, PBDEs were phased out of production in the United States over the past decade, so furniture that has been manufactured more recently will be less likely to contain these chemicals. Therefore, caution should be exercised in generalizing these findings broadly across the U.S. fire service.

This study has some limitations. Most of the firefighters participating in this study were from the Midwest (i.e., Illinois, Wisconsin, Indiana) so a comparison with NHANES, a nationally representative sample, could overlook geographic differences. However, NHANES is the best comparison group available as regionally representative data for Midwest residents does not exist for these compounds. Although most of the urinary metabolites are specific for the parent compounds, it is important to note that some OPFRs have other metabolites (e.g., hydroxyl triphenyl phosphate for TPhP, 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate for TCPP) not included in this study. Additionally, DPhP is a metabolite for several other compounds including isopropylphenyl diphenyl phosphate, t-butylphenyl diphenyl phosphate, and 2-ethylhexyl diphenyl phosphate (Nishimaki-Mogami et al., 1988; Phillips et al., 2020; Shen et al., 2019). However, the metabolites

included in this study are those included in NHANES (Ospina et al., 2018), which allowed comparisons to concentrations found in the general population. We did not restrict firefighters from responding to fires as part of their occupation prior to the scenarios (or during the time period between scenarios) and it is possible participants recently responded to fires as part of their occupation (although this was not documented). Given the extended half-lives (i.e., several days) of several of these chemicals (e.g., DPhP, BDCPP, BDE-209), we cannot rule out the possibility that the firefighters' occupation or other non-occupationally related sources of exposure contributed to their metabolite levels even before the fire scenarios and specimen collections in this study. In fact, the data support that the previous fire-scenario assignment (at least 7 days prior) may have contributed to the pre-fire concentrations of BDCPP for some firefighters. Despite this potential confounder, we found post-fire urinary concentrations for several OPFR metabolites that were higher than pre-fire urinary concentrations. Additionally, the parent compounds (TPhP, TDCPP, BDE-209) of the most abundant metabolites (BDCPP, DPhP, BDE-209) were also the most abundant chemicals detected in air and deposited on turnout gear (as reported previously). BDE-209 concentrations were statistically significantly higher than the general population, suggesting firefighters may be chronically exposed to low levels of this chemical as part of their occupation.

This study provides further evidence that firefighters in full protective turnout gear can biologically absorb compounds that are produced or released during fires. While inhalation exposure is possible for firefighters on the exterior of the structure, interior firefighters wore SCBA throughout the response and overhaul firefighters donned SCBA before entering the structure post suppression. Hence, the dermal route likely played an important role in the absorption of the OPFRs. Participants in this study used commercial skin-cleansing wipes (Essendant baby wipes NICA630FW) and showered shortly after completing the scenarios, which likely removed some of the dermal contamination. While the impact of these measures should be further evaluated, higher biological levels may have been experienced if skin cleansing was delayed, which is often the case during emergency fire responses.

5. Conclusions

Firefighters can be exposed to certain PBDEs, OPFRs, and brominated and chlorinated furans and chlorinated dioxins when responding to structure fires containing modern home furnishings. Several FR biomarkers (BDE-209, DPhP, and BDCPP) were consistently detected in biological specimens at concentrations above the general population levels, and other compounds (23478-PeCDF) were above the general population levels during at least one collection period. Urinary concentrations of DPhP increased significantly from pre- to post-fire, suggesting absorption of the parent compound (TPhP) during the fire response. BCEtP concentrations were not above general population levels but did increase significantly pre- to post-fire. Job assignment appears to play an important role, as those who previously worked interior response had higher pre-fire BDCPP concentrations than those who had previously worked exterior operations. That the previous scenario occurred at least 7 days prior to the specimen collection suggests that BDCPP will remain in the body for several days following exposure. Future work should further investigate how job assignment and control interventions (e.g., routine laundering of turnout gear) impact the biological absorption of FRs during structural firefighting.

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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.2021.113782.

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Chemical prioritisation strategy in the European Human Biomonitoring Initiative (HBM4EU) – Development and results

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ABSTRACT

The European Human Biomonitoring Initiative (HBM4EU[1]) has established a European Union-wide human biomonitoring (HBM) programme to generate knowledge on human internal exposure to chemical pollutants and their potential health impacts in Europe, in order to support policy makers' efforts to ensure chemical safety and improve health in Europe.

A prioritisation strategy was necessary to determine and meet the most important needs of both policy makers and risk assessors, as well as common national needs of participating countries and a broad range of stakeholders. This strategy consisted of three mains steps: 1) mapping of knowledge gaps identified by policy makers, 2) prioritisation of substances using a scoring system, and 3) generation of a list of priority substances reflective of the scoring, as well as of public policy priorities and available resources.

For the first step, relevant ministries and agencies at EU and national levels, as well as members of the Stakeholder Forum each nominated up to 5 substances/substance groups of concern for policy-makers. These nominations were collated into a preliminary list of 48 substances/substance groups, which was subsequently shortened to a list of 23 after considering the total number of nominations each substance/substance group received and the nature of the nominating entities.

For the second step, a panel of 11 experts in epidemiology, toxicology, exposure sciences, and occupational and environmental health scored each of the substances/substance groups using prioritisation criteria including hazardous properties, exposure characteristics, and societal concern. The scores were used to rank the 23 substances/substance groups. In addition, substances were categorised according to the level of current knowledge about their hazards, extent of human exposure (through the availability of HBM data), regulatory status and availability of analytical methods for biomarker measurement.

Finally, in addition to the ranking and categorisation of the substances, the resources available for the project and the alignment with the policy priorities at European level were considered to produce a final priority list of 9 substances/substance groups for research activities and surveys within the framework of the HBM4EU project.

1. Introduction

Human biomonitoring (HBM) measures levels of chemicals directly in human biological samples (e.g. blood, urine, hair). This type of measurement aggregates exposure from all relevant routes and sources and, as such, is a powerful tool for tracing the uptake of chemicals in the human body (Angerer et al., 2007). Assessment of human exposure provides important information for health risk assessments, but it requires considerable coordination efforts, harmonised and comparable methods and financial investment. Therefore, the selection of substances

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for inclusion in HBM surveys needs to be well thought out. The identification of policy-relevant chemicals to be included in the Human Biomonitoring Initiative in Europe (HBM4EU) is a crucial step towards achieving these goals (Apel et al., 2020; Buekers et al., 2018; David et al., 2020; Louro et al., 2019).

HBM4EU is a joint effort of 30 countries, the European Environment Agency (EEA) and the European Commission (EC), which takes part in the project through the European Union (EU) Policy Board. The initiative aims to generate knowledge on human exposure to specific chemicals and chemical groups in Europe and on the human health impacts of this exposure. Running from 2017 to 2021, HBM4EU is co-funded by participating partners and the EC's Horizon 2020 research programme. It is organised to answer policy-relevant questions for priority chemicals, as identified by the partner countries' National Hubs (i.e. bodies set up at national level representing the national network of HBM activities), the EU Policy Board (representatives of EC services and EU agencies dealing with chemicals) and a Stakeholder Forum (comprised of non-governmental organisations (NGOs), industry and trade unions). The generated knowledge will support the efforts of policy makers to enhance chemical safety in Europe, as well as to set research priorities at the European level.

The selection of the first list of high-priority substances for action in HBM4EU was undertaken in 2016, at the stage of developing the proposal for this H2020 initiative. This step involved consortium partners and representatives from EU institutions (General Directorates and EU agencies in charge of chemical policy, monitoring and regulation), and took into account both national and EU level policy needs to better understand chemical exposure and health outcomes. This first prioritisation exercise resulted in 9 substances or substance groups: phthalates and Hexamoll® DINCH, bisphenols, per-/polyfluoroalkyl substances (PFAS), flame retardants, cadmium and chromium VI, polycyclic aromatic hydrocarbons (PAHs), anilines, chemical mixtures and emerging substances.

Because two additional rounds of chemical prioritisation were planned during the course of the project, a prioritisation strategy was developed to make the process more accountable, transparent, legitimate and useful for the next two rounds. This strategy consisted in a multi-step approach involving the consultation of the EU Policy Board, the National Hubs, and members of the HBM4EU Stakeholder Forum, which we present here. Its actual use for the first time and its results, namely the second list of HBM4EU priority substances, are also described. In addition, we offer recommendations for further improvement, emerging from the feedback of the various entities that participated in this second HBM4EU prioritisation round.

2. Chemical prioritisation strategy developed and applied in HBM4EU

In the first instance, a review of the literature was performed to collect criteria used for chemical prioritisation in HBM programmes worldwide. Prioritisation criteria used in the following HBM programmes were identified: the United States (US) National Health And Nutrition Examination Survey (NHANES), the Canadian Health Measures Survey (CHMS), the German Environmental Survey (GerES), the French Longitudinal Study of Children (ELFE), the French cross-sectional health survey (Esteban) and the Flemish Environment and Health Study (FLEHS) (Casteleyn et al., 2015; CDC 2002; 2003; 2006; Fillol et al., 2014; Fréry et al., 2012; Haines et al., 2017; Health Canada 2010; 2013; 2015; Kolossa-Gehring et al., 2012a; Schoeters et al., 2017). The prioritisation criteria were assessed in relation to their relevance to the objectives and specificities of the HBM4EU project (Ganzleben et al., 2017). The selected criteria related to hazardous properties, exposure characteristics, regulatory status, public concern and technical feasibility for HBM (HBM4EU 2017).

In brief, the strategy consisted of the implementation of three successive main steps, as described in Fig. 1. The first step was to *map knowledge needs* (nomination of substances along with their policy and knowledge needs; holding of a stakeholder workshop) and *initiate the prioritisation* (based on relatively simple frequency weightings to produce a short list of substances). The second step was to *rank nominated substances/substance groups* from the short list according to a priority score reflective of their level of concern and thus the relevancy to have them prioritised. In addition, a category (from A to D) was allocated to each substance to inform on the current level of knowledge on the substance, mainly from the perspective of HBM research. These substance categories aimed to support the prioritisation process by indicating the information gaps that research activities in HBM4EU could target. The third step consisted of consulting with the EU Policy Board and the HBM4EU Management Board to agree on a list of proposed priority substances, based on the ranked list of substances but also according to resources and policy considerations. Each of these steps is described in more detail below. The second priority list of substances, proposed according to this methodology, was finally approved by the HBM4EU Governing Board.

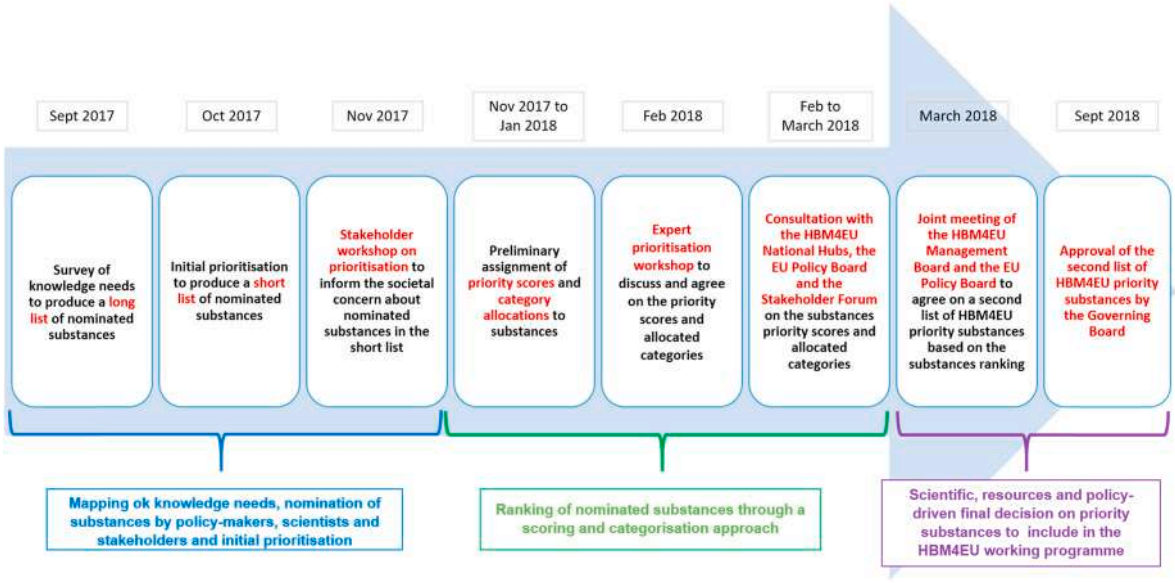

Fig. 1. Overview of the chemical prioritisation strategy under HBM4EU.

2.1. Mapping of knowledge needs for substances and initial prioritisation

The objective of the *mapping of knowledge needs* was to identify the activities and research needed on specific substances, as expressed by the EU Policy Board, the National Hubs and the HBM4EU Stakeholder Forum. These three groups of participants ensured an overarching knowledge input that would benefit the results generated under HBM4EU. It was therefore necessary to gain a comprehensive understanding of the specific needs of these groups of participants.

The key steps for determining the knowledge needs were to: 1) run an online survey for the nomination of substances; 2) produce a short list of nominated substances and 3) organise a stakeholder workshop to collect more input.

2.1.1. Online survey for the nomination of substances: polling European chemical research needs under HBM4EU

The online survey was structured around the selected set of prioritisation criteria consisting of the hazardous properties, exposure characteristics, regulatory status, public concern, and technical feasibility for HBM measurement. The complete online survey with questions is included in Supplementary Material - Annex 1. It ran from July to September 2017 and was opened to the following key entities: members of the EU Policy Board, the participating countries represented by their National Hubs and members of the HBM4EU Stakeholder Forum (Fig. 2).

Each entity could nominate up to 5 substances (or groups of substances) by completing the full online survey for each nominated substance/substance groups, as well as by defining the policy-related questions they thought HBM4EU research activities should address for each substance and how new knowledge generated under the project could benefit society. They were required to submit any available information and evidence on the nominated substance/substance groups involving questions related to the prioritisation criteria. Respondents could re-nominate substances that were already on the first list of HBM4EU prioritised substances, to emphasise their high priority and the fact that HBM-related research is still needed. These substances were communicated directly to the respective chemical group leaders (CGL) of the HBM4EU first priority list of substances (CGLs are tasked with developing scoping documents on current policy questions and proposals for relevant monitoring and research activities for the substances under their remit) and were not considered further in the process for obtaining the second HBM4EU priority list.

The survey participants nominating groups of substances were asked to provide a rationale for grouping these substances together. This included common analytical methods for measuring a panel of substances in a single biological sample, substances with similar use with possibility of substitution within the group or substances with similar toxicological profiles.

The survey results were collated to produce a long list of nominated substances and substance groups. This involved consolidating multiple nominations for individual substances and for groups of substances, where there was an overlap.

2.1.2. Producing a short list of substances and drafting background documents

Because the developed methodology for prioritising chemicals requires assessing information on the substances' hazards, exposures, regulations and HBM analytical feasibility, it was necessary to have a manageable number of substances to assess within the established timeframe. Therefore, the next step consisted in shortening the long list of all nominated substances down to a shorter list of approximately 25 substances/substance groups. The initial criterion for including a substance in the short list was to consider the number of nominations, on the basis of having been nominated by at least one member of the EU Policy Board and/or 9 National Hubs (representing just over one-third of the participating countries). In practice, there was less commonality across the nominations than expected, which meant the above criteria had to be adapted by including:

- all substances and groups prioritised by the EU Policy Board (with the objective of meeting EU knowledge needs for policy support), as well as
- substances nominated by two or more National Hubs, or by at least one National Hub and one member of the Stakeholder Forum.

For each substance/substance group on the short list, informative so-called draft background documents were produced with the information provided in the online survey, including details on toxicological information, effects on human health and exposure characteristics required in the later stages of the prioritisation process, as also knowledge gaps and proposed research efforts.

2.1.3. Stakeholder workshop on chemical prioritisation: including stakeholder needs in the process

A stakeholder workshop on prioritisation was held in November 2017 to apprehend stakeholders' perspectives on the societal relevance of new HBM4EU-generated knowledge on the substances from the short list, and to better understand stakeholders' substance priorities and the reasoning behind these priorities. Stakeholders were asked to vote for the three substances/substance groups from the short list that they considered as most important to include in the HBM4EU project activities. The number of votes obtained for each substance was converted into a score, which was later used for scoring the substances in light of the public concern criterion (one of the selected prioritisation criteria).

2.2. Ranking of nominated substances through a scoring and categorisation approach

The second main step of the strategy consisted of *ranking the substances* from the short list based on their priority score, which was calculated against a set of prioritisation criteria according to the methodology described in more detail below. The category allocated to the substances (reflective of the availability of HBM data in Europe, current analytical capacity to measure them in HBM studies, their current EU regulatory status and the level of knowledge about their hazards) was also used to propose an alternative ranking, i.e. according to the substance's priority score but this time among the substances in the different categories (A to D).

This step involved a panel of 11 experts, from a wide variety of fields such as epidemiology, toxicology, exposure sciences, and occupational and environmental health (details available in Supplementary Material - Annex 2). As the data and information provided in the online nomination survey (and gathered in draft background documents) formed the basis for the substance's scoring and categorisation, these experts were first tasked with reviewing and, if necessary, supplementing these documents with any missing important information. Each draft background document was reviewed by two experts, who were also asked to propose a priority score (according to a previously defined methodology) and a category to the substance(s) covered by the document they had to review. In order to reach consensus priority scores and categories among the 11 experts, these were then presented and discussed by the expert panel during a two-day scoring workshop held in February 2018, especially in case of divergent proposals from the two experts. Agreed priority score and category were included in the revised background document for each substance/substance groups.

2.2.1. Scoring the substances or substance groups included in the short list of nominations

The scoring of the substances involved a three-step process, which included: 1) setting a consensus weighting value to be applied to each prioritisation criterion; 2) scoring the substances against each chosen prioritisation criterion and 3) calculating the substance's overall score. The process is further described below.

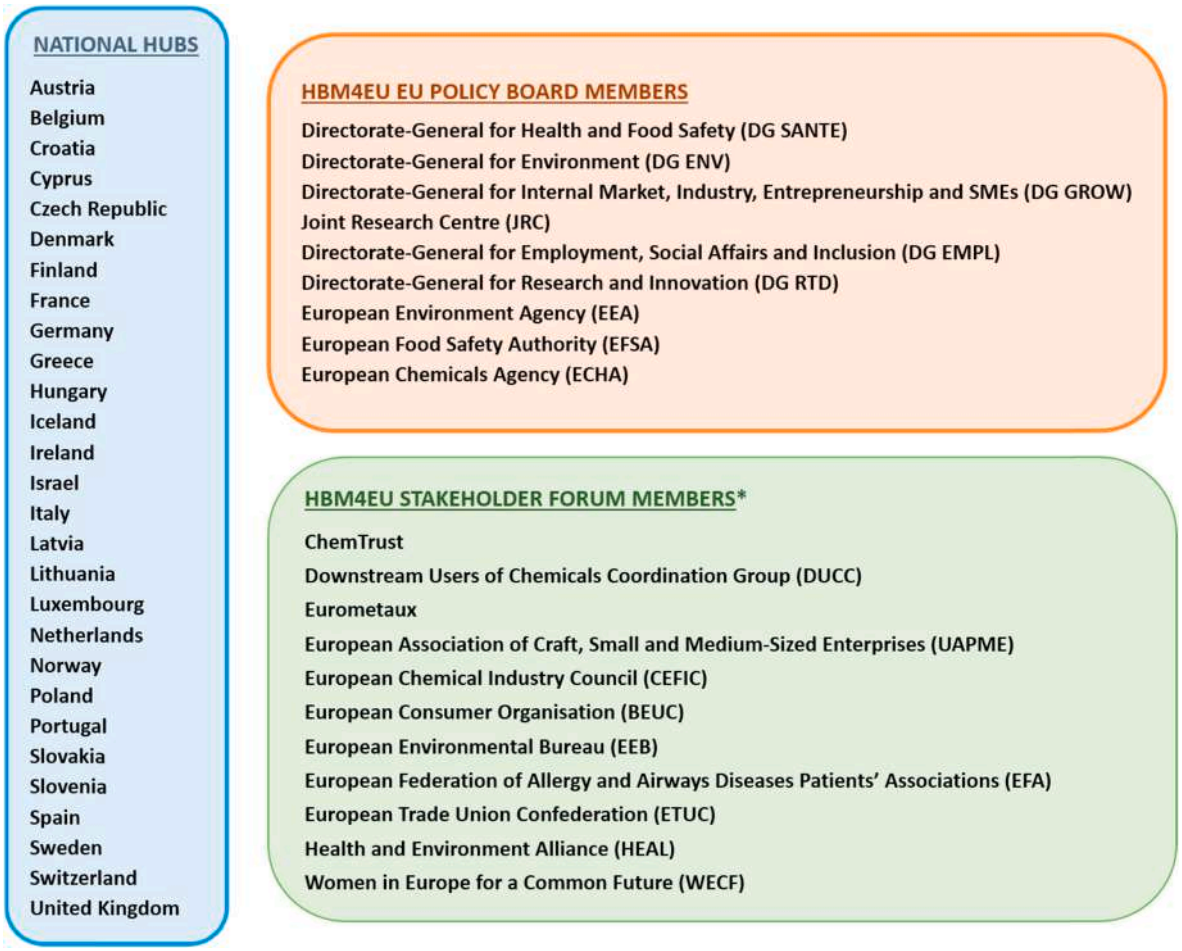

Fig. 2. Entities invited to nominate substances for prioritisation under HBM4EU.
* In 2019, two new stakeholders joined the Stakeholder Forum: Pesticide Action Network (PAN) and Plastics Europe. They did not take part in the 2017 round of chemical prioritisation.

2.2.1.1. Setting a consensus weighting value to be applied to each prioritisation criterion. An adapted Delphi method was adopted to weight the prioritisation criteria (hazards, exposure characteristics, regulatory status, public concern and technical feasibility for HBM measurement) according to their relative importance in the prioritisation process. The adapted Delphi method involved two rounds of consultation:

1) During the first round, a questionnaire was sent to the 11 experts, asking them to assign a percentage to each criterion to reflect its relative weight for the scoring step (i.e., their estimated importance for the priority setting). The sum of the weighting value assigned to each prioritisation criterion had to reach 100%. Experts had the possibility of justifying the given weighting values. Considering all expert responses, the median and boundary (minimum and maximum) values were calculated for each prioritisation criterion and were shared with all experts.
2) A conference call was organised to discuss the weighting values given during the first consultation round. Experts could explain the rationale for giving their values. Following these discussions, a second consultation round was organised during with each expert had the opportunity to modify the weighting values that he had given in the first round.

For each prioritisation criterion, the median of the weighing value given by the experts during the second consultation round was finally retained.

2.2.1.2. Scoring the substances against each selected prioritisation criterion. In order to score the substances against the criteria "hazardous properties" and "exposure characteristics", a systematic approach was implemented through the scoring rules that are summarised in Table 1 (scoring rules for different hazard endpoints) and Table 2 (scoring rules for different exposure characteristics). As informed by the information included in the substance's revised background documents, scores of 6, 3 or 1 were given, corresponding to, respectively, a High, Moderate or Low category of severity (towards hazard endpoints) or level (towards exposure characteristics). This approach is based on the GreenScreen® for Safer Chemicals method,[2] which has been designed to identify chemicals of high concern and safer alternatives using criteria to classify the human health and environmental hazard level for a chemical.

If no information was available to score a hazard endpoint/an exposure parameter or if the available data was considered inadequate, a Data Gap score of 2 was given. If available toxicological data did not suggest any related effect or mode of action toward an endpoint, a Low severity score of 1 was assigned for that endpoint instead of a Null score. This conservative approach is justified by the fact that toxicological tests may not currently be sensitive enough to detect effects.

Scores could deviate from the rules only if justified and accepted by the expert group involved in the scoring.

The scoring of the "public concern" criterion was informed in particular by the results of the vote held during the stakeholder

[2] https://www.greenscreenchemicals.org/learn/full-greenscreen-method.

Table 1
Scoring rules for endpoints of the *hazardous properties* criterion.

Hazardous properties						
Endpoint	Source of information	Severity level				Highest score by endpoint
		High (score of 6)	Moderate (score of 3)	Low (score of 1)	Data gap (score of 2)	
Carcinogenicity	EU – CLP regulation (H-Statements)	Carc. 1A or 1B (H350 or H350i)	Carc. 2 (H351)			
	EU – SVHC List	Candidate List				
	IARC classification	Group 1 or 2A	Group 2B	Group 4 (or Group 3 in specific cases)	Group 3	
	Peer-reviewed literature					
Mutagenicity	EU – CLP regulation (H-Statements)	Muta. 1A or 1B (H340)	Muta. 2 (H341)			
	EU – SVHC List	Candidate List				
	Peer-reviewed literature					
Reproductive toxicity	EU – CLP regulation (H-Statements)	Repr. 1A or 1B (H360F, H360FD, H360Fd)	Repr. 2 (H360Df, H361f, H361fd)			
	Peer-reviewed literature					
Developmental toxicity	EU – CLP regulation (H-Statements)	Repr. 1A or 1B (H360D, H360FD, H360Df, H362)	Repr. 2 (H360Fd, H361d, H361fd)			
	Peer-reviewed literature					
Endocrine activity	EU – SVHC List (article 57f)	Candidate List				
	EU - BKH List US - TEDX List		Cat. 1 or cat. 2 Inclusion in the list	Cat. 3		
	Peer-reviewed literature					
STOT RE (Systemic toxicity after repeated exposure)	EU – CLP regulation (H-Statements)	STOT -RE 1 (H373)	STOT-RE 2 (H373)			
	Adverse effects (e.g. on liver, kidneys, cardiovascular function) after chronic exposure to the substance, indicated from peer-reviewed literature	Yes	Suspected	Not identified		
Neurotoxicity[a]	Chemical Scorecard[b] Peer-reviewed literature	Strong evidence	Suspected	Not identified		
Immunotoxicity[a]	Chemical Scorecard [b] Peer-reviewed literature	Strong evidence	Suspected	Not identified		
Respiratory sensitiser[a]	EU – CLP regulation (H-Statements)	Resp. Sens. 1A or 1B (H334)				
	EU – SVHC List (article 57f) Peer-reviewed literature	Candidate List				
Skin sensitiser[a]	EU – CLP regulation (H-Statements)	Skin. Sens. 1A or 1B (H317)				
	Peer-reviewed literature					
Total score[c]						x
Adjusted total score for the hazardous properties criterion[d]						x/60 = X

CLP: Classification, Labelling and Packaging; EU: European Union; IARC: International Agency for Research on Cancer; STOT-RE: Specific target organ toxicity - repeated exposure; SVHC: Substances of Very High Concern; US: United States.

[a] Considered only if not yet addressed in another entry (to avoid double counting).

[b] Chemical Scorecard is an information service provided by the US Environmental Defense Fund that uses data from the US Environmental Protection Agency's Toxic Release Inventory plus other governmental and scientific agencies. Health effects are provided for more than 5000 chemicals.

[c] For each endpoint, the highest score was used to calculate the total score, which constitutes a conservative approach.

[d] The adjusted total score for the hazardous properties criterion was obtained by dividing the calculated total score by the highest possible score of 60 (10 endpoints of high severity category).

workshop on prioritisation. The number of stakeholder votes for each substance/substance group on the short list was translated into a corresponding score (e.g. a score of 4 for 4 votes). In addition, information on whether the substance is included in the SIN list[3] and/or in the Trade Union List for REACH authorisation[4] and whether NGO campaigns have been conducted regarding the substance were also considered (Table 3).

2.2.1.3. Calculating the overall score for each substance. The adjusted total score for each selected criterion (hazardous properties, exposure characteristics and the public concern) was multiplied by its respective median weighting value. Finally, the sum of the products resulted in the overall priority score for each substance (Table 4).

2.2.2. Categorising the substances or substance groups included in the short list of nominations

The categorisation step aimed to assign a category to each substance to reflect the level of knowledge on the availability of HBM data in

[3] http://sinlist.chemsec.org/.
[4] https://www.etuc.org/IMG/pdf/TUListREACH.pdf.

Table 2
Scoring rules for the parameters of the *substance exposure characteristics* criterion.

Exposure characteristics							
Parameter		Source or information	Severity level High (score of 6)	Moderate (score of 3)	Low (score of 1)	Data gap (score of 2)	Highest score per parameter
Persistency and/or bioaccumulation potential		EU – SVHC List (articles 57d and 57e for PBT and vPvB)	Candidate List				
		Peer-reviewed literature/ Institutional report	Persistent and evidence of bioaccumulation or significant biological half-life in mammals	Persistent (without evidence of bioaccumulation) or significant biological half-life in mammals			
Tonnages		ECHA	>1000 tpa	10-1000 tpa	<10 tpa		
Extent of exposure		ECHA	EU wide	Country/regional	Hotspot		
Routes of exposure		Peer-reviewed literature/ Institutional report	Multipathway exposure (oral, inhalation, dermal)	Multipathway (two routes of exposure only)	One route of exposure		
Passage of placental barrier		Peer-reviewed literature/ Institutional report	Strong evidence	Limited evidence	No		
Exposed populations	Workers	ECHA/Peer-reviewed literature/ Institutional report	Widespread (professional use and use in different industrial sectors	Some professional/industrial use	Intermediate use only		
	General population		Evidence of wide exposure (multiple media) - dispersive use	Limited evidence of exposure through external media	No significant exposure		
	Vulnerable groups exposed		Neonates, children, pregnant women				
Level of concern of the exposure	HBM data	ECHA/EFSA/Peer-reviewed literature/ Institutional report	Recent HBM data above or close to an established health-based HBM guidance value		Recent HBM data well below an established health-based HBM guidance value		
	External exposure data		Recent external exposure data above or close to a regulatory reference value		Recent external exposure data well below a regulatory reference value		
Total score[a]							y
Adjusted total score for the exposure characteristics criterion[b]							y/60 = Y

ECHA: European Chemicals Agency; EFSA: European Food Safety Authority; EU: European Union; PBT: Persistent, Bioaccumulative and Toxic; SVHC: Substances of Very High Concern; tpa: tonnes per annum; vPvB: very Persistent and very Bioaccumulative.

[a] For all parameters, the highest score was used to calculate the total score, which constitutes a conservative approach.

[b] The adjusted total score for the exposure characteristics criterion was obtained by dividing the calculated total score by the highest possible score of 60 (10 parameters of high severity category).

Table 3
Scoring rules for the *public concern* criterion.

Level of public concern	Related score
Stakeholder votes reflecting their interest in certain substances (or groups) from the short list of nominated substances during the stakeholder workshop on prioritisation	Number of votes translated into scores (maximum score = 9)
Inclusion in the SIN List and/or the Trade Union Priority List for REACH Authorisation	No = 0; Yes = 1
Recent NGO campaigns/media coverage	No = 0; Yes = 1
Total score	z
Adjusted total score for the public concern criterion[a]	z/11 = Z

[a] The adjusted total score was obtained by dividing the total score by the highest possible score.

Table 4
Calculation method of the overall priority score for substances included in the short list of nominations.

Prioritisation criterion	Criterion adjusted total score	Weighting value (W_i)	Product of the criterion adjusted total score by W_i
Hazardous properties	X	W_1	$X*W_1$
Exposure characteristics	Y	W_2	$Y*W_2$
Public concern	Z	W_3	$Z*W_3$
Overall priority score of the substance			$\Sigma[X*W_1 + Y*W_2 + Z*W_3]$

particular and, to a lesser extent, on the hazards, regulatory status or analytical capabilities for implementing HBM. Five substance categories (A to E) were defined in the HBM4EU project, as informed in Table 5.

Because category E substances are not yet constitutively identified substances, none of the substances nominated during the prioritisation process would belong to this category. Hence, substances were classified into the categories A to D, based on the information contained in the revised background document. For substance groups, categorisation was performed either for several substances included in the group or at least for an identified representative substance of the group.

The aim of allocating the substances to these categories was to ensure a balanced workload across the different areas of activity of the HBM4EU project by including substances from categories A to D in the

Table 5
Definition of category A to E substances under HBM4EU.

Substance's category within HBM4EU	Definition
Category A	Substances for which HBM data are sufficient to provide an overall picture of exposure levels across Europe, and interpretation of biomonitoring results in terms of health risks is possible. Risk management measures have been implemented at national or European level. Improvement of knowledge for these substances will therefore focus on policy-related research questions and evaluation of the effectiveness of existing regulatory measures.
Category B	Substances for which HBM data exists, but not sufficiently to have a clear picture across Europe. Also, knowledge on the extend of exposure, levels and impact on the human health should be improved, in order to give policy makers relevant and strategic data to establish appropriate regulations and improve chemical risk management. Analytical method and capacities to monitor the substances across Europe might have to be improved.
Category C	Substances for which HBM data scarcely or does not exists. Efforts to develop an analytical method to obtain relevant HBM results need to be done. Hazardous properties of the substances are identified, yet greater knowledge on toxicological characteristics and effects on the human health is needed. Interpretation of HBM data is not possible, due to the lack of HBM guidance values.
Category D	Substances for which a toxicological concern exists but HBM data are not available. HBM4EU research may be focused on the development of suspect screening approaches permitting to generate a first level of data enabling to document the reality of human exposure and better justify further investment in a full quantitative and validated method development.
Category E	Substances not yet identified as of toxicological concern and for which no HBM data are available. A bottom-up strategy will be applied, consisting to non-targeted screening approaches coupled to identification of unknowns capabilities for revealing, and further identifying, new (i.e. not yet known) markers of exposure related to chemicals of concern for HBM (parent compound or metabolite).

final list of priority substances. Indeed, according to the definitions of the substance categories, substances classified as A or B would require more public policy-oriented work, whereas C or D substances would require more research efforts such as developing HBM analytical methods or identifying biomarkers of effect.

2.3. Scientific, resources and policy driven final decision on the second HBM4EU priority list

The ranked list of substances was sent to the HBM4EU Management Board and the EU Policy Board, allowing them to weigh the scientific and policy merit of conducting research on each substance. Both boards met separately to discuss their priorities for action. Finally, a joint discussion with members of each of the two boards took place to agree on the final list of HBM4EU priority substances, considering also the resources available to conduct research activities during the 2019–2021 period.

2.4. After chemical prioritisation: feedback survey and suggestions for improvement

A survey was sent out in June 2019 to obtain feedback from the different participants who contributed to the second round of HBM4EU chemical prioritisation. This survey was designed to garner suggestions on how to further refine and streamline the overall strategy, including the scientific aspects of the elaborated method. The feedback received was taken into account to improve the overall process ahead of the third round of HBM4EU chemical prioritisation, which will take place in 2020–21.

3. Results

3.1. Mapping of knowledge needs for substances and initial prioritisation

3.1.1. Results of the online survey for the nomination of substances for research under HBM4EU

One hundred and thirty-two substances with policy needs were nominated in the online survey (this initial list is available here). Respondents were from 24 National Hubs, 4 members of the Stakeholder Forum and 6 members of the EU Policy Board.

As mentioned in section 2.1.1, re-nominations of substances that were part of the first list of HBM4EU priority substances were communicated to the respective CGLs and consequently removed to produce a 92-nomination list (cf. Annex 3 - Supplementary Material). This list was further refined by looking for overlaps across nominations:

- Single substances were grouped when they were associated to each other. For example, "mercury" was combined with "mercury and its compounds".
- Substance groups were used when consolidating some of the nominated single substances or smaller groups into larger groups of substances.

Finally, 23 single substances and 25 substance groups were obtained in the so-called "long list" of nominated substances, which is available here.

3.1.2. Results from the initial prioritisation to produce a short list of substances

Following application of the established criteria to reduce the long list of nominations (see section 2.1.2), a "short list" of 23 substances/substance groups was produced. This was a manageable number of substances in order to apply the further steps of the prioritisation strategy (i.e. the scoring and categorisation) in the allotted time. This short list is available in Annex 4 of the Supplementary Material.

Fig. 3 summarizes the overall process starting from all the nominations received up to the short list of nominated substances.

3.2. Ranking of nominated substances included in the short list through the scoring and categorisation approach

3.2.1. Consensual weighting values for the prioritisation criteria

When implementing the adapted Delphi approach, the 11 experts agreed that two of the five prioritisation criteria (i.e. regulatory status, and technical feasibility for HBM measurement) were not of highest relevance for the scoring step. Indeed, scoring the substances according to their current regulatory status was not considered appropriate, because this would produce a bias towards already regulated substances for which more follow-up activities are needed instead of gaining new knowledge. Likewise, as the HBM4EU project entails activities aiming to develop analytical methods for measuring biomarkers, it was not considered informative to de-prioritise lesser-known substances for which no analytical biomonitoring methods currently exist (C or D category substances). While finally not used for the scoring step, these two prioritisation criteria were nonetheless useful for the categorisation step, as this defines the type of work to be perform in HBM4EU.

Table 6 indicates the weighting values assigned to each of the three selected prioritisation criteria (hazardous properties, exposure characteristics and the public concern) during the second round of consultation with the 11 involved experts. The median values were considered thereafter as the consensus weighting value to be applied to the adjusted score of each prioritisation criterion.

Fig. 3. Overview of the process to obtain the short list of substances/substance groups in the second HBM4EU prioritisation round.

Table 6
Weighting values assigned to the prioritisation criteria by experts during a second round of consultation as part of an adapted Delphi method and resulting consensus weighting values.

Prioritisation criterion	Weighting values (%) assigned by the experts											Resulting consensus weighting value (median)
	Expert number											
	1	2	3	4	5	6	7	8	9	10	11	
Hazardous properties	30	35	50	40	40	40	30	40	40	50	25	40%
Exposure characteristics	50	45	40	40	40	40	50	40	40	30	50	40%
Public concern	20	20	10	20	20	20	20	20	20	20	25	20%

3.2.2. *Ranking the substances/substance groups according to their priority score (and category)*

The substance's/substance group's scores against the hazard, exposure and public concern criteria that were discussed and approved by the expert panel involved in the scoring and categorisation steps, have been used to calculate the substance's/substance group's overall priority score following the approach described in section 2.2.1. These scores (individual score against each prioritisation criterion and overall priority score) and the category assigned by the experts to each substance/substance groups are available in the substance's revised background documents under https://www.hbm4eu.eu/prioritisation-step-5/. As an example, the scoring and categorisation of acrylamide is also provided in Supplementary Material - Annex 5.

The substances (or selected "lead" substances representing a nominated substance group) from the short list were then ranked according to their overall priority score (Table 7). As several single "lead" substances belonging to wide groups of substances have been scored (e.g. nano titanium dioxide, nano silver and carbon nanotubes were scored within the nominated nanomaterials group), 29 substances were ranked although only 23 substances/substance groups were included in the short list of substances' nominations. The substance's category, as indicator of the type of activity to be undertaken in the project for each substance (either policy-oriented activities as for example derivation of HBM guidance values or rather research activities as development of analytical methods) was specified next to the priority score. An alternative way of ranking the substances consisted in considering the priority score but this time among the substances from a certain category (ranking not shown).

3.3. *Finalisation and approval of the second HBM4EU prioritisation list*

The EU Policy board and the HBM4EU Management Board agreed on a final substances priority list after having engaged discussions on the substance's ranking and on the priorities for action from a policy perspective. The resources available for the project were considered in order to calibrate the number of substances to be included in the final list. This consideration also explains why few substances that had been ranked highly were not included in the final list. As an example, nanomaterials were not included due to the complexity to characterize them and the considerable resources that would be required for the development of analytical methods to measure them in biological matrices.

The second HBM4EU substance priority list, later approved by the HBM4EU Governing Board, is presented in Table 8.

3.4. *Results of the feedback survey and recommendations for improvement of the chemical prioritisation strategy*

In June 2019, a survey for the refinement of the prioritisation strategy was sent in anticipation of the third round of prioritisation under HBM4EU. Detailed information on this survey is available in the Annex 6 of the Supplementary Material. Main suggestions and improvements made are described below and will be taken into account for future rounds of prioritisation.

First, considering the nomination of groups of substances, the question was raised if it had to remain an option, considering the methodological difficulties that have been encountered to score such groups. The majority of respondents were still in favour of keeping this possibility. However, the nomination of a group should be manageable with regard to the resources of the programme. Substances of a given group

Table 7
Ranking of nominated substances or representative substance(s) for a substance group based on their overall priority score, along with the allocated knowledge-level representing category (A to D).

Rank	Substance (substance group)	Hazard score	Exposure score	Public concern score	Overall score[a]	Substance category (A to D)[b]
1	Arsenic (and its inorganic compounds)	27.2	38.0	9.0	74.2	B
2	Lead	25.3	36.0	9.0	70.3	A
3	Acrylamide	27.2	36.8	5.4	69.4	B
4	Aflatoxin B1 (*Mycotoxins*)	30.8	27.2	5.4	63.4	B
5	Chlorpyrifos (*Pesticides*)	13.3	29.2	20.0	62.5	B
6	Dimethoate (*Pesticides*)	12.4	31.2	18.0	61.6	C
7	Pyrethroids (*Pesticides*)	16.0	27.2	18.0	61.2	B
8	Permethrin (*Pesticides*)	14.0	28.0	18.0	60.0	B
9	Mercury (*Mercury & its organic compounds*)	17.2	28.0	10.8	56.0	A
10	DDAC (*Quaternary ammonium salts*)	9.2	32.8	12.8	54.8	C
11	Methylmercury (*Mercury & its organic compounds*)	22.0	23.2	9.0	54.2	B
12	Nano titanium dioxide (*Nanomaterials*)	16.0	26.8	10.8	53.6	D
13	4,4-MDI, 2,4-TDI & 2,6-TDI (*Diisocyanates*)	18.0	28.0	7.2	53.2	C
14	Glyphosate (*Pesticide*)	7.2	32.0	12.8	52.0	B
15	Deoxynivalenol (DON) (*Mycotoxins*)	18.0	28.0	5.4	51.4	C
16	BP-3 (*UV filters-Benzophenones*)	12.8	29.2	9.0	51.0	B
17	D4 (*Cyclic Siloxanes*)	5.6	33.2	11.0	49.8	C
18	N,N-dimethylformamide (DMF) (*Reprotoxic aprotic solvents*)	16.0	30.0	3.6	49.6	B
19	Nano silver (*Nanomaterials*)	14.0	26.0	9.0	49.0	D
20	BHT (2,6-di-tert-butyl-p-cresol)	14.0	32.8	1.8	48.6	C
21	Fumonisin B1 (*Mycotoxins*)	18.0	24.0	5.4	47.4	C
22	Fipronil (*Pesticide*)	16.8	25.2	3.6	45.6	C
23	Perchlorate	13.2	30.0	1.8	45.0	C
24	1-methyl-2-pyrrolidone (NMP) (*Reprotoxic aprotic solvents*)	12.0	27.2	3.6	42.8	B
25	UV-328 (*Phenolic benzotriazoles*)	12.0	27.2	3.6	41.0	C
26	Carbon nanotube (*Nanomaterials*)	12.8	18.8	9.0	40.6	D
27	BENPAT (*Substituted phenylenediamines*)	15.2	24.8	0	40.0	D
28	POE-tallow amine	12.0	20.0	3.6	35.6	C
29	N,N-diethyl-m-toluamide (*Pesticides*)	7.2	25.2	0.0	32.4	C

[a] The overall priority score was obtained by adding the weighted prioritisation criteria scores.

[b] An A to D category was allocated to the substance considering its knowledge level as informed in the revised background document and according to the definitions of the categories.

Table 8
Approved second HBM4EU list of priority substances.

No	Single substance or group of substances	Substance(s) considered for the scoring	Overall priority score	Substance Category
1	Lead (and its compounds)	Lead	70.3	A
2	Mercury (and its organic compounds)	Mercury	56.0	A
		Methylmercury	54.2	B
3	Arsenic inorganic compounds	Inorganic arsenic compounds, including diarsenic trioxide	74.2	B
4	Acrylamide	Acrylamide	69.4	B
5	Mycotoxins	Aflatoxin B1	63.4	B
		Deoxynivalenol	51.4	C
		Fumonisin B1	47.4	C
6	Pesticides	Chlorpyrifos	62.5	B
		Dimethoate	61.6	C
		Pyrethroids	61.2	B
		Permethrin	60.0	B
		Glyphosate	52.0	B
		Fipronil	45.6	C
7	UV filters - Benzophenones	Benzophenone-3	51.0	B
8	Aprotic solvents	N,N-dimethylformamide	49.6	B
		1-methyl-2-pyrrolidone	42.8	B
9	Diisocyanates	4,4-MDI, 2,4-TDI & 2,6-TDI	53.2	C

may have structural similarities or the same analytical methods, offering the possibility for work packages to include a set of substances (i.e. a group) in their programmes in a rational way. The online survey for nominating substances in the second prioritisation round suggested that a possible rationale for including several substances in a single group may be based on a similar toxicological profile or similar uses. However, a rationale for grouping substances based on similar uses can possibly lead to the nomination of a wide group of substances of very different types, making it difficult to work on all of them within the project. Therefore, more relevant rationales for grouping substances, e.g. common analytical methods is recommended (e.g. all species of inorganic arsenic such as arsine, arsenate, dimethylarsinic acid (DMA), methylarsonic acid (MMA) may all be measured by a single speciation method).

Secondly, regarding the scoring method for groups of substances, a legitimate discussion was raised on the scoring of a "representative" lead substance of the group. An alternative approach consists in considering a "worst-case scoring", performed on the most harmful characteristics identified for substances within the group. Because there may be significant differences across substances within a group, due for example to the heterogeneity in data availability, the most cautious approach should always prevail when considering risk assessment. This enhances the need of a robust and well-justified rationale behind the nominations of groups, as mentioned above.

Thirdly, the likelihood that a specific substance is part of a mixture of daily life exposure could additionally be taken into account as a parameter to score the exposure criterion.

Finally, regarding the overall nomination process, interesting suggestions have been made to improve its efficiency. Indeed, each entity nominating substances had to complete the entire online survey for each substance/substance groups (with an upper limit of 5 nominations). The survey is a time-consuming procedure that had to be completed even if the nominated substance may not be selected in the final prioritisation process. It was therefore suggested that each nominating entity first

provides initial expression of interest. Then, a short list of substances would be set on the basis of the interest of the nominating entities and shared with them, to allow coordinating efforts on providing the relevant documentation for a substance or group of this list.

4. Discussion

It is the first time that a pan-European HBM project has been implemented, with currently 30 countries being part of the initiative already (North Macedonia and Estonia joined the initiative in 2020). The prioritisation process had to propose a method allowing the selection of substances of common priority across participating countries, the EC and a range of stakeholders, but also to determine the research efforts needed at national levels.

4.1. Comparison with other chemical prioritisation process

The HBM4EU prioritisation process can be compared to other substance prioritisation activities at the EU level, for example the EU Directive 2000/60/EC, commonly known as the EU Water Framework Directive (WFD) (Daginnus et al., 2011). This was also a policy-oriented prioritisation process for monitoring chemicals in water bodies. The process for the WFD was finalised with Decision 2455/2001/EC on the first list of priority substances and is revised and updated every 6 years. Under the WFD, priority substances are identified based on a two-year consultation process for which a combined monitoring-based and modelling-based priority-setting procedure has been developed. Background documents are produced for the priority substances as well as a procedure for the identification of priority hazardous substances. An expert advisory group, consisting of experts from Member States, European Free Trade Association (EFTA) countries, the Scientific Committee on Toxicity, Ecotoxicity and the Environment (SCTEE), the European Chemicals Bureau and stakeholders from industry, water suppliers and environmental groups, are consulted. In the WFD, substances are prioritised taking into account (Bodar et al., 2003): 1) risk assessments carried out under existing chemically relevant EU Directives and Regulations, e.g. ECHA and the European Food Safety Authority (EFSA) (Bodar et al., 2003; ECHA 2016; EFSA 2009; EU Regulation No 528/2012); 2) targeted risk-based assessments focusing on aquatic ecotoxicity and human toxicity *via* the aquatic environment; 3) simplified risk-based assessments based on intrinsic hazards, widespread environmental contamination, production volumes and use patterns. The priority list of substances under the WFD focuses only on single substances (Daginnus et al., 2011; Faust et al., 2019; INERIS 2009).

Similar to the WFD, the time for implementation of the proposed HBM4EU prioritisation process is quite long (approximately 1 year). The HBM4EU prioritisation process also includes consulting an expert group, as well as with partners participating in the project, i.e. policy makers, scientists and stakeholders who nominated substances to be included for monitoring and research activities within the project. This approach aims to ensure the legitimacy, credibility and societal relevance of the process. Within HBM4EU, a stakeholder workshop was also conducted to address public concerns, a step that was not included in the WFD priority-setting process.

In contrast to the WFD, the HBM4EU priority-setting process did not include any type of risk assessment studies, nor did it focus only on single substances, cf. group of priority substances on mixtures, emerging chemicals, flame retardants, PAHs and pesticides.

In Europe, other national biomonitoring programmes are also known to have a priority strategy for substances that share some similarities with that of HBM4EU.

In France, the National Nutrition and Health Survey (ENNS) was conducted in 2006–2007 to meet the objectives on biomonitoring, chronic disease surveillance and nutritional surveillance. More recently, the Grenelle I Act (No, 2009–967 of August 3, 2009) led to the development of a French National Biomonitoring programme, in which two distinct studies were designed: 1) the Health Study on Environment, Biomonitoring, Physical Activity and Nutrition (called "Esteban"), which is a nationwide cross-sectional survey of the mainland population aged 6–74 years and 2) the ELFE cohort (Longitudinal Study from Childhood) constituting the perinatal component of the French National Biomonitoring programme (Balicco et al., 2017; Dereumeaux et al., 2016; Fillol et al., 2014; Fréry et al., 2012). The prioritisation process relies on members of government agencies to validate an initial list of pollutants and on a group of French-speaking and international HBM experts to establish the selection criteria, to rate the chemicals using a graded score and to review, validate and establish a provisional final list. The final list is reviewed, revised and recommended by an "emerging risk" group of the National Environmental Health Plan (PNSE).

In Belgium, Flanders has established the Flemish Environment and Health Study (FLEHS) (Schoeters et al. 2012, 2017). The prioritisation of chemicals was based on international lists and expert advice using weighted scoring, followed by a step-by-step procedure implemented to first categorise criteria and then select and score the chemicals. Scientific experts and the strategic advisory board for the Ministry of Environment, Health and Energy and the Socio-economic Board (representatives of employers and employees) were asked to make recommendations.

Germany has one of the longest running HBM programmes in Europe. The German Environmental Survey (GerES) has been running since 1985, and its prioritisation strategy is based on existing international lists and further information on hazardous chemicals and the degree of exposure of the general public in Germany and expert judgments (government authorities, industry and science sectors) (Kolossa-Gehring et al. 2012b, 2017; Schulz et al. 2007, 2017). The selection of chemicals for GerES also focuses on a cooperation project between the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) and the German Chemical Industry Association (VCI) to select new substances and to develop new analytical HBM methods.

In Canada, the Canadian Health Measures Survey (CHMS) prioritisation approach is based on expert advice (workshop of experts), stakeholder consultations *via* questionnaires, and the Health Canada regulatory programme needs mandated by the Chemicals Management Plan. The process was adapted and adjusted during each of the first three cycles of the CHMS (Haines et al., 2017).

In the US, the main HBM programme is NHANES, running since the 1970s (CDC 2009, 2019). It comprises a participatory approach led by the Center for Disease Control (CDC) *via* notices in the Federal Register to establish criteria for inclusion or removal of chemicals and for the nomination of chemicals to measure in the biomonitoring program as part of NHANES. An expert panel of outside reviewers and CDC scientists scores nominated individual chemicals or categories of chemicals using weighted criteria to categorise these into five priority groups. Another recent initiative is the Environmental Influences on Child Health Outcomes (ECHO) by the US National Institutes of Health. With multiple cohorts of participating children, it will take into account longitudinal studies to investigate environmental exposures (including chemicals) on child health and development. It will investigate the exposure to about 200 chemicals already present in NHANES as well as new chemicals. ECHO's prioritisation strategy is based on database and literature research for chemicals in environmental media and in consumer products that are potentially toxic, and that have not been measured in NHANES (Pellizzari et al., 2019).

4.2. Specificity and limitation of the HBM4EU chemical prioritisation process

With different types of approaches for each country and HBM programme, there are some commonalities that were taken into account and adapted to the objectives of HBM4EU. The aim of the HBM4EU initiative is not only to measure the internal exposure to well-known

substances in the European population, but also to focus on human exposure to lesser known or emerging substances for which analytical methods are not yet available, and toxicological and exposure data is currently insufficient.

As an H2020 project, HBM4EU addresses societal challenges to health and well-being for European citizens. It is a main objective of the project to bridge the divide between science and policy at the European level and to generate results that meet the knowledge needs of EU policy makers. Priority was therefore given to the nomination of substances by members of the EU Policy Board, with the aim of delivering on this key objective. Input from the National Hubs was also highly valued and helped to ensure that the project also serves the knowledge needs of national policy makers and to determine whether national and European level priorities are aligned. Selected substances are the subject of research at European level. It is therefore important that HBM4EU addresses knowledge gaps on chemical exposure and resulting health impacts that have relevance at the European level and that can generate results that benefit the pan-European population. Substances that are exclusively of local or national concern were therefore not considered. Input from the Stakeholder Forum made it possible to assess the social relevance of research activities on nominated substances and drew in additional evidence and knowledge. As such, the strategy for the prioritisation of substances was not based entirely on scientific evidence. It was also guided by an imperative to produce knowledge in support of policy making at European level.

A literature review was carried out to discern and adapt the prioritisation strategy from international experiences. The prioritisation criteria that were selected and used for the scoring and the whole prioritisation process needed to be streamlined, also including time restrictions. During the prioritisation process, substances were allocated to categories reflecting the availability of existing data on hazard and exposure. The substance's regulatory status and availability of analytical methods to perform HBM were also taken into account. The aim was to ensure that HBM4EU focused not just on well-known and -studied substances, but also covered ones with limited data. Indeed, the particularity of this project in comparison to other biomonitoring programmes is that it includes also research activities dedicated, for example, to the identification of biomarkers of effect or the development of analytical methods. Therefore also, when data gaps have been identified for certain hazards or exposure parameters during the scoring step, a Low score was attributed instead of a null score to avoid "disadvantaging" lesser-known substances during the ranking of the substances. This special attention to have lesser-known substances included in the second priority list has the merit to constitute an anticipatory approach by generating knowledge on data poor substances.

Despite efforts made to implement an objective and systematic scoring method, expert judgment was inevitable to score some parameters, in particular for data-poor substances. For example, the CLP classification was considered for scoring some endpoints (i.e. an objective score relying on solid evidence), but where no official classification has been proposed, then evidence identified in the peer-reviewed literature were considered. In this case, the expert's judgement has a more prominent influence on the given score. Nevertheless, each given score was discussed with a panel of experts from a variety of fields of expertise, in order to reduce the subjectivity in the scoring. The evaluation of societal concern can also be liable to some subjectivity. Scoring this criterion relied mainly on the stakeholder's reflections and information about the substances captured during a stakeholder workshop (including whether NGO campaigns related to the substances were recently conducted), but also on lists established by diverse stakeholders. In this consideration however, the weight given under the prioritisation process to the scoring of societal concern was lower (20%) than the ones given to the scoring of hazards and exposure characteristics (40% each). However, to help define this societal concern, it may worth better considering differences in terms of occupational health aspects versus the general population or environmental aspects. Specific questions on such aspects can be asked before prioritisation in the survey used by the entities to nominate substances, to provide further arguments in support of the nomination of substances or groups of substances. Finally, every step of the process, justifications of the choices made and results are fully documented on the HBM4EU webpages, in order to account for the choices made in a completely transparent way.

4.3. Reflections for a further refined chemical prioritisation process

Steps for a further refined prioritisation strategy are presented below:

1) Pre-nomination step: the EU Policy Board, the Stakeholder Forum as well as the National Hubs provide an initial expression of interest for up to 5 substances or groups of substances together with a rationale for nominating them.
2) Long list of nominated substances/groups: this list will be publicly available and will collate all the nominated substances/groups of substances and the nominating parties who nominated them.
3) Short list of nominated substances: based on the long list of nominated substances/groups of substances, partners involved in the prioritisation process will produce a short list of approximately 25 substances/groups, ranked according to the number of times they were nominated and to who nominated them (i.e. following the same criteria used during the second round of prioritisation, as mentioned above).
4) Compiling data: after producing and sharing the short list, nominating parties will have 2 months to complete the online survey requesting information related to the prioritisation criteria. Based on the information provided in the survey on each nominated substances/groups of substances, background documents will be produced within 3 months. In the meantime, a Stakeholder Forum workshop will be organised to evaluate the societal concern of the substances/groups of substances on the short list.
5) Scoring and ranking process: the substances/groups of substances of the short list will be scored and categorised by experts, on the basis of the information gathered in the background documents, allowing for the elaboration of a ranked list of prioritised substances.

Before the end of the HBM4EU project, a third round of prioritisation will be run based on the feedback and suggestions for improvement presented in this paper. This work will then benefit any follow-up HBM initiative that takes place. Currently, discussions are ongoing for the set-up of a partnership under Horizon Europe, the European Partnership for the Assessment of Risks from Chemicals (PARC).

5. Conclusion

To prioritise chemicals for inclusion in a European-wide HBM initiative, a structured and transparent process was developed using a participatory approach. Prioritisation must reflect the selection of chemicals of interest considering the diversity of needs for such initiative, as also the policy needs from the participating countries and agencies and the current concerns of European citizens. The prioritisation strategy for substances and substance groups developed in HBM4EU served to guide biomarker selection for the biomonitoring studies initiated in HBM4EU and the associated research to improve interpretation of biomarker data in terms of health risks and exposure sources. The process was considered to be transparent and science-based, as shown by the feedback that was obtained.

The strategy for the second round of prioritisation of HBM4EU priority substances was implemented over a one-year period (from July 2017 to June 2018). The list of prioritised substances and their respective selected CGLs was approved by the HBM4EU Governing Board composed of the programme sponsors in the participating countries, the European Chemicals Agency (ECHA), the EEA and EFSA in July 2018,

after which research activities could start. The second list included acrylamide, aprotic solvents, arsenic, diisocyanates, lead, mercury, mycotoxins, pesticides (including chlorpyrifos, dimethoate, pyrethroids, glyphosate and polyethoxylated (POE)-tallow amine, and fipronil), and a type of UV filters (benzophenones).

To further maintain harmonised and comparable results of prioritisation processes, we recommend that any future HBM project take this prioritisation process into account. The prioritisation strategy developed under HBM4EU, as well as the third list of priority substances, can be used in any follow-up HBM initiative.

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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.2021.113778.

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Combined effects of chronic $PM_{2.5}$ exposure and habitual exercise on renal function and chronic kidney disease: A longitudinal cohort study

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ABSTRACT

Background: We investigated the combined effects of chronic $PM_{2.5}$ exposure and habitual exercise on the decline of renal function and the incidence of chronic kidney disease (CKD) in a large cohort in Taiwan.

Methods: The present data analysis included a total of 108,615 participants aged 18 years or above who were recruited between 2001 and 2016. All participants underwent at least two medical examinations. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation. The incident of eGFR decline ≥30% was defined as a decline in eGFR of ≥30% during the study period, while the incident CKD was defined as an eGFR <60 mL/min/1.73 m^2 or a newly self-reported physician-diagnosed CKD in the subsequent visits. The satellite-based spatiotemporal model was used to estimate $PM_{2.5}$ exposure at each participant's address. Information on habitual exercise was collected using a standard self-administered questionnaire. The Cox regression model with time-dependent covariates was used for data analyses.

Results: Higher habitual exercise was associated with lower risks of renal function decline and CKD development, whereas higher $PM_{2.5}$ exposure was associated with higher risks of renal function decline and CKD development. We found no significant interaction effect between $PM_{2.5}$ and habitual exercise, with an HR (95% CI) of 1.02 (0.97, 1.07) for incident eGFR decline ≥30% and 1.00 (0.95, 1.05) for CKD development. Compared to participants with inactive-exercise and high-$PM_{2.5}$, participants with high-exercise and low-$PM_{2.5}$ had 74% and 61% lower risks of renal function decline and CKD development, respectively.

Conclusion: Increased habitual exercise and reduced $PM_{2.5}$ exposures are associated with lower risks of renal function decline and CKD development. Habitual exercise reduces risks of renal function decline and CKD development regardless of the levels of chronic $PM_{2.5}$ exposure. Our study suggests that habitual exercise is a safe approach for kidney health improvement even for people residing in relatively polluted areas and should be promoted.

1. Introduction

Chronic kidney disease (CKD) is a global public health challenge. In 2017, there were 697 million CKD patients worldwide, increasing by 27% over past 10 years (James et al., 2018). CKD contributed to 1.2 million deaths and was ranked as the 12th leading cause of global death in 2017 (Roth et al., 2018). The most severe stage of CKD, end-stage renal disease, requires costly dialysis or transplant, seriously affects patients' quality of life, and results in an enormous economic burden.

Regular exercise may improve kidney function and reduce the risk of

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CKD progression (Castaneda et al., 2001; Guo et al., 2020b; Jafar et al., 2015; Robinson-Cohen et al., 2009, 2014). The World Health Organization (WHO) recommends an adult to undertake at least 150 min of moderate-intensity physical activity per week to prevent non-communicable diseases (WHO, 2010). However, exercise may increase the inhalation of air pollutants due to higher ventilation. Air pollution has been shown as a novel risk of reduced renal function and CKD development by increasing evidences (Bowe et al., 2017, 2018; Chan et al., 2018; Mehta et al., 2016). Thus, there is an emerging public concern whether the increased intake of air pollutants due to exercise may exacerbate the adverse health effects on kidney health.

There are more than 91% of world population lives in a place where air quality does not meet the WHO guideline (WHO, 2016). The risk-benefit relationship between air pollution and exercise needs to be addressed urgently to inform people whether it is safe to perform habitual exercise in polluted regions. Few studies have investigated the combined effects of habitual exercise and air pollution exposure on hypertension, respiratory diseases, and mortality (Andersen et al., 2015; Fisher et al., 2016; Guo et al., 2020a, 2020c; Kubesch et al., 2018; McConnell et al., 2002; Sun et al., 2020). There is little information on the combined effects on kidney health so far. We previously investigated the association between chronic exposure to $PM_{2.5}$ and incident CKD using a large longitudinal cohort. We observed that every 10 $\mu g/m^3$ increase in $PM_{2.5}$ was associated a higher risk of 6% in CKD development in that study [Hazard Ratio (HR): 1.06; 95% confident interval(CI): 1.02, 1.10] (Chan et al., 2018). We have also investigated the association between habitual exercise and incident CKD using the same cohort and our results show habitual exercise was associated with a lower risk of CKD and impaired renal function (Guo et al., 2020b). We therefore extended our previous research to investigate the combined effects of habitual exercise and chronic $PM_{2.5}$ exposure on reduced renal function and development of CKD based on the same longitudinal cohort in Taiwan, where the annual $PM_{2.5}$ concentration was 2.6 times of the limit recommended by the WHO (WHO, 2006).

2. Methods

2.1. Study design and participants

The present study was based on an ongoing longitudinal cohort in Taiwan. Details of the cohort have been described in our previous publications (Guo et al., 2020a; Lao et al., 2019b). In brief, a private firm, the MJ Health Management Institution, has provided a standard medical screening program for Taiwan residents since 1994 (Chang et al., 2016). Residents who joined the program were encouraged to visit clinics on a yearly basis and to receive a series of standard medical examinations and to complete an extensive self-administrated questionnaire. Between 1996 and 2016, around 0.60 million Taiwan residents were recruited in the program and 44% of them had at least two medical visits. Each participant was required to sign an informed consent form prior to each medical examination authorizing the institution to release the data for research purpose. Ethical approval for this study has been obtained from the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee. Details of participant selection were described in supplementary file (Online Resource 1 & 2). Finally, a total of 108,615 participants and 104,092 participants with a follow-up period of ≥3 years were included in analysis to investigate the combined effect on incidence of eGFR decline ≥30% and incidence of CKD, respectively.

2.2. Air pollution exposure assessment

The details of estimating $PM_{2.5}$ exposure have been published elsewhere (Lao et al., 2019b; Li et al., 2005; Lin et al., 2015; Zhang et al., 2017). Briefly, We developed a satellite-based spatiotemporal model based on the aerosol optical depth (AOD) data at a resolution of 1 km^2 (Li et al., 2005; Lin et al., 2015). The AOD data was derived from the Moderate Resolution Imaging Spectroradiometer (MODIS) carried on Terra and Aqua satellites of the National Aeronautics and Space Administrations (NASA) (Li et al., 2005; Lin et al., 2015). The model was validated by comparing the estimated $PM_{2.5}$ exposure with data from more than 70 monitoring stations across Taiwan. The correlation coefficients for yearly average concentration ranged from 0.72 to 0.83 (Zhang et al., 2017).

The address of each participant was geocoded into latitude and longitude data and the estimated $PM_{2.5}$ exposure was matched with the individual address. The 2-year average of $PM_{2.5}$ concentrations was used as a proxy of chronic exposure, which corresponds to the average of yearly $PM_{2.5}$ concentrations of the calendar year of medical examination and the previous year. Both the continuous (per 10 $\mu g/m^3$) and category (participants were grouped into three categories based on the tertile cut-off points of $PM_{2.5}$, i.e. low: ≤22.40, moderate: 22.40–26.0, and high: >26.01 $\mu g/m^3$) of $PM_{2.5}$ were used for data analysis.

2.3. Assessment of habitual exercise

Details of assessing habitual exercise have been described elsewhere (Guo et al., 2020a, 2020b; Lao et al., 2019a; Wen et al., 2011; Zhang et al., 2018b). First, a standard self-administrated questionnaire was used to collect information on weekly exercise that participants generally engaged during the month before medical examination. Weekly exercise was classified into four intensity categories with examples provided under each category: light (e.g. walking), moderate (e.g. brisk walking), medium-vigorous (e.g. jogging), and high-vigorous (e.g. rope skipping). A standard metabolic equivalent of task (MET; 1 MET = 1 kcal/kg/hour) value was assigned to four intensity categories: 2.5 (light), 4.5 (moderate), 6.5 (medium-vigorous), and 8.5 (high-vigorous), respectively(Ainsworth et al., 2000; Wen et al., 2011). If participants reported activities in more than one category, a weighted MET was calculated based on time spent in each category. Afterwards, the weekly exercise volume (MET-h) of each participant was calculated as the product of intensity (MET) and duration (hours) of exercise. Participants were then classified into three exercise groups based on the tertile cut-off point of exercise-volume (MET-h): inactive (0 MET-hour), moderate (0–8.75 MET-hour), and high (>8.75 MET-hour) for data analysis. We did not use the continuous MET-h for data analysis because 0 MET-h was assigned to all participants in inactive group.

2.4. Outcome ascertainment

Glomerular filtration rate (GFR) is regarded as the best overall index of kidney function (KDIGO, 2013), while estimated glomerular filtration rate (eGFR) is widely used as an alternative for GFR in research (KDIGO, 2013). We used the following two health outcomes based on eGFR in this study.

- Incidence of eGFR decline ≥30%: in the cohort of 108,615 participants with 468,154 medical examination records, a participant was defined as an incident of eGFR decline ≥30% if s/he has a decline of eGFR ≥30% during the study period. The decline of eGFR was calculated using the formula: $\frac{(baseline\ eGFR - follow\text{-}up\ eGFR)}{baseline\ eGFR} \times 100\%$. We used the cutoff point of ≥30% for decline in eGFR because it was reported that an eGFR decline ≥30% was more strongly associated with the risk of end-stage renal disease (ESRD), an end point of CKD progression (Coresh et al., 2014). It is also widely used as a parameter of renal function outcomes in previous studies (Bowe et al., 2017, 2018).
- Incidence of CKD: in the cohort of 104,092 participants with 446,119 medical examination records, a participant was defined as an incident CKD if s/he had an eGFR <60 mL/min/1.73 m^2 or self-reported physician-diagnosed CKD in the subsequent visits. eGFR <60 mL/

min/1.73 m^2 was widely used as the diagnosing criteria of CKD in many studies and guidelines (Bowe et al., 2017; 2018; Chan et al., 2018; KDIGO, 2013).

The end point of the health outcome of CKD was defined as the first occurrence of CKD or the final visit if CKD did not occur over the study period. Similarly, the endpoint of eGFR decline ≥30% was defined as the first occurrence of eGFR decline ≥30% or the final visit if eGFR decline ≥30% did not occur constantly over the study period.

To calculate eGFR, overnight fasting venous blood samples were drawn in the morning and serum creatinine was analyzed through uncompensated Jaffe method containing an alkaline picrate kinetic test on a HITACHI 7150 (before 2005) or a TOSHIBA C8000 (after 2005) analyzer. Based on serum creatinine level, age, and gender, eGFR was calculated using the following Modification of Diet in Renal Disease (MDRD) equation:

$$186.3 \times (serum\ creatinine)^{-1.154} \times age^{-0.203} \times (0.742\ for\ women)$$

where serum creatinine is in mg/dL.

2.5. Covariates

Details about the procedure of medical examination and quality-control measures have been described in previous studies (Chan et al., 2018; Chang et al., 2016; Guo et al., 2020b; Zhang et al., 2018a). Weight and height were measured by an auto-anthropometer (KN-5000A, Nakamura) with participants wearing light clothes. Body mass index (BMI) was calculated as the weight (kg) divided by the square of the height(m). Seated blood pressure including systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by a computerized auto-mercury sphygmomanometer (CH-5000, Citizen). Overnight fasting venous blood samples were drawn to measure glucose and lipids (total cholesterol (TC), triglycerides (TG), high lipoprotein cholesterol (HDL-C), and low lipoprotein cholesterol (LDL-C)) on an automated biochemical analyzer (HITACHI 7150 before 2005 or TOSHIBA C8000 since 2005). Urinary total protein was analyzed using a ROCHE Miditron or ROCHE Cobas U411semi-automated computer-assisted urinalysis system.

Besides, a standard self-administered questionnaire was used to collect information on the participants' demographic characteristics, behavioral and lifestyle factors, and medical history.

Based on previous literature, we included the following covariates in the present study: age (years), gender (male or female), education levels (lower than high school, high school, college or university, or post-graduate), smoking status (never, ever[smoked at least once but quit later], or current[more than once a week]), alcohol drinking (seldom [<once/week], occasional[1–3 times/week], or regular[>3 times/week]), occupational exposure to dust or solvent in the workplace (yes or no), physical labor at work (sedentary jobs [e.g. clerk], jobs that require approximately half sedentary and half standing/walking [e.g. nurse], jobs that mostly require walking and standing [e.g. retail salesperson], or jobs that require vigorous physical activity[e.g. porter]), BMI (kg/m2), diabetes (defined as fasting blood glucose ≥126 mg/dL or self-reported physician-diagnosed diabetes), hypertension (defined as an SBP ≥140 mm Hg or a DBP ≥90 mm Hg, or self-reported physician-diagnosed hypertension), dyslipidemia (defined as TC ≥ 240 mg/L, TG ≥ 200 mg/dL, HDL- C < 40 mg/dL, or LDL-C ≥160 mg/dL), self-reported CVD or stroke (yes or no) and any self-reported form of cancer (yes or no), baseline eGFR, urinary total protein (negative[<0.1 g/L], trace [0.1~0.2 g/L], 1 plus [0.2~1.0 g/L], 2 plus [1.0~2.0 g/L], 3 plus [2.0~4.0 g/L], and 4 plus [>4.0 g/L]), season (spring, summer, fall, or winter) and calendar year of baseline visit.

2.6. Statistical analysis

Cox regression models with time-dependent covariates was used to investigate the effects of $PM_{2.5}$ or exercise on incidences of eGFR decline ≥30% and CKD, respectively, with a city-level random intercept added to account for within-city clustering. The time-scale used in the Cox regression model was time-in-study (i.e. follow-up time). The $PM_{2.5}$ concentration, habitual exercise and all covariates were treated as time-dependent variables in the models except for gender, baseline eGFR, and baseline calendar year. Three statistical models were developed with gradual addition of aforementioned covariates: Crude Model: without adjustment; Model 1: adjusted for age, gender, education, season, baseline calendar year, smoking status, alcohol drinking, occupational exposure, and physical labor at work; Model 2 further adjusted for BMI, diabetes, hypertension, dyslipidemia, self-reported cardiovascular disease, self-reported cancer, baseline eGFR, and urinary total protein. A trend test was performed across exercise and $PM_{2.5}$ categories. Hazard ratios (HRs) with 95% confident interval (CI) were presented with the inactive-exercise group or the low-$PM_{2.5}$ as the reference. Mutual adjustments for exercise and $PM_{2.5}$ were performed for comparison (i.e. we further included exercise in the model for assessing main effect of $PM_{2.5}$ or $PM_{2.5}$ in the model for assessing the main effect of exercise). We subsequently evaluated the potential interaction effect of $PM_{2.5}$ and exercise by adding a product term of "continuous $PM_{2.5}$(every 10 $\mu g/m^3$) × category-exercise" into the fully adjusted model.

We then performed subgroup analyses stratified by $PM_{2.5}$ or exercise categories, separately, to assess the effects of habitual exercise or $PM_{2.5}$ in each stratum. To examine the combined effects of $PM_{2.5}$ and exercise, participants were classified into nine groups based on their $PM_{2.5}$ and exercise categories, and those with inactive-exercise and high-$PM_{2.5}$ were served as the reference group.

A series of sensitivity analyses were performed. 1) We excluded participants with baseline diabetes, cardiovascular diseases, or cancer to eliminate the potential comorbidity effects (for the outcome eGFR decline ≥30%, participants with baseline CKD or those reported physician-diagnosed kidney disease were also excluded); 2) We used the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (KDIGO, 2013) to calculate eGFR for comparison; 3) We used annual average $PM_{2.5}$ concentration at the previous year of medical examination to examine the stability of $PM_{2.5}$ effects; 4) We restricted the analysis within the elderly aged ≥65 years to examine whether the combined effects of $PM_{2.5}$ and exercise on renal function or CKD were different in the elderly; 5) We used the inverse probability weight method to control for potential bias caused by missing values.

Statistical analyses were performed using R 3.2.5 (R Core Team, Vienna, Austria). A two-tailed P value < 0.05 defined the statistical significance.

3. Results

A total of 108,615 participants with 468,154 observations were included in the study to investigate the combined effects of habitual PA and $PM_{2.5}$ on the incidence of eGFR decline≥30%. Around 76.2% of the participants underwent more than two medical examinations with a median number of 4 (ranged from 2 to 26). The median interval of examinations was 17 months (IQR, 12–29 months). We identified 4,825 participants whose eGFR declined more than 30% during the study period. The mean follow-up duration was 6.7 years (standard deviation (SD: 3.2)).

A total of 104,092 participants without CKD at baseline were included in the analysis to investigate the combined effects of habitual exercise and $PM_{2.5}$ on the incidence of CKD. Similarly, around 76.2% of the participants underwent more than two medical examinations with a median number of 4 (ranged from 2 to 26). The median interval of examinations was 17 months (IQR, 12–29 months). We identified 4,850 incident cases of CKD. The mean follow-up duration was 6.7 years (SD:

3.2).

The general characteristics of participants at baseline and all observations are summarized in Table 1. Slightly more than half of participants were males. The majority had higher education level, never smoked, and seldom drank. More than 60% participants had a mostly sedentary job. Fig. 1 shows the temporal and spatial distribution of $PM_{2.5}$ concentrations by year. $PM_{2.5}$ concentration reached the peak in 2004 and declined gradually in the following years. The majority of participants lived in the western parts of Taiwan.

Table 2 shows the main effects of habitual exercise and $PM_{2.5}$ on the incidences of eGFR decline ≥30% and CKD respectively. Participants who undertook a moderate/high volume of exercise were associated with a lower risk of incidences of eGFR decline ≥30% and CKD. However, exposure to a moderate/high level of $PM_{2.5}$ was associated with a higher risk of incident eGFR decline ≥30% and CKD. All estimates remained stable to additional adjustment for $PM_{2.5}$ or exercise. Significant trends for associations were shown across exercise or $PM_{2.5}$ levels. Besides, Overall interactions between habitual exercise and ambient $PM_{2.5}$ were not statistically significant with an HR of 1.02 (0.97, 1.07) for the incidence of eGFR decline ≥30% and 1.00 (95% CI: 0.95, 1.05) for the incidence of CKD.

Beneficial effects of exercise were observed in subgroup analysis stratified by $PM_{2.5}$ categories. In contrast, harmful effects of $PM_{2.5}$ exposure were observed in each exercise stratum (Table 3). The

Table 1
Characteristics of the participants.

Characteristic	Incidence of eGFR decline ≥30%				Incidence of CKD			
	Baseline [a] (n = 108,615)		All visits [b] (n = 468,154)		Baseline [c] (n = 104,092)		All visits [d] (n = 446,119)	
Age (year)	39.1	(11.8)	43.4	(12.0)	38.4	(11.3)	42.5	(11.4)
Male (n, %)	56,600	(52.1)	255,743	(54.6)	54,017	(51.9)	241,194	(54.1)
Education (n, %)								
Lower than high school	14,672	(13.5)	59,105	(12.6)	12,910	(12.4)	50,535	(11.3)
High school	22,046	(20.3)	88,588	(18.9)	21,218	(20.4)	84,987	(19.1)
College or university	59,335	(54.6)	255,569	(54.6)	57,732	(55.5)	247,730	(55.5)
Postgraduate	12,562	(11.6)	64,892	(13.9)	12,232	(11.8)	62,867	(14.1)
Smoking status (n, %)								
Never	81,389	(74.9)	354,265	(75.7)	78,127	(75.1)	338,050	(75.8)
Former	5,995	(5.5)	30,264	(6.5)	5,578	(5.4)	27,927	(6.3)
Current	21,231	(19.6)	83,625	(17.9)	20,387	(19.6)	80,142	(18.0)
Alcohol consumption (n, %)								
Seldom	93,428	(86.0)	397,059	(84.8)	89,661	(86.1)	378,774	(84.9)
Occasional	10,436	(9.6)	48,457	(10.4)	9,944	(9.6)	46,019	(10.3)
Regular	4,751	(4.4)	22,638	(4.8)	4,487	(4.3)	21,326	(4.8)
Physical labor at work (n, %)								
Sedentary jobs	69,454	(64.0)	316,238	(67.6)	66,522	(63.9)	300,656	(67.4)
Jobs that require approximately half sedentary and half standing/walking	28,667	(26.4)	113,040	(24.2)	27,513	(26.4)	108,301	(24.3)
Jobs that mostly require walking and standing	8,562	(7.9)	32,240	(6.9)	8,195	(7.9)	30,782	(6.9)
Jobs that require vigorous physical activity	1,932	(1.8)	6,636	(1.4)	1,862	(1.8)	6,380	(1.4)
Habitual exercise (n, %)								
Inactive (0 MET-h)	49,486	(45.6)	160,613	(34.3)	47,951	(46.1)	155,718	(34.9)
Moderate (≤8.75 MET-h)	32,600	(30.0)	152,539	(32.6)	31,307	(30.1)	146,468	(32.8)
High (>8.75 MET-h)	26,529	(24.4)	155,002	(33.1)	24,834	(23.9)	143,933	(32.3)
Occupational exposure (n, %) [e]	9,127	(8.4)	36,581	(7.8)	8,806	(8.5)	35,342	(7.9)
BMI (kg/m^2)	23.0	(3.5)	23.3	(3.5)	22.9	(3.5)	23.3	(3.5)
Urinary total protein (n, %)								
negative or normal (<0.1 g/L)	103,422	(95.2)	451,082	(96.4)	99,484	(95.6)	431,548	(96.7)
trace (0.1–0.2 g/L)	4,423	(4.1)	13,241	(2.8)	4,058	(3.9)	11,905	(2.7)
1 plus (0.2–1.0 g/L)	583	(0.5)	2,729	(0.6)	429	(0.4)	1,945	(0.4)
2 plus (1.0–2.0 g/L)	187	(0.2)	886	(0.2)	121	(0.1)	592	(0.1)
3 plus (>2.0 g/L)			216	(0.05)			129	(0.03)
Diabetes(n, %)	3,689	(3.4)	22,622	(4.8)	3,288	(3.2)	20,066	(4.5)
Hypertension (n, %)	14,483	(13.3)	75,679	(16.2)	12,760	(12.3)	65,617	(14.7)
Dyslipidemia (n, %)	42,559	(39.2)	198,118	(42.3)	40,033	(38.5)	185,872	(41.7)
Cardiovascular disease (n, %)	2,524	(2.3)	14,874	(3.2)	2,153	(2.1)	12,343	(2.8)
Cancer (n, %)	1,127	(1.0)	7519	(1.6)	1,002	(1.0)	6,476	(1.5)
$PM_{2.5}$ (ug/m^3) [f]	26.8	(7.9)	26.3	(7.4)	26.8	(7.8)	26.3	(7.4)
$PM_{2.5}$ by exercise categories (ug/m3)								
Inactive (0 MET-h)	26.8	(7.8)	26.6	(7.5)	26.8	(7.8)	26.6	(7.5)
Moderate (≤8.75 MET-h)	26.9	(7.8)	26.3	(7.4)	26.9	(7.8)	26.3	(7.4)
High (>8.75 MET-h)	26.9	(7.9)	26.0	(7.4)	26.8	(7.9)	26.0	(7.4)
Season (n, %)								
Spring	26,532	(24.4)	114,240	(24.4)	25,561	(24.6)	109,028	(24.4)
Summer	30,837	(28.4)	135,582	(29.0)	29,233	(28.1)	128,502	(28.8)
Fall	28,837	(26.6)	127,662	(27.3)	27,673	(26.6)	121,844	(27.3)
Winter	22,409	(20.6)	90,670	(19.4)	21,625	(20.8)	86,745	(19.4)

Abbreviations: CKD, chronic kidney disease; BMI, body mass index.
Values are presented as mean (standard deviation) for continuous variables and count (%) for categorical variables.
[a] Baseline characteristics of the 108,615 participants for analysis of the combined effects of $PM_{2.5}$ and habitual exercise on eGFR decline ≥30%.
[b] Characteristics of the 468,154 observations from the 108,615 participants.
[c] Baseline characteristics of the 104,092 participants for analysis of the combined effects of $PM_{2.5}$ and habitual exercise on CKD development.
[d] Characteristics of the 446,119 observations from the 104,092 participants.
[e] Classified as exposure to dust or organic solvents in the workplace, established by asking, 'Are there any occupational hazards in your workplace?'
[f] Refers to the average $PM_{2.5}$ levels of the year of the visit and the year before the visit.

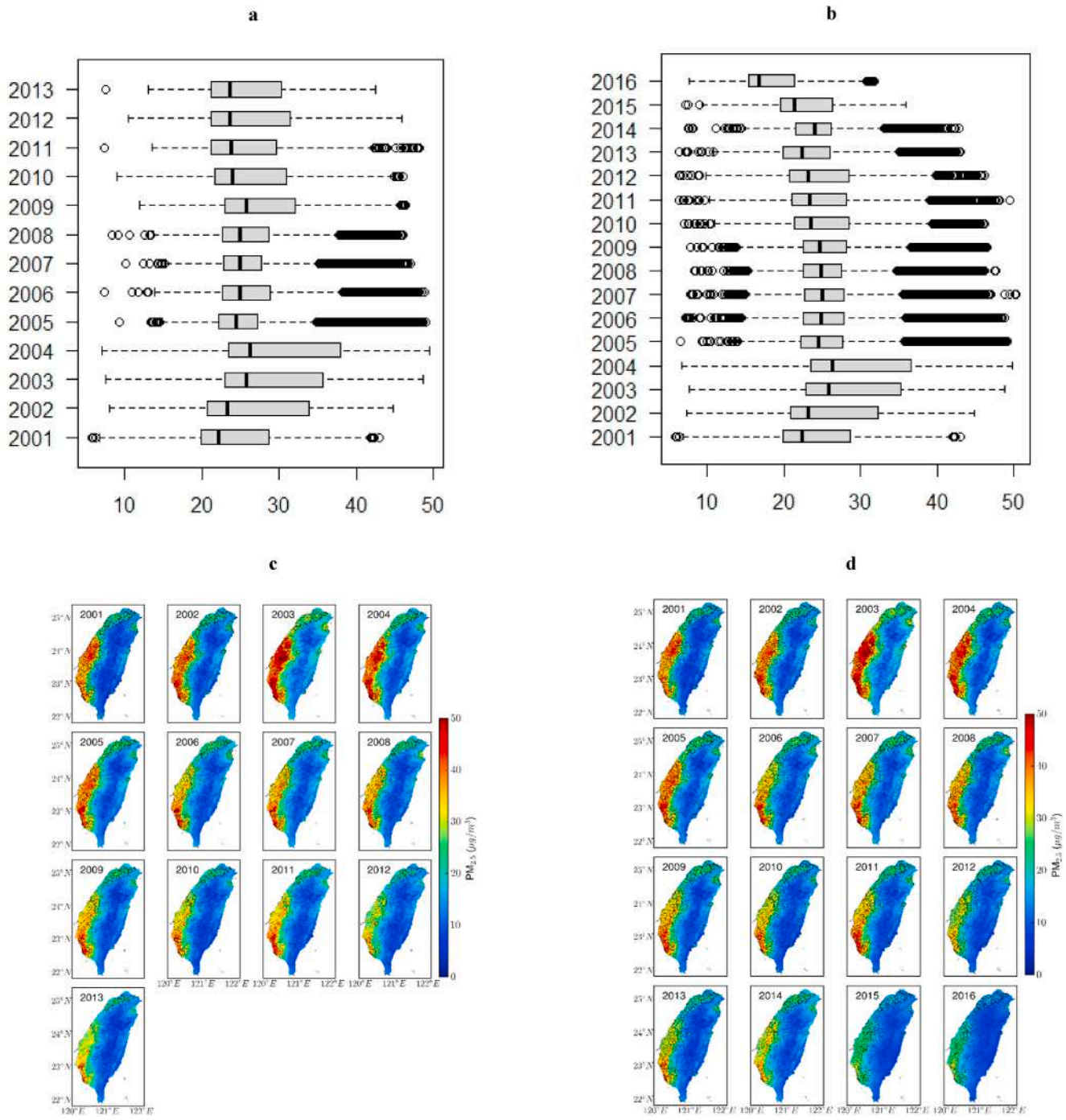

Fig. 1. The spatial and temporal distribution of the two-year average of $PM_{2.5}$ concentrations by year in Taiwan.
Panels a and b represent the temporal distributions of the 2-year average $PM_{2.5}$ concentrations by year. Boxes cover the 25–75th percentiles (IQR) with centre lines indicating the median concentration. Whiskers extend to the highest observations within three IQRs of the box, with more extreme observations shown as circles. Panels a illustrates the distribution of baseline $PM_{2.5}$ concentrations from 108,615 participants. Panels b shows the distribution of $PM_{2.5}$ exposure of 468,154 medical visits from the 108,615 participants. Panel c and d represent the spatial distributions of the participants. Circles indicate the locations of the participants. Panel c depicts the address locations (circles) of the 108,615 participants at baseline by year; Panel d depicts the address locations (circles) of the 108,615 observations from the 468,154 participants by year.

combined effects of habitual exercise and $PM_{2.5}$ are shown in Fig. 2 and Fig. 3 for the incidences of eGFR decline ≥30% and CKD, respectively. Overall, participants with low level of $PM_{2.5}$ exposure combined with high-volume of exercise had the lowest risk of renal function decline and CKD development. There was a prominently downward trend in the risk of developing eGFR decline ≥30% and CKD, with exposure to lower level of $PM_{2.5}$ in each stratum of exercise. The decreasing trend patterns of the effects of habitual exercise on these two outcomes were relatively flat across $PM_{2.5}$ stratums.

Sensitivity analyses generally yielded similar results (Online Resource 3–7).

4. Discussion

In this large Taiwanese cohort study, we found that habitual exercise significantly reduced the risk of renal function decline and CKD

Table 2
Associations of eGFR decline and CKD development with habitual exercise or $PM_{2.5}$ exposure in Taiwanese adults.

Models	Association with eGFR decline ≥30%															
	Main effects of exercise								Main effects of $PM_{2.5}$ exposure							
	HR (95% CI)		P		HR (95% CI) [a]		P		HR (95% CI)		P		HR (95% CI) [a]		P	
Crude model																
Moderate-exercise/$PM_{2.5}$	0.75	(0.70, 0.80)	<	.001	0.76	(0.70, 0.81)	<	.001	1.36	(1.26, 1.46)	<	.001	1.34	(1.24, 1.43)	<	.001
High-exercise/$PM_{2.5}$	0.63	(0.59, 0.67)	<	.001	0.65	(0.60, 0.69)	<	.001	2.31	(2.09, 2.56)	<	.001	2.24	(2.02, 2.48)	<	.001
Test for trend	0.79	(0.76, 0.82)	<	.001	0.80	(0.77, 0.83)	<	.001	1.48	(1.41, 1.55)	<	.001	1.45	(1.38, 1.53)	<	.001
Per 10 μg/m^3									2.05	(1.88, 2.24)	<	.001	1.98	(1.81, 2.16)	<	.001
Model 1																
Moderate-exercise/$PM_{2.5}$	0.78	(0.72, 0.83)	<	.001	0.79	(0.73, 0.84)	<	.001	1.51	(1.41, 1.63)	<	.001	1.50	(1.39, 1.61)	<	.001
High-exercise/$PM_{2.5}$	0.64	(0.60, 0.69)	<	.001	0.66	(0.61, 0.71)	<	.001	2.80	(2.52, 3.11)	<	.001	2.75	(2.48, 3.06)	<	.001
Test for trend	0.80	(0.77, 0.83)	<	.001	0.81	(0.78, 0.84)	<	.001	1.63	(1.55, 1.72)	<	.001	1.62	(1.54, 1.70)	<	.001
Per 10 μg/m^3									2.90	(2.63, 3.19)	<	.001	2.85	(2.59, 3.14)	<	.001
Model 2																
Moderate-exercise/$PM_{2.5}$	0.87	(0.81, 0.93)	<	.001	0.87	(0.81, 0.94)	<	.001	1.54	(1.44, 1.66)	<	.001	1.54	(1.43, 1.66)	<	.001
High-exercise/$PM_{2.5}$	0.70	(0.65, 0.75)	<	.001	0.71	(0.66, 0.76)	<	.001	2.86	(2.58, 3.18)	<	.001	2.84	(2.56, 3.15)	<	.001
Test for trend	0.84	(0.81, 0.87)	<	.001	0.84	(0.81, 0.87)	<	.001	1.65	(1.57, 1.74)	<	.001	1.65	(1.57, 1.73)	<	.001
Per 10 μg/m^3									3.18	(2.88, 3.50)	<	.001	3.15	(2.86, 3.47)	<	.001
	Association with CKD development															
	Main effects of exercise								Main effects of $PM_{2.5}$ exposure							
	HR (95% CI)		P		HR (95% CI) [a]		P		HR (95% CI)		P		HR (95% CI) [a]		P	
Crude model																
Moderate-exercise/$PM_{2.5}$	1.06	(0.98, 1.15)		.13	1.07	(0.99, 1.16)		.07	1.29	(1.20, 1.39)	<	.001	1.30	(1.21, 1.40)	<	.001
High-exercise/$PM_{2.5}$	1.30	(1.21, 1.40)	<	.001	1.33	(1.24, 1.43)	<	.001	1.77	(1.59, 1.96)	<	.001	1.81	(1.63, 2.01)	<	.001
Test for trend	1.15	(1.11, 1.19)	<	.001	1.16	(1.12, 1.21)	<	.001	1.32	(1.26, 1.38)	<	.001	1.34	(1.27, 1.40)	<	.001
Per 10 μg/m^3									1.90	(1.75, 2.06)	<	.001	1.95	(1.80, 2.12)	<	.001
Model 1																
Moderate-exercise/$PM_{2.5}$	0.96	(0.88, 1.03)		.25	0.96	(0.89, 1.04)		.36	1.42	(1.32, 1.53)	<	.001	1.41	(1.31, 1.52)	<	.001
High-exercise/$PM_{2.5}$	0.81	(0.75, 0.87)	<	.001	0.82	(0.76, 0.89)	<	.001	2.17	(1.95, 2.41)	<	.001	2.15	(1.93, 2.39)	<	.001
Test for trend	0.89	(0.86, 0.93)	<	.001	0.90	(0.87, 0.93)	<	.001	1.46	(1.39, 1.53)	<	.001	1.45	(1.38, 1.53)	<	.001
Per 10 μg/m^3									2.70	(2.46, 2.96)	<	.001	2.68	(2.45, 2.93)	<	.001
Model 2																
Moderate-exercise/$PM_{2.5}$	0.93	(0.86, 1.01)		.07	0.94	(0.87, 1.01)		.11	1.40	(1.30, 1.50)	<	.001	1.39	(1.29, 1.50)	<	.001
High-exercise/$PM_{2.5}$	0.79	(0.73, 0.85)	<	.001	0.80	(0.75, 0.87)	<	.001	2.16	(1.94, 2.40)	<	.001	2.14	(1.92, 2.37)	<	.001
Test for trend	0.89	(0.85, 0.92)	<	.001	0.89	(0.86, 0.93)	<	.001	1.45	(1.38, 1.53)	<	.001	1.44	(1.37, 1.52)	<	.001
Per 10 μg/m^3									2.66	(2.43, 2.90)	<	.001	2.63	(2.41, 2.88)	<	.001

Abbreviations: CKD, chronic kidney disease; HR, hazard ratio.
The low, moderate, and high level of $PM_{2.5}$ was ≤22.40, 22.40–26.01, and >26.01 μg/m^3, respectively.
The inactive, moderate, and high volume of exercise was 0, 0–8.75, and >8.75 MET-h, respectively.
Crude Model: without adjustment; Model 1: adjusted for age, gender, education, season, baseline calendar year, smoking status, alcohol drinking, occupational exposure, and physical labor at work; Model 2 further adjusted for BMI, diabetes, hypertension, dyslipidemia, self-reported cardiovascular disease, self-reported cancer, baseline eGFR, and urinary total protein.
[a] Further adjusted for $PM_{2.5}$ (for the association between exercise and eGFR decline ≥30%/CKD development) or exercise (for the association between $PM_{2.5}$ and eGFR decline ≥30%/CKD development).

development even at high level of $PM_{2.5}$, whereas ambient $PM_{2.5}$ was associated with higher risk of decreased renal function and CKD development at all levels of habitual exercise. We present a novel finding of no significant interaction between ambient $PM_{2.5}$ and habitual exercise, which suggests that the level of ambient air pollution did not significantly modify beneficial effects of exercise on renal function exacerbation and CKD development.

We found that regular exercise was associated with lower risk of renal function decline and CKD development, which corroborates existing evidence (Guo et al., 2020b; Robinson-Cohen et al., 2009, 2014). Exercise brings benefits on cardiovascular health via improving cardiovascular endothelial function, insulin sensitivity, lipidic dysmetabolism, and anti-inflammation, while reducing plasma viscosity and insulin resistance (Evensen et al., 2018; Linke et al., 2008; Schauer et al., 2020; Wang et al., 2017). Because there is a close relationship between CKD and cardiovascular diseases, where the disease of one organ leads to the abnormality of the other (Di Lullo et al., 2015; Subbiah et al., 2016), similar biological mechanisms are conceivable to be extended to renal vasculature.

Our findings of adverse effects of long-term exposure to ambient $PM_{2.5}$ on renal function decline and incident CKD are in agreement with previous cohort studies (Bowe et al., 2017, 2018; Chan et al., 2018; Mehta et al., 2016). Inflammation and oxidative stress are hypothesized as principal biological mechanisms to explain the positive association between air pollution and kidney diseases (Webster et al., 2017), which have been demonstrated in prior studies (Sørensen et al., 2003; Zhang et al., 2017). However, the HR for the association of ambient $PM_{2.5}$ with incident CKD in this study was greater than the one reported in our previous study (2.66 vs. 1.06) (Chan et al., 2018). It is possible that previous study did not consider the city-level random effects (Beelen et al., 2014) and did not use time-dependent covariates in the cox models (Bellera et al., 2010). The $PM_{2.5}$ concentration increased before 2005 and declined since then (Fig. 1). Another previous cohort study also showed that risk of $PM_{2.5}$ on incidence of CKD increased when using time-varying exposure in analysis compared with using baseline exposure (Bowe et al., 2018).

To our knowledge, current evidence exploring combined effects of air pollution and habitual exercise on kidney diseases are limited. Our study, showing that healthy benefits of a higher level of habitual exercise with respect to renal function and CKD in Asian adults were not statistically significantly moderated by higher levels of chronic $PM_{2.5}$ exposure, are novel. Some previous studies have investigated the effect modification of the association between exercise and other diseases by different levels of air pollution. Studies from Danish Diet, Cancer, and

Table 3
Subgroup analyses stratified by habitual exercise or $PM_{2.5}$ categories in Taiwanese adults.

eGFR decline ≥30%									
Stratified by $PM_{2.5}$	Low-$PM_{2.5}$			Moderate-$PM_{2.5}$			High-$PM_{2.5}$		
	HR (95% CI)		*p*	HR (95% CI)		*p*	HR (95% CI)		*p*
Habitual Exercise									
Moderate	0.87	(0.77, 0.99)	.03	0.89	(0.79, 1.00)	.05	0.89	(0.79, 1.01)	.08
High	0.75	(0.66, 0.85)	<.001	0.71	(0.62, 0.80)	<.001	0.65	(0.57, 0.74)	<.001
Trend test	0.87	(0.82, 0.92)	<.001	0.84	(0.79, 0.90)	<.001	0.81	(0.76, 0.87)	<.001
Stratified by exercise	Inactive-exercise			Moderate-exercise			High-exercise		
	HR (95% CI)		*p*	HR (95% CI)		*p*	HR (95% CI)		*p*
$PM_{2.5}$									
Moderate	1.49	(1.32, 1.69)	<.001	1.51	(1.33, 1.72)	<.001	1.55	(1.36, 1.76)	<.001
High	2.57	(2.18, 3.03)	<.001	2.81	(2.34, 3.37)	<.001	2.58	(2.13, 3.14)	<.001
Trend test	1.58	(1.46, 1.71)	<.001	1.63	(1.49, 1.78)	<.001	1.59	(1.45, 1.74)	<.001
Per 10 μg/m^3	3.02	(2.57, 3.55)	<.001	2.67	(2.26, 3.16)	<.001	3.36	(2.86, 3.94)	<.001
CKD development									
Stratified by $PM_{2.5}$	Low-$PM_{2.5}$			Moderate-$PM_{2.5}$			High-$PM_{2.5}$		
	HR (95% CI)		*p*	HR (95% CI)		*p*	HR (95% CI)		*p*
Habitual exercise									
Moderate	0.96	(0.83, 1.10)	.55	0.86	(0.75, 0.98)	.03	1.00	(0.87, 1.13)	.94
High	0.88	(0.78, 1.01)	0.07	0.76	(0.67, 0.87)	<.001	0.76	(0.67, 0.87)	.001
\	0.94	(0.88, 1.00)	0.05	0.87	(0.82, 0.93)	<.001	0.87	(0.81, 0.92)	<.001
Stratified by exercise	Inactive-exercise			Moderate-exercise			High-exercise		
	HR (95% CI)		*p*	HR (95% CI)		*p*	HR (95% CI)		*p*
$PM_{2.5}$									
Moderate	1.47	(1.26, 1.70)	<.001	1.28	(1.12, 1.47)	<.001	1.37	(1.23, 1.53)	<.001
High	1.87	(1.54, 2.27)	<.001	2.03	(1.70, 2.42)	<.001	1.96	(1.68, 2.29)	<.001
Trend test	1.39	(1.26, 1.53)	<.001	1.39	(1.27, 1.51)	<.001	1.39	(1.29, 1.50)	<.001
Per 10 μg/m^3	1.88	(1.60, 2.22)	<.001	2.44	(2.08, 2.85)	<.001	2.83	(2.48, 3.23)	<.001

Abbreviations: CKD, chronic kidney disease; HR, hazard ratio.
The low, moderate, and high level of $PM_{2.5}$ was ≤22.40, 22.40–26.01, and >26.01 μg/m^3, respectively.
The inactive, moderate, and high volume of exercise was 0, 0–8.75, and >8.75 MET-h, respectively.
All results were fully adjusted for age, gender, education, season, baseline calendar year, smoking status, alcohol drinking, occupational exposure, physical labour at work, BMI, diabetes, hypertension, dyslipidemia, self-reported cardiovascular disease, self-reported cancer, baseline eGFR, and urinary total protein.

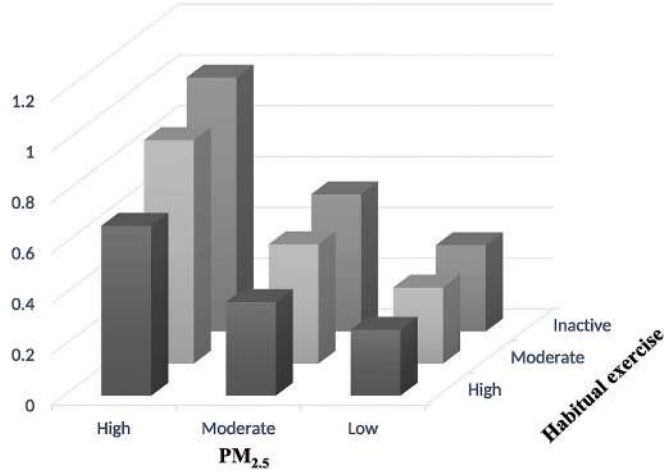

Fig. 2. Combined effects of habitual exercise and $PM_{2.5}$ on eGFR decline ≥30% among adults in Taiwan.
The low, moderate, and high level of $PM_{2.5}$ was ≤22.40, 22.40–26.01 and > 26.01 μg/m^3, respectively. The inactive, moderate, and high volume of exercise was 0, 0–8.75 and > 8.75 MET-h, respectively. All results were fully adjusted for age, gender, education, season, baseline calendar year, smoking status, alcohol drinking, occupational exposure, physical labor at work, BMI, diabetes, hypertension, dyslipidemia, self-reported cardiovascular disease, self-reported cancer, baseline eGFR and urinary total protein. Participants were classified into nine groups according to $PM_{2.5}$ and exercise categories with inactive-exercise exposed to the High-$PM_{2.5}$ comprising the reference group. Chart's data source (Online Resource 8).

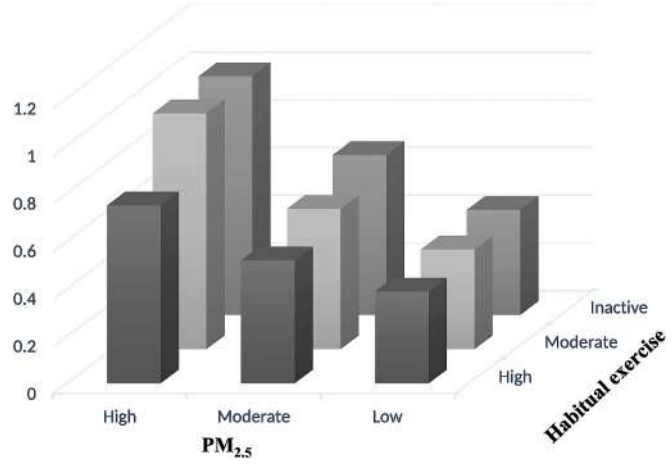

Fig. 3. Combined effects of habitual exercise and $PM_{2.5}$ on incident CKD among adults in Taiwan.
Abbreviations: CKD, chronic kidney disease. The low, moderate and high level of $PM_{2.5}$ was ≤22.40, 22.40–26.01 and > 26.01 μg/m^3, respectively. The inactive, moderate and high volume of exercise was 0, 0–8.75 and > 8.75 MET-h, respectively. All results were fully adjusted for age, gender, education, season, baseline calendar year, smoking status, alcohol drinking, occupational exposure, physical labor at work, BMI, diabetes, hypertension, dyslipidemia, self-reported cardiovascular disease, self-reported cancer, baseline eGFR and urinary total protein. Participants were classified into nine groups according to $PM_{2.5}$ and exercise categories with inactive-exercise exposed to the high-$PM_{2.5}$ comprising the reference group. Chart's data source (Online Resource 9).

Health cohort have also observed no statistically significant interaction between exercise and air pollution on incident asthma/COPD hospitalizations, myocardial infarction and all-cause mortality except for respiratory mortality (Andersen et al., 2015; Fisher et al., 2016; Kubesch et al., 2018) and suggested that participation in exercise may reduce but not reversing the benefits of exercise on respiratory mortality. A cohort study done in Hong Kong elderly reported no interaction effects of exercise and chronic exposure to $PM_{2.5}$ on respiratory mortality while it found some evidence of reducing cardiovascular benefits of walking slowly in higher polluted areas (Sun et al., 2020). Another study including 3,535 children reported that participating in sports was associated with increasing risk of asthma in areas with high level of ozone, but not in those with low-level exposure (McConnell et al., 2002). Our results may not be directly comparable with mentioned studies because of younger or older aged groups and different health outcomes and levels of air pollution in these studies.

The possible reason is that the excess inhaled air pollution results from exercise is only a small fraction of the total inhaled air pollution (Rojas-Rueda et al., 2011). Another potential reason is that acute harmful effects caused by excess dose of inhaled air pollution during sports do not outweigh long-term beneficial health effects of regular exercise (Andersen et al., 2015). In addition, no significant interaction between habitual exercise and $PM_{2.5}$ exposure on systemic inflammation was also reported in our previous study (Zhang et al., 2018b), which indirectly support our findings because systemic inflammation is one of the major pathway of the exercise/PM-renal function association.

Although the measurement of exercise and $PM_{2.5}$ were not directly comparable (i.e., exercise was measured in MET-h and $PM_{2.5}$ in $\mu g/m^3$), our results based on tertile categories indicate that the numerical values of the HRs for the associations with $PM_{2.5}$ were larger than those with exercise. Table 2 shows that each categorical increment in $PM_{2.5}$ was associated with a 65% higher risk of renal function reduction and a 44% higher risk of developing CKD, respectively, whereas each categorical increment in exercise was associated with a 16% lower risk of renal function reduction and a 11% lower risk of developing CKD, respectively. Similar patterns were observed in Table 3 (stratified analysis) and Figs. 2 and 3 (combinations of different exercise and $PM_{2.5}$ categories). Further studies are warranted to investigate whether air pollution mitigation is more effective in kidney health improvement compare with habitual exercise.

This study has several important strengths. Firstly, the large prospective cohort design enabled us to obtain stable and precise estimates. The large sample size also enabled us to conduct a series of subgroup and sensitivity analyses to clarify outcomes' associations with chronic $PM_{2.5}$ exposure and habitual exercise. Second, repeated medical examinations allowed us to use a certain drop in renal function (i.e. eGFR decline $\geq$30%) as one of our health outcomes. This is more meaningful in clinical practice, as a certain drop in eGFR are closely associated with the risk of ESRD and mortality (Coresh et al., 2014). Finally, we used validated spatiotemporal models based on satellite-data for assessment of $PM_{2.5}$. This enabled us to overcome the spatial coverage problems that occur by using data from monitoring stations. This approach also enabled us to monitor the changes of $PM_{2.5}$ exposure over time.

Several limitations in our study should be noted. First, information was not available on whether participants did exercise outdoors or indoors, we could not solely investigate the effects of outdoor exercise. Nevertheless, outdoor exercise was the major mode of exercise for Taiwanese. A national survey shows that around 80% of residents chose outdoor exercise as their most frequent exercise from 2005 to 2016 ("Sports Administration: Report of Active Cities," 2016). Second, instead of direct-measured exercise, information on habitual exercise was collected from a self-administered questionnaire which is commonly used in large-scale epidemiological studies. However, the validity and reliability of the questionnaire have been tested previously (Wen et al., 2008). Third, single measurement of eGFR was used to define incident CKD in this study. According to the clinical practice, diagnosis of CKD needs two separately measurements of eGFR <60 mL/min/1.73 m^2 with an interval of 90 days (KDIGO, 2013). Single measurement of eGFR <60 mL/min/1.73 m^2 indicates that patients might have CKD or acute kidney diseases (including acute kidney injury (AKI)). Fourth, we only evaluated the effects of $PM_{2.5}$ because of the lack of information on other air pollutants, like nitrogen dioxide and ozone (Bowe et al., 2017). However, the collinear issue between pollutants suggests we should analyze each pollutant separately. Finally, our study was conducted in a moderately polluted area. Further studies are warranted in more serious polluted regions to verify our findings.

5. Conclusion

In conclusion, we found that a high habitual exercise combined with a low chronic $PM_{2.5}$ exposure is associated with lower risk of renal function decline and CKD development, whereas a low level of habitual exercise combined a high chronic $PM_{2.5}$ exposure is associated with higher risk of renal function decline and CKD development. Habitual exercise reduces the risk of renal function decline and CKD development regardless of the levels of chronic $PM_{2.5}$ exposure. Chronic $PM_{2.5}$ exposure increased the risk of renal function decline and CKD development regardless of the levels of habitual exercise. Our study suggests that exercise is a safe approach for kidney health improvement for people residing in relatively polluted areas. Our study reinforces the importance of air pollution mitigation for kidney health.

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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.2021.113791.

Author contributions

Dr Lao conceived and designed the study. Drs Chang, Lau, and Lao acquired the data. Yiqian Zeng, Dr Guo, Dr Lin, Yacong Bo, and Dr Yu searched the literature. Yiqian Zeng and Dr Lao did data analysis and interpretation. Yiqian Zeng and Dr Lao drafted the first version of manuscript. All authors critically revised the manuscript. Dr Lao acquired the funding. Drs Chang, Lau, Tam, and Lao supervised this study.

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Comparison of lipid-normalised concentrations of persistent organic pollutants (POPs) between serum and adipose tissue

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ABSTRACT

Human biomonitoring of persistent organic pollutants (POPs) is typically based on serum analysis and for comparison and modelling purposes, data are often normalised to the lipid content of the serum. Such approach assumes a steady state of the compound between the serum lipids and for example lipid-rich adipose tissue. Few published data are available to assess the validity of this assumption. The aim of this study was to measure concentrations of POPs in both serum and adipose tissue samples from 32 volunteers and compare the lipid-normalised concentrations between serum and adipose tissue. For p,p'-DDE, PCB-138, PCB-153 and PCB-180, lipid-normalised adipose tissue concentrations were positively correlated to the respective serum concentrations but generally were more highly concentrated in adipose tissue. These results suggest that the investigated legacy POPs that were consistently found in paired samples may often not be in a steady state between the lipid compartments of the human body. Consequently, the analysis of serum lipids as a surrogate for adipose tissue exposure may more often than not underestimate total body burden of POPs. Further research is warranted to confirm the findings of this study.

1. Introduction

Persistent organic pollutants (POPs) include a range of lipophilic chemicals that are persistent and bioaccumulate in animal and human lipid-rich tissues and fluids (Jones and de Voogt 1999). Examples of POPs are dioxin-like and other polychlorinated biphenyls (PCBs), legacy organochlorine pesticides, and polybrominated diphenyl ethers (PBDEs) (Artacho-Cordón et al., 2015). Most of these POPs are associated with immunologic, teratogenic, reproductive, carcinogenic, and neurological effects, although specific exposure-response relationships vary (Kodavanti et al., 1998).

Despite widespread regulatory bans or use restrictions in most countries, POPs remain detectable in the environment (Syed et al., 2013). The primary route of external exposure for most POPs in the general population is via accumulation in the food chain, including breast milk (Artacho-Cordón et al., 2015). After absorption into the bloodstream, POPs are distributed throughout the body (Lee et al., 2017). Due to their lipophilic character, POPs prefentially partition into lipid-rich tissues and adipose tissue has accordingly been identified as a major storage compartment for these compounds (Patterson et al., 1986).

Biomonitoring for these lipophilic POPs to characterize human body burdens has been conducted using a variety of matrices including adipose tissue samples, blood serum or plasma, and human milk. Due to relative ease of collection, its less invasive nature and/or availability, blood serum or plasma has been preferred to adipose tissue or human milk (Pauwels et al., 2000; Ryan and Mills 1997).

Typically, it is assumed that POPs are in a steady state between the lipid compartments of the human body, such as blood and adipose tissue (Lee et al., 2017; Pauwels et al., 2000; Phillips et al., 1989). Patterson et al. (1988) investigated use of serum lipid as a surrogate for lipid-adjusted concentrations in adipose tissue for measurement of 2,3,7,8-tetrachlorodibenzo-p-dioxin and found a ratio of approximately 1:1, supporting the idea that such highly lipophilic compounds are in a

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steady state in lipid stores throughout the body. For other POPs, such partitioning dynamics have not been clearly demonstrated (Pauwels et al., 2000). Yu et al. (2011) analysed various adipose tissues and serum for various POPs in small set of subjects and found that only <30% of participants had similar concentrations in the various matrices indicating a steady state while the remaining participants' adipose tissue and serum concentrations varied widely. Other work comparing POPs concentrations among human lipid-rich matrices, including serum versus breast milk (LaKind et al., 2009), suggests that the assumption of a consistent relationship between lipid-adjusted POPs concentrations among different matrices is likely too simplistic (Botella et al., 2004; Waliszewski et al., 2003).

While there is a body of work comparing concentrations of lipophilic POPs in different human lipid compartments (see for example Kanja et al., 1992; Stellman et al., 1998; and Pauwels et al., 2000 and more recently Yu et al., 2011), uncertainty remains whether lipid-normalised concentrations of POPs in serum accurately predict lipid-normalised concentrations in other lipid compartments. For example, the analysis of Mannetje et al. (2012) suggests that there is a relationship between increased molecular size and the ratio of concentration in serum versus breast milk which may indicate a kinetic effect.

The aim of this study is to compare lipid-adjusted concentrations between serum and adipose tissue in order to (1) add to the body of evidence assessing the assumption of a steady state of POPs between serum and adipose tissue and to (2) examine the use of serum lipids as a surrogate for body burden. We present the results of analysis of paired adipose tissue and serum samples collected from patients undergoing abdominal surgery.

2. Material and methods

2.1. Sample characteristics

We invited patients undergoing laparoscopic surgery over a four-month period in 2016 to participate in the study. Ethical approval was granted by the Wesley Hospital Ethics Committee (No. 2016.02.180). Participants that consented to participate in the study provided a sample from subcutaneous adipose fat tissue (<1 g) and a serum sample. Adipose tissue samples were obtained during surgery. Blood samples were collected on the same day as the adipose tissue samples. The surgical patients were all fasting for at least 6 h before the operation. Overall, 32 participants (14 females, 18 males) contributed samples. The mean age of all participants was 55 years (52 for females, 57 for males) with a range from 20 to 89 years.

2.2. Lipid extraction of adipose tissue

Between 0.05 and 0.10 g of each adipose tissue sample was accurately weighed (W1), before being homogenised with 1 g of hydromatrix (diatomaceous earth) with a pestle and mortar. The homogenised adipose tissue samples were spiked with internal standards (500 pg each of $^{13}C_{12}$-transChlordane, $^{13}C_{12}$-p,p'-DDE, $^{13}C_{12}$-PCB-81, $^{13}C_{12}$-PCB-123, $^{13}C_{12}$-PCB-167, $^{13}C_{12}$-PCB-189 and 250 pg each of $^{13}C_{12}$-BDE47, $^{13}C_{12}$-BDE99, $^{13}C_{12}$-BDE100, $^{13}C_{12}$-BDE153, $^{13}C_{12}$-BDE154 and $^{13}C_{12}$-BDE183) and transferred to a 100 mL pre-packed Accelerated Solvent Extraction (ASE) cell containing (from the bottom upwards): Two cellulose filters, 30 g anhydrous Na_2SO_4, 10 g hydromatrix. ASE cells were loaded onto an ASE 350 (Thermo Fisher Scientific) and target compounds were extracted using hexane:DCM (3:2 v/v ratio) at 90 °C and 1500 psi (heating time 5 min, static time 5 min, 2 static cycles, rinse volume - 50%, purge time - 120 s). The extract was transferred to a pre-weighed tube (W2) and concentrated under a gentle stream of nitrogen at 40 °C until all traces of solvent were removed. The weight of the tube was recorded again (W3) for lipid determination before crude extracts were reconstituted in 5 mL hexane:DCM 3:2.

2.3. Sample clean-up

Serum samples underwent a combined extraction and clean up where samples (1–5 g) were weighed and spiked with internal standards. The serum samples and the in hexane:DCM 3:2 reconstituted adipose tissue lipid samples were added to the top of 100 mL pre-packed ASE cells containing (from the bottom upwards): two cellulose filters, 5 g silica gel, 2 g hydromatrix, cellulose filter, 12 g of acid impregnated silica (44% sulphuric acid), 5 g florisil and 20 g of hydromatrix. Serum samples were spiked with internal standards and extracted using hexane:DCM (3:2, v/v ratio) at 90 °C and 1500 psi (heating time 5 min, static time 5 min, 3 static cycles, rinse volume - 50%, purge time - 120 s). The extracts were concentrated using a gentle stream of nitrogen at 40 °C to near dryness and then reconstituted to a final volume of approximately 20 μL with *n*-nonane.

2.4. Instrumental analysis

The instrumental analysis methods have previously been described in-depth by He et al. (2018) and Wang et al. (2019). A-chlordane, g-chlordane, p,p'-DDE, PCB-52, PCB-118, PCB-138, PCB-153, PCB-180, BDE28, BDE47, BDE100, BDE99, BDE154, BDE153, BDE183 were determined in all samples using a TRACE GC Ultra equipped with a TriPlus Autosampler, coupled with a TSQ Quantum XLS triple quadrupole mass spectrometer. Separation was achieved using a DB-5MS column (30 m × 0.25 mm i.d.; 0.25 μm film thickness, J&W Scientific) with the following GC programme: initial temperature of 80 °C and held for 2 min, and increased to 180 °C at 20 °C/min, and held for 0.5 min, then increased to 300 °C at 10 °C/min, and held for 5 min. Helium was used as the carrier gas at constant flow rate of 1.0 mL/min. The volume injected was 1.0 μL, in splitless mode. The QqQ mass spectrometer was operated in electron ionization (EI) mode using the multiple reactions monitoring (MRM) mode with an emission current set at 20 μA.

2.5. Determination of lipid content in samples

The lipid content of adipose tissue was determined gravimetrically by applying the weights recorded in the lipid extraction from adipose tissue samples to the following equation:

$$Lipid\ Content\ (\%) = \frac{W3 - W2}{W1} \times 100$$

As blood samples were stored at −20 °C before being spun down to serum, they haemolysed and lipid content for each sample could not be measured serologically. As a result, total serum lipid content was estimated based on measurements of total cholesterol (CHOL) and triglyceride (TG) that were available for some individuals. CHOL and TG data was obtained from independent serology tests that were done by participants shortly before or after their hernia keyhole surgery in which adipose tissue samples were collected. For the lipid content estimation, we employed following formula put forward by Covaci et al. (2006):

$$TL(g/L) = 1.12 \times CHOL + 1.33 \times TG + 1.48$$

For 15 of 32 patients, cholesterol data was available and for 5 of those, there was available triglyceride data. For patients missing CHOL data, we used the mean of the 15 patients with available data (i.e., $\overline{X}_{CHOL} = 1.90\,g/L$) while for patients missing TG data, we used the average of the 5 with available TG data (i.e., $\overline{X}_{TG} = 1.50\,g/L$).

2.6. Quality assurance and quality control (QA/QC)

Laboratory blank samples of hydromatrix were prepared and analysed alongside adipose tissue samples (n = 4), matrix blank (bovine calf serum) samples were analysed alongside serum samples (n = 4). The blank samples were extracted and analysed in each batch of samples.

Method detection limits (MDLs) were defined as the average blank concentrations plus three times their standard deviations (SDs). When concentrations of an analyte were not detected in blank samples, a value of 5 pg/sample were used to calculate the MDL, as this was four times lower than the lowest calibration standard used. The MDLs for the individual chemicals in each experiment are listed in Table 1. Lipid-adjusted limits of quantification (LOQs) for each sample were calculated from:

$$LOQ(ng/g\ lipid) = \frac{0.001 \times MDL}{sample\ volume \times lipid\ content}$$

LOQs in Table 1 are expressed as a range as the MDL differed from sample to sample due to varying lipid contents. Accordingly, the samples with the minimum and maximum lipid content within each matrix correspond to the minimum and maximum LOQ for each chemical, respectively. Duplicate serum sample pairs (n = 5) were included in the analysis to assess the reproducibility of the analytical methods. For replicates A and B and with both replicates above the MDL ($n = 2$), the mean normalised difference (expressed in %) was calculated according to $[value(A - B)/(value(A + B)/2)] \times 100$. The average normalised difference ranged from 15 to 56% (see Table 1).

2.7. Statistical analysis

For the calculation of the geometric mean and determination of the median (see Table 2), non-detected sample concentrations were substituted as ½ the sample-specific limit of quantification (LOQ) when the detection frequency (DF) for that compound was >50%. DF cut-off values for geometric mean and median calculation were chosen at <60% and <50%, respectively. For the rank-order correlation analysis, the regression analysis toolkit included in Excel (2016) was used. A p-value of <0.05 served as the criteria for statistical significance while a p-value of <0.001 was interpreted as high statistical significance.

3. Results

3.1. Summary statistics and detection frequencies

POPs were detected in all serum and adipose tissue samples. An overview of the lipid-adjusted concentrations of detected POPs are presented in Table 2.

For organochlorine pesticides, we report high detection rates for p, p'-DDE at 97% in both adipose tissue and serum. In contrast, g-chlordane and a-chlordane could be detected in 78% and 75%, respectively, of all samples in adipose tissue while both were detectable in less than 15% of all serum samples. PCBs 138, 153, and 180 were detected consistently (i.e., in more than two thirds of all samples in both matrices) whereas PCBs 52 and 118 were less frequently detected and quantified. While all BDEs were detected in adipose tissue, with a frequency ranging from 50% to 100%, detection frequency of BDEs in serum was much lower. Only BDE47, BDE99, and BDE153 could be quantified in any of the serum samples. This limits the usefulness of BDE results when comparing data obtained for samples from adipose tissue with those from serum.

3.2. Correlations between concentrations of POPs in adipose tissue and serum

Rank-order (i.e., Spearman) correlations between quantifiable adipose tissue and serum concentrations were assessed for each analyte with at least 10 available paired measurements (see Table 2). Adipose tissue and serum concentrations of p,p'-DDE were strongly and positively correlated ($R_S = 0.82; p < 0.001$). For PCBs, we found moderate positive rank-order correlations for PCBs 138 and 153, while PCB-180 concentrations were highly correlated between adipose and serum ($R_S = 0.75$, $p < 0.001$).

3.3. Ratios of lipid-adjusted adipose tissue and serum concentrations

Evaluation of the ratios of lipid-adjusted concentrations in adipose tissue and serum samples allows an analysis of the relative distribution among different lipid compartments. Fig. 1 illustrates the distribution of concentration ratios for p,p'-DDE, PCB-138, PCB-153 and PCB-180 based on the ratios calculated from paired samples for each individual (Table 3). We limited our overall ratio presentation to these compounds as they have the highest frequency of paired, quantifiable adipose tissue and serum concentrations. While the ranges of individual ratios encompass 1 for all four of these analytes, the overall picture does not

Table 1
Summary of minimum detection limit (MDL) for the adipose tissue and calf serum blanks and lipid-adjusted limit of quantification (LOQ) ranges, as well as serum duplicates. Blank cells indicate that the analyte was not above the MDL in any duplicate samples.

	Adipose Tissue		Serum		Serum
	4 Laboratory Blanks		4 Matrix Blanks		5 Duplicate Samples
	MDL	LOQ	MDL	LOQ	ND
	pg/sample	ng/g lipid	pg/sample	ng/g lipid	%
g-chlordane	5	0.01–0.13	31	1.2–6.5	
a-chlordane	9.4	0.02–0.25	8.1	0.32–1.7	
p,p'-DDE	960	1.5–26	24	0.95–5.0	21[d]
PCB-52	81	0.13–2.1	5	0.20–1.1	
PCB-118	140	0.22–3.7	5	0.20–1.1	
PCB-138	150	0.23–3.9	27	1.1–5.6	15[b]
PCB-153	100	0.16–2.7	30	1.2–6.3	47[c]
PCB-180	74	0.12–2.0	5	0.20–1.1	56[b]
BDE28	6.8	0.01–0.18	5	0.20–1.1	
BDE47	69	0.11–1.8	5	0.20–1.1	
BDE100	5	0.01–0.13	5	0.20–1.1	
BDE99	26	0.04–0.70	55	2.2–12	
BDE154	5	0.01–0.13	5.6	0.22–1.2	
BDE153	5	0.01–0.13	5	0.20–1.1	44[a]
BDE183	5	0.01–0.13	5	0.20–1.1	

[a] One set of duplicate samples was above MDL for the analyte.
[b] Two sets of duplicate samples were above MDL for the analyte.
[c] Three sets of duplicates were above MDL for the analyte.
[d] Four sets of duplicate samples were above MDL for the analyte.

Table 2
Geometric mean (GM), standard deviation (SD) and median in parentheses, range and percentage of samples above LOQ (%>LOQ) for POPs concentrations (ng/g lipid) within adipose tissue and serum samples of 32 individuals, as well as Spearman (R_S) correlation coefficients between both matrices for statistically significant correlations.

	Adipose Tissue			Serum			Correlation
	GM (SD) [median]	Range	%>LOQ	GM (SD) [median]	Range	%>LOQ	R_S
g-chlordane	0.16 (0.69) [0.16]	<LOQ-3.5	78	NC	<LOQ-10	9	NA
a-chlordane	0.26 (0.82) [0.21]	<LOQ-3.7	75	NC	<LOQ-11	13	NA
p,p'-DDE	190 (810) [180]	<LOQ-3900	97	96 (340) [85]	<LOQ-1500	97	0.82
PCB-52	NC	<LOQ-4.5	13	NC	<LOQ-13	6	NA
PCB-118	NC (34) [1.7]	<LOQ-190	53	NC	<LOQ-7.3	9	NA
PCB-138	12 (290) [13]	<LOQ-1700	94	4.2 (7.7) [4.9]	<LOQ-33	75	0.61
PCB-153	16 (390) [17]	<LOQ-2200	94	6.0 (11) [6.3]	<LOQ-51	88	0.57
PCB-180	13 (280) [15]	<LOQ-1600	94	3.0 (9.2) [4.8]	<LOQ-39	69	0.75
BDE28	NC (0.44) [0.08]	<LOQ-0.88	50	NC	–	0	NA
BDE47	1.9 (3.1) [2.2]	<LOQ-16	78	NC	<LOQ-11	22	NA
BDE100	0.56 (0.71) [0.55]	<LOQ-3.4	97	NC	–	0	NA
BDE99	0.71 (3.0) [0.76]	<LOQ-17	81	NC	<LOQ-9.6	6	NA
BDE154	NC (0.21) [0.06]	<LOQ-0.96	50	NC	–	0	NA
BDE153	4.4 (11) [4.4]	1.0–64	100	NC	<LOQ-6.6	13	NA
BDE183	NC (0.53) [0.08]	<LOQ-2.8	56	NC	–	0	NA

NC: Not calculated due to low rate of detections. NA: Not assessed due to limited number of paired samples with detected concentrations. NS: Not significant.

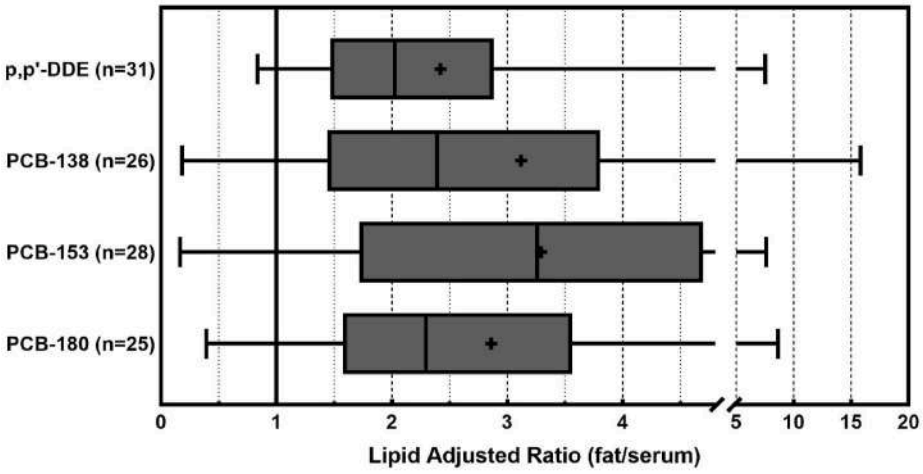

Fig. 1. Ratios of adipose tissue to serum concentrations for selected frequently-detected POPs. Boxes extend from the median to the 25th and 75th percentiles. Whiskers extend to the minimum and maximum. The arithmetic mean is represented by crosses. The black line at X = 1 represents perfect steady state between adipose tissue and serum concentrations.

support a generic assumption that these compounds are in a steady state between lipid stores in the body.

The majority of paired samples for p,p'-DDE and PCB congeners 138, 153 and 180 showed adipose tissue lipid-adjusted concentrations more than two-fold higher than in serum lipid. Furthermore, adipose:serum concentration ratios for the four analytes showed no significant correlation with age ($p > 0.3$, data not shown). It should be noted that Fig. 1 excludes one patient for better graphical representation that had extraordinarily high adipose:serum ratios, ranging from 31 for p,p'-DDE, 42 for PCB-180, 60 for PCB-153 to 63 for PCB-138. Minima for p,p'-DDE and PCB-180 stem from one individual while the minima for PCB-138 and PCB-153 come from another.

3.4. Individual data

As only the four analytes p,p'-DDE and PCB congeners 138, 153 and 180 were detected frequently in both matrices, this section will focus these four analytes. Given the limitations of serum lipid analysis, data for the group of individuals for which serum lipid was estimated from both CHOL and TG measurements rather than from averages are highlighted where appropriate.

Our data show that adipose:serum ratios for different legacy POPs were relatively consistent for a given individual, with relatively low standard deviation (see Table 3). Moreover, we observed very strong Spearman correlations ($R_S = 0.99$) among the individual adipose:serum distribution ratios across the four compounds, each with high statistical significance ($p < 0.001$), indicating that an adipose:serum distribution ratio for one of the four legacy POPS is indicative of the adipose:serum ratios of the other compounds.

4. Discussion

By analysing a range of legacy and emerging POPs in 32 paired serum and subcutaneous adipose tissue samples, this study aims to investigate the assumption that POPs are in a steady state between different human lipid compartments and whether lipid-normalised serum concentrations of POPS can reliably be used as a surrogate for body burden.

In this study, p,p'-DDE and PCB congeners 138, 153 and 180 were detected most frequently and lipid-adjusted adipose tissue and serum concentrations of those four compounds were positively correlated (see Table 2). Furthermore, our data showed that lipid-normalised adipose:serum concentration ratios for different POPs for a given individual were

Table 3
Lipid-normalised adipose:serum concentration ratios for all participants for the legacy POPs p,p'-DDE and PCBs 138, 153, together with the age and sex of the individual. The five individuals with the serum lipid estimation from CHOL and TG measurements as well as the ten individuals with serum lipid estimation from CHOL measurement and TG average are highlighted distinctly from the 17 individuals for which no serum lipid data was available. Furthermore, arithmetic mean and coefficient of variation (CV), calculated as standard deviation divided by mean and displayed as percentage, are provided.

Sample information			Serum lipid data[a]		Adipose:serum ratio				Statistics	
Patient	Age	Sex	CHOL	TG	p,p'-DDE	PCB-138	PCB-153	PCB-180	Mean	CV
Patients with both CHOL and TG measurements										
FAB_002	63	F	2.3	1.5	1.6	NA	2.2	1.3	1.7	27
FAB_008	51	M	2.4	1.4	1.8	2.3	2.4	3.5	2.5	30
FAB_010	58	F	2.7	1.5	1.5	0.18	0.16	1.1	0.8	91
FAB_012	57	M	2.4	0.5	2.0	2.1	2.0	2.2	2.1	5.5
FAB_032	74	M	1.3	2.0	2.6	4.0	3.4	2.4	3.1	24
Patients with CHOL measurement and imputed average TG										
FAB_001	82	F	2.1	NM	3.2	4.4	5.0	4.2	4.2	18
FAB_005	63	F	1.6	NM	1.5	2.3	2.7	2.2	2.2	24
FAB_009	32	F	2.0	NM	2.1	1.4	NA	NA	1.8	27
FAB_014	71	M	1.7	NM	3.2	NA	6.6	5.4	5.1	34
FAB_018	60	M	1.8	NM	2.1	4.0	2.9	1.9	2.7	35
FAB_023	41	F	2.0	NM	NA	0.78	0.68	1.1	0.85	26
FAB_025	37	M	1.4	NM	1.6	1.1	1.7	1.5	1.5	17
FAB_026	74	F	1.9	NM	2.8	3.4	3.5	2.4	3.0	17
FAB_028	42	F	1.8	NM	0.84	0.61	0.98	0.39	0.70	37
FAB_031	40	M	1.6	NM	3.1	2.3	2.2	NA	2.5	21
Patients with both CHOL and TG imputed with average values										
FAB_003	89	M	NM	NM	2.5	2.8	3.5	2.0	2.7	23
FAB_004	63	M	NM	NM	1.9	3.1	4.0	3.0	3.0	29
FAB_006	50	F	NM	NM	1.3	3.3	3.3	5.5	3.3	50
FAB_007	68	M	NM	NM	2.3	3.6	3.6	3.3	3.2	19
FAB_011	59	F	NM	NM	3.9	NA	6.0	3.3	4.4	32
FAB_013	33	F	NM	NM	2.0	2.4	4.4	3.6	3.1	36
FAB_015	55	M	NM	NM	2.4	2.5	5.6	NA	3.5	53
FAB_016	59	F	NM	NM	1.0	1.7	1.4	1.8	1.5	23
FAB_017	79	M	NM	NM	3.8	4.6	4.7	5.3	4.6	13
FAB_019	70	M	NM	NM	31	63	61	42	49	32
FAB_020	49	M	NM	NM	7.2	16	7.6	8.7	9.8	41
FAB_021	41	F	NM	NM	1.4	7.5	6.4	2.1	4.4	70
FAB_022	35	M	NM	NM	1.5	1.5	1.5	NA	1.5	1.9
FAB_024	20	M	NM	NM	1.4	NA	NA	NA	NC	NC
FAB_027	29	F	NM	NM	0.95	NA	NA	NA	NC	NC
FAB_029	60	M	NM	NM	1.5	0.46	0.54	0.78	0.81	56
FAB_030	40	M	NM	NM	7.5	NA	NA	NA	NC	NC

NM = no measurement available; imputed with average values from those with measurements (CHOL: 1.90 g/L; TG: 1.50 g/L).
NA = no ratio available due to a non-detect either in adipose tissue or serum.
NC = not calculated as only one adipose:serum ratio is available.
[a] Serum lipid data is presented in g/L.

relatively similar (see Table 3). These findings are in agreement with the broader literature (Artacho-Cordón et al., 2015; Pauwels et al., 2000; Whitcomb et al., 2005; Arrebola et al., 2012a; Mussalo-Rauhamaa 1991).

Between individuals, the median lipid-normalised adipose:serum ratios were greater than 2 for the same four legacy POPs (see Fig. 1). These results indicate that p,p'-DDE and PCB congeners 138, 153 and 180 are two times more concentrated in adipose tissue than in serum for the majority of participants. The findings from this study are generally consistent with previous observations in the literature, with several studies reporting ranges of lipid-normalised adipose:serum concentration ratios from 1 to 4 (Artacho-Cordón et al., 2015; Arrebola et al. 2012a, 2012b; Mussalo-Rauhamaa 1991; Whitcomb et al., 2005). Since a large part of the body lipids are associated with adipose tissue, an estimation of exposure based on lipid-normalised serum in the assumption of a steady state between serum and adipose tissue which is commonly used in exposure modelling (Czub and McLachlan 2004; Ritter et al., 2009; Quinn and Wania 2012) may result in an underestimation of the amount of POPs that have accumulated in a body and thus may also underestimate overall past exposure.

Notably, the range of lipid-normalised adipose:serum ratios we report spans over more than two orders of magnitude (see Fig. 1). This substantial variance of concentration ratios between individuals poses questions about what could affect such inter-individual differences. One partial explanation may lie in continuously decreasing human exposure to POPs that is demonstrated in temporal trend studies in Australia and globally (Mueller and Toms 2010; Croes et al., 2014; Stubleski et al., 2018; Hardell et al., 2010; Nøst et al., 2013; Zietz et al., 2008). As a result, higher adipose tissue concentrations may reflect stored POPs while low serum concentrations arise from low external exposure. However, more work is needed to fully understand the toxicokinetics of POPs in the human body.

The dataset presented here has several limitations. The number of participants (n = 32) was a reasonable sample size to assess patterns in analyte concentrations. However, LOQs for many analytes in serum samples were quite high compared to the LOQs for adipose tissue samples, resulting in relatively fewer detected and quantified samples for serum than adipose. As a result, the number of samples available for assessing paired correlations and ratios were limited for most analytes. A further limitation to this study was the serum lipid content determination. Only for approximately 15% of participants, we could estimate serum lipid content based on measurements of both serum cholesterol and serum triglycerides with a formula retrieved from the literature (Covaci et al., 2006). For more than half of the participants for which no measurement of either serum cholesterol or serum triglycerides was available, average lipid content was ascribed to samples due to a lack of

sample-specific data on serum lipids. In the future, blood samples must not be frozen before being spun down to serum as this makes lipid content determination difficult. Moreover, the large age range of participants (20–89 years) may present a further limitation.

In conclusion, there has been a general assumption that measuring POPs in serum lipids is a good surrogate for concentrations in lipid compartments throughout the body. Our preliminary results show that lipid-normalised concentrations of legacy POPs such as p,p'-DDE and several PCBs are in some individuals more than a factor of 2, and in few individuals more than a factor of 4, higher in adipose tissue than in serum. Even though the findings from this study cannot fully rule out the possibility of a lipid-normalised serum concentration overestimating lipid-normalised adipose tissue levels, given the large variance of two orders of magnitude shown in Fig. 1, our dataset indicates that the lipid-normalised concentrations in serum for these POPS may more often than not underestimate the lipid-normalised concentrations in adipose tissue in individuals. As a result, serum measurements normalised to serum lipid content may underestimate the actual body burden of POPs in the population. Further research is warranted to confirm the findings of this study.

Declaration of competing interest

The authors declare that no known conflicts of interest have impacted the quality and validity of the conducted research.

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Concentrations of perfluoroalkyl substances in donor breast milk in Southern Spain and their potential determinants

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ABSTRACT

Background: Breast milk is considered to offer the best nutrition to infants; however, it may be a source of exposure to environmental chemicals such as perfluoroalkyl compounds (PFAS) for breastfeeding infants. PFAS are a complex group of synthetic chemicals whose high stability has led to their ubiquitous contamination of the environment.

Objective: To assess the concentrations and profiles of PFAS in breast milk from donors to a human milk bank and explore factors potentially related to this exposure.

Methods: Pooled milk samples were collected from 82 donors to the Human Milk Bank of the Virgen de las Nieves University Hospital (Granada, Spain). Ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) was applied to determine milk concentrations of 11 PFAS, including long-chain and short-chain compounds. A questionnaire was used to collect information on donors' socio-demographic characteristics, lifestyle, diet, and use of personal care products (PCPs). Factors related to individual and total PFAS concentrations were evaluated by multivariate regression analysis.

Results: PFAS were detected in 24–100% of breast milk samples. PFHpA was detected in 100% of samples, followed by PFOA (84%), PFNA (71%), PFHxA (66%), and PFTrDA (62%). Perfluorooctane sulfonate (PFOS) was detected in only 34% of donors. The median concentrations ranged from <0.66 ng/dL (perfluorohexane sulfonic acid [PFHxS]) to 19.39 ng/L (PFHpA). The median of the sum of PFAS concentrations was 87.67 ng/L and was higher for short-chain than long-chain PFAS. Factors most frequently associated with increased PFAS concentrations included intake of creatin animal food items and use of PCPs such as skin care and makeup products.

Conclusions: Several PFAS, including short-chain compounds, are detected in pooled donor milk samples. Breast milk may be an important pathway for the PFAS exposure of breastfed infants, including preterm infants in NICUs. Despite the reduced sample size, these data suggest that various lifestyle factors influence PFAS concentrations, highlighting the use of PCPs.

Abbreviations: PFCAs, Perfluoroalkyl carboxylic acids; PFSAs, Perfluoroalkane sulfonic acids; SC PFAS, Short-chain PFAS; LC PFAS, Long-chain PFAS; NICU, Neonatal intensive care unit; PCPs, Personal care products.

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1. Introduction

Breast milk is considered the best food for infants in general and for high-risk premature infants in particular, offering proper nutrition, immunological benefits, and growth-promoting components and reducing the risk of complications (American Academy of Pediatrics [AAP], 2012). When preterm infants cannot receive breast milk from their mothers, included those admitted to a neonatal intensive care unit (NICU), the World Health Organization (WHO) and AAP recommend the administration of pasteurized human milk from a milk bank rather than artificial infant formula (AAP, 2012; WHO United Nations Children's Fund [UNICEF], 2003). Donated breast milk delivers essential nutrients and therapeutic benefits to the preterm infant but also has the potential to transmit infectious diseases and transfer toxic chemicals from exposed mothers (Carroll, 2014; Lehmann et al., 2018). Consequently, the European Human Milk Banking Association (Weaver et al., 2019) and other international milk banks have established guidelines for donor selection to ensure the safety of the milk (Clifford et al., 2020). These take account of pathogenic microorganisms and certain toxic substances (*e.g.*, tobacco, alcohol, medications, caffeine, and drugs of abuse) but do not consider occupational or environmental exposure to hazardous chemicals.

Per- and polyfluoroalkyl substances (PFAS) are a group of thousands of synthetic chemicals that are widely used in commercial and industrial products. They serve as polymerization aids in the production of fluoropolymers, as surfactants in fire-fighting foams, as anti-mist agents in chromium plating, and as water and oil repellents in textiles, leather, food contact materials, and cosmetics. PFAS are also employed in the production of semiconductors, medical devices, plant protection products, biocides, feed additives, pharmaceuticals, and paints (Glüge et al., 2020). Hydrogen atoms are entirely or partially replaced by fluorine atoms in these aliphatic substances (Buck et al., 2011) and the bond between carbon and fluorine is extremely strong and stable; hence, PFAS are highly resistant to thermal, chemical, and biological degradation and can accumulate in living organisms and biomagnify in food webs (Pérez et al., 2013). The degree of bioaccumulation generally increases with greater length of perfluoroalkyl carbon chain, and the elimination kinetics are highly species-dependent, with humans showing the longest PFAS half-lives, reaching 8.5 years for perfluorohexane sulfonic acid (PFHxS) (Olsen et al., 2009). Over the past decade, exposure to certain PFAS has been associated with lipid and insulin dysregulation (Sinisalu et al., 2020; Sun et al., 2018), infertility (Bach et al., 2016), reduced fetal growth (Kashino et al., 2020), increased miscarriage risk (Liew et al., 2020), obesity (Braun, 2017), impaired cognitive development (Vuong et al., 2019), and altered thyroid (López-Espinosa et al., 2012; Preston et al., 2020) and immune (Abraham et al., 2020; Grandjean et al., 2012) functions. These associations are supported by animal studies indicating that some PFAS are endocrine and metabolic disruptors, immunotoxic, reproductively toxic, and/or carcinogenic (ATSDR, 2018; Fenton et al., 2020; Street et al., 2018).

The most widespread PFAS in the environment are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), long-chain PFAS that are frequently detected in sera from populations worldwide (Bartolomé et al., 2017; Calafat et al., 2007; Kannan et al., 2004; Lewis et al., 2015; Thépaut et al., 2021). Current regulations in the European Union (EU) and elsewhere mainly address PFOS and PFOA, which are listed under the Stockholm Convention on Persistent Organic Pollutants (POPs) (Regulation (EU) 2019/1021; UNEP, 2009) and have been phased out in the EU since 2008 (European Directive, 2006/112/EC). Restrictions are also in place or planned under EU chemical legislation for other PFAS, including short-chain compounds such as PFHxS and perfluorohexanoic acid (PFHxA) (ECHA, 2019). These are less bioaccumulative than long-chain PFAS but are equally persistent in the environment and may exert similar toxicity (Nian et al., 2020).

Food, drinking water, and the indoor environment are considered to be the principal sources of human exposure to PFAS (Cornelis et al., 2012; Haug et al., 2011), which also include cosmetics and all-weather textiles, among other products made from PFAS (EFSA, 2020; Schultes et al., 2018). Seafood, meat, and dairy products may be the major sources of dietary exposure, especially to PFOA and PFOS (Domingo and Nadal, 2017; Titlemier et al., 2007). Socio-demographic factors have also been related to a greater internal PFAS burden, including occupation, male sex, higher age, and low parity (Bartolomé et al., 2017; Colles et al., 2020; Guzman et al., 2016).

Several PFAS have been detected in umbilical cord blood, placenta, breast milk, and plasma samples from breastfed infants, indicating that placental transfer and breastfeeding are both potential routes of PFAS exposure (Abraham et al., 2020; Cariou et al., 2015; Lien et al., 2013; Vela-Soria et al., 2021). It has been reported that a substantial proportion of PFAS in the mother is transferred to the infant during breastfeeding, which may contribute to reduce maternal serum and breast milk concentrations over the lactation period (Bartolomé et al., 2017; Macheka-Tendenguwo et al., 2018; Mondal et al., 2014; Thomsen et al., 2010). Identification of breastfeeding as an important pathway for the exposure to PFAS of breastfed infants (Haug et al., 2011) has been supported by findings of their wide presence in breast milk samples from mothers worldwide (Hu et al., 2021; Lee et al., 2018; Macheka--Tendenguwo et al., 2018).

Human milk banks provide milk for very premature, fragile, and sometimes medically compromised infants who are especially vulnerable to the effects of toxic chemicals. The present study is part of a wider project that aims to assess the potential adverse health impact on neonates in a NICU of exposure to endocrine-disrupting chemicals (EDCs) from their medical care, diet, and environment (Iribarne-Duran et al., 2019). The purpose of this study was to evaluate the concentrations and profiles of eleven long-and short-chain PFAS in milk samples from donors to a human milk bank and to explore factors that influence their concentrations.

2. Material and methods

2.1. Study population

Between 2015 and 2018, 82 donor mothers were recruited from the Regional Human Milk Bank of the Virgen de las Nieves University Hospital in Granada (Southern Spain). In general, donor women are registered at the milk bank after breastfeeding is well-established (*i.e.*, 2–3 weeks post-delivery). Exclusion criteria for donor milk selection include: positive serology for HIV, syphilis, or hepatitis B or C; risk factor for sexual transmitted disease (*e.g.,* unstable partner, non-utilization of condom, tattooing/piercing in previous three months, acupuncture, and blood transfusion); transplantation in previous 6 months; current smoking or drug habit; and high consumption of alcohol (>2 drinks/day or >20 g/day) or caffeine-containing drinks (>3 cups/day or >30 g/day). All local donors supplying the milk bank between 2015 and 2018 (n = 446) were invited to participate in the study and were fully informed of its nature and purpose. Donors who agreed to participate (18.4%) were asked to donate a milk sample for the analysis of environmental chemicals and to complete a structured questionnaire on socio-demographic and reproductive characteristics, lifestyle, diet, and use of personal care products (PCPs). Information on dietary habits and PCP use was available for a subsample of 77 donors. An informed consent form was signed by the donors before collecting personal information and biological samples. The research protocol was approved by the Biomedical Research Ethics Committee of Granada.

2.2. Milk sample collection

Participating donors were asked by the milk bank to collect mature milk over a minimum of 1 week and a maximum of 4 weeks by manual expression and/or breast pump and to keep them frozen (−20 °C) until delivery to the bank. On their arrival at the bank, samples were stored at

−30 °C without breaking the cold chain at any time. Before their pasteurization (done within 2 weeks), samples from each donor were thawed and pooled, obtaining an aliquot of 5–30 mL of the pooled milk. This was then stored at −20 °C until analysis at the "UNETE research unit" of the Centro de Investigación Biomédica (University of Granada). The day of pasteurization was recorded as the donation date. Hence, the interval between the start of milk collection by the mother and the donation date never exceeded 6 weeks.

2.3. Laboratory analysis

A modification of a validated ultra-high performance liquid chromatography-with tandem mass spectrometry method (Vela-Soria et al., 2020) was used (see Supplementary material) to determine the concentrations of eleven PFAS in pooled milk samples, including: seven long-chain PFAS, *i.e.*, six perfluoroalkyl carboxylic acids (PFCAs) with >7 perfluorinated carbons (PFOA, perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], perfluoroundecanoic acid [PFUnDA], perfluorododecanoic acid [PFDoDA] and perfluorotridecanoic acid [PFTrDA]), and one perfluoroalkane sulfonic acid (PFSA) with ≥6 perfluorinated carbons (PFOS); and four short-chain PFAS, *i.e.*, two PFCAs (PFHxA and perfluoroheptanoic acid [PFHpA]), and two PFSAs (perfluorobutane sulfonic acid [PFBS] and PFHxS) (Buck et al., 2011).

Milk aliquots used for the determination of PFAS had not been analyzed before. Quality control (QC) procedures included the use of blanks, low and high-concentration QC materials prepared from a fortified breast milk pool, analytical standards, and reagent and matrix blanks to ensure the accuracy and precision of the data. We also performed repeated measurements of breast milk QC pools, reflecting inter- and intraday variations. Relative standard deviation (%RSD) values were calculated as a measure of the precision of the method. Table S2 summarizes the mean accuracy and %RSD values obtained. The accuracy of the method was also verified by injecting QCs of different concentrations every 20 samples. Limits of detection (LD) ranged between 0.66 and 0.86 ng/L and limits of quantification (LQ) between 2.19 and 2.87 ng/L (Table S2).

2.4. Explanatory variables

The questionnaire administered by the milk bank to prospective milk donors and an *ad hoc* questionnaire were used to gather the following socio-demographic, reproductive, and lifestyle data: age (years), parity (multiparous or primiparous), lifetime duration of breastfeeding, either exclusive or mixed (<1, 1–10, or >10 months), birth weight and length and gestational age of the most recent newborn, schooling (university education or not), current occupation (unemployed, manual worker, or non-manual worker), area of residence (urban, sub-urban, or rural), smoking habit (ever smoked in the past or not), and current body mass index (BMI, kg/m^2) categorized as underweight/normal (<25 kg/m^2) or overweight/obese (≥25 kg/m^2). Women were also asked about their weight gain during the most recent pregnancy (kg) and weight change from before pregnancy (gain, loss, or no change). The number of days post-delivery was calculated as the difference between milk donation and birth dates. Dietary information was collected on the main origin of drinking water and the average consumption frequency (servings per day or week) in the previous 12 months of seafood, fish (oily and lean fish), dairy products (yoghurt, milk, butter, cheese), meat (red meat and cold meats), pulses, eggs, bread, chocolate, cereals, rice, pasta, fruit, vegetables (raw and cooked), fried food, canned food, coffee, and alcoholic beverages (Table 2). Data were also gathered on the frequency with which the women used sun screen, lip protector, face treatments (cream, tonic, milk), body lotion, hand cream, hair mask, makeup products (foundation, lipstick, eyeliner, and eye shadow), nail polish, hair dye, shampoo, shower cream, deodorant, hairspray/mousse/gel, perfume, toothpaste, and mouth wash and received manicure and pedicure treatments in the previous 12 months (Table 3).

Table 1
General characteristics of milk donors (n = 82).

Variables	n (%)	Median	Range
Age (years)		33	19–42
Year of sample collection			
2015	25 (30.5)		
2016	27 (32.9)		
2017	23 (28.0)		
2018	7 (8.5)		
Multiparous	37 (45.1)		
Lifetime duration of breastfeeding (months)			
<1	41 (50.0)		
1–10	22 (26.8)		
>10	19 (23.2)		
Time since delivery (days)		71	20–273
Length of gestation (weeks)		39	26–41
Birth weight (g)		3130	840-4500
Birth length (cm)		50	16–56
Current BMI (kg/m^2)		22.86	17.30–36.09
Overweight/obese	26 (33.8)		
Weight gain during pregnancy (kg)		12	1–36
Weight change from before pregnancy			
Weight loss	19 (22.1)		
Weight gain	39 (49.4)		
No weight change	24 (28.6)		
Area of residence			
Rural	26 (31.2)		
Sub-urban	24 (29.9)		
Urban	32 (39.0)		
Maternal university education	51 (66.2)		
Occupation			
Unemployed	6 (6.3)		
Manual worker	22 (26.3)		
Non-manual worker	54 (67.5)		
Ex-smoker	39 (47.6)		

BMI: Body mass index.

In addition, the protein content of unpasteurized pooled milk samples (g/100 mL) was determined as a potential explanatory variable, given evidence that perfluorinated compounds are mainly transported bound to human serum albumin (Luo et al., 2012) and their lactational transfer is produced by binding to milk protein (Fromme et al., 2010). The total lipid, lactose (g/100 mL), and caloric (kcal/100 mL) contents of samples were also measured as independent variables.

2.5. Statistical analysis

The detection frequency of PFAS in milk samples and 50th, 75th, and 95th percentiles of their concentrations were calculated, including the total concentration of all PFAS (∑PFAS), the most abundant PFAS commonly found in human blood samples (∑4 PFAS = [PFOA + PFOS + PFNA + PFHxS] and ∑5 PFAS = [∑4 PFAS + PFHpA]) (Cousins et al., 2020; EFSA, 2020), long-chain PFAS (∑LC PFAS), short-chain PFAS (∑SC PFAS), PFSAs (∑PFSAs), and PFCAs (∑PFCAs). Total concentrations were calculated as the sum of molar concentrations of the compounds based on molecular weight and were expressed as PFOA (∑PFAS, ∑4 PFAS, ∑5 PFAS, ∑LC PFAS, ∑PFCAs), PFOS (∑PFSAs), or PFHpA (∑SC PFAS). When PFAS were detected in at least 70% of samples, concentrations below the LD were assigned a value of LD/$\sqrt{2}$ and were treated as continuous variables, as were the sums of the different PFAS groups. PFAS detected in less than 70% of the milk samples were categorized as detected or non-detected (binary variables). Spearman's correlation test was used to assess relationships between PFAS concentrations (Fig. 1).

Multivariate regression analyses were performed with natural-logarithm-transformed continuous (linear regression) or binary (logistic regression) PFAS concentrations as dependent variables. A forward stepwise procedure was used to enter independent variables in the models. All variables described in section 2.4, and the year of sample collection (2015, 2016, 2017, or 2018), were tested as potential

Table 2
Food intake frequency of milk donors (n = 77).

Variables	n (%)	Variables	n (%)
Coffee intake = 1 cup/day	17 (20.7)	**Pulse**	
Alcohol intake ≥ 1 drink/ month	4 (4.9)	1 sv/week	13 (16.9)
Origin of drinking water		2 sv/week	29 (37.7)
Tap water	53 (68.8)	>2 sv/week	35 (45.5)
Bottled water	24 (31.2)	**Eggs**	
Seafood		1 sv/week	16 (20.8)
<1 sv/week	11 (14.3)	2 sv/week	28 (36.4)
1 sv/week	19 (24.7)	>2 sv/week	33 (42.9)
>1 sv/week	47 (57.3)	**Bread**	
Lean fish		<1 sv/day	15 (19.5)
<1 sv/week	18 (23.4)	1 sv/day	25 (32.5)
1 sv/week	37 (48.1)	>1 sv/day	37 (48.1)
>1 sv/week	22 (28.6)	**Chocolate**	
Oily fish		Never	10 (13.0)
<1 sv/week	29 (37.7)	<1 sv/day	44 (57.1)
1 sv/week	34 (44.2)	≥1 sv/day	23 (28.0)
>1 sv/week	14 (18.2)	**Cereals**	
Yoghurt		Never	27 (35.1)
<1 sv/day	31 (40.3)	<1 sv/day	35 (45.5)
≥1 sv/day	46 (59.7)	≥1 sv/day	15 (19.5)
Milk		**Rice**	
<1 glass/day	14 (18.2)	1 sv/week	66 (85.7)
≥1 glass/day	63 (81.8)	>1 sv/week	11 (14.3)
Cheese		**Pasta**	
Never/rarely	22 (28.6)	1 sv/week	66 (85.7)
>2 sv/week	34 (44.2)	>1 sv/week	11 (14.3)
≥1 sv/day	21 (27.3)	**Fruit**	
Butter		≤2 sv/week	12 (15.6)
Never	23 (29.9)	>2 sv/week	65 (84.4)
1 sv/week	36 (46.8)	**Raw vegetables**	
>1 sv/week	18 (23.4)	≤2 sv/week	15 (19.5)
Meat		>2 sv/week	62 (80.5)
≤1 sv/week	11 (14.3)	**Cooked vegetables**	
2 sv/week	13 (16.9)	≤2 sv/week	17 (22.1)
>2 sv/week	53 (68.8)	>2 sv/week	60 (77.9)
Cold meat		**Fried food**	
<2 sv/week	43 (55.8)	<1 sv/week	37 (48.1)
2 sv/week	34 (44.2)	1 sv/week	25 (32.5)
Red meat		>1 sv/week	15 (19.5)
Never	20 (26.0)	**Canned food (ever)**	62 (80.5)
<1 sv/week	33 (42.9)		
≥1 sv/week	34 (31.2)		
sv: serving			

Table 3
Use of personal care products among milk donors (n = 77).

Variables	**n (%)**	**Variables**	**n (%)**
Sunscreen (ever)	37 (48.1)	**Eye shadow**	
Sunscreen application		Rarely/never	49 (63.6)
None	40 (51.9)	<once a day	18 (23.4)
Face	26 (33.8)	≥once a day	10 (13.0)
Entire body	11 (14.3)	**Nail polish (traditional)**	
Sunscreen protection factor		Rarely/never	65 (84.4)
None	40 (51.9)	≥once a week	12 (15.6)
<50	12 (15.6)	**Acrylic nail polish**	
50	25 (32.5)	<once a month	70 (90.9)
Lip protector (ever)	30 (39.0)	once a month	7 (9.1)
Face cream		**Manicure**	
<once a day	24 (31.2)	<once a month	67 (87.0)
once a day	31 (40.3)	once a month	10 (13.0)
>once a day	22 (28.6)	**Pedicure**	
Face tonic		<once a month	66 (85.7)
Rarely/never	60 (77.9)	once a month	11 (14.3)
≥once a week	17 (22.1)	**Hair dye**	
Face milk		Never	36 (46.8)
Rarely/never	70 (90.9)	<once a month	23 (29.9)
≥once a week	7 (9.1)	once a month	18 (23.4)
Face treatment		**Shampoo**	
Never	59 (76.6)	<3 times/week	25 (32.5)
<once a month	12 (15.6)	≥3 times/week	52 (67.5)
Once a month	6 (7.8)	**Shower cream**	
Body lotion		<once a day	6 (7.8)
Rarely/never	28 (36.4)	≥once a day	71 (92.2)
<once a day	15 (19.5)	**Hairspray/mousse/gel**	
≥once a day	34 (44.2)	Rarely/never	58 (75.3)
Hand cream		≥once a day	19 (24.7)
<once a day	43 (55.8)	**Deodorant**	
once a day	19 (24.7)	<once a day	8 (10.4)
>once a day	15 (19.5)	once a day	52 (67.5)
Hair mask		>once a day	17 (22.1)
Rarely/never	38 (49.4)	**Perfume**	
≥once a week	39 (50.6)	Rarely/never	12 (15.6)
Foundation makeup		<once a day	24 (31.2)
Rarely/never	41 (53.2)	≥once a day	41 (53.2)
<once a day	19 (24.7)	**Toothpaste**	
≥once a day	17 (22.1)	≤once a day	17 (22.1)
Lipstick		>once a day	60 (77.9)
Rarely/never	39 (50.6)	**Mouthwash**	
<once a day	24 (31.2)	Rarely/never	47 (61.0)
≥once a day	14 (18.2)	<once a day	11 (14.3)
Eyeliner		≥once a day	18 (23.4)
Rarely/never	36 (46.8)		
<once a day	21 (27.3)		
≥once a day	20 (26.0)		

explanatory variables). Given the modest sample size, the p-value threshold of 0.10 was selected to retain explanatory variables in the model. Associations were expressed as exponentiated regression coefficients (exp [β]) or odds ratios (OR) with 95% confidence intervals (CI). The overall R-squared for each model was calculated to determine the percent variability in exposure explained by explanatory variables. R version 4.0.4 (SAS Institute Inc., Cary, NC, USA) was used for data analyses.

3. Results

General characteristics of the study participants are displayed in Table 1. Donors had a median age of 33 years and 45% were multiparous (33 mothers had 1 previous birth). Most of the milk samples were collected in 2015–2017, with only 8% being collected in 2018. The lifetime breastfeeding duration was <1 month for 50% and >10 months for 23%. The median interval between delivery and milk donation was 98 days (3.3 months), ranging from 20 days (<1 month) to 273 days (9 months). In their most recent pregnancy, the birth was preterm (<37 weeks) in 23% of deliveries and the infant had low birth weight (<2500 g) in 18%. Around one-third of donors were overweight or obese; 49% gained weight from before pregnancy and 22% lost weight. More than one-third of the donors resided in the metropolitan urban area of Granada, 66% had completed university education, 26% were manual workers, and 48% were ex-smokers. Most donors drank tap water and consumed >2 servings/week of meat and >1 serving/week of seafood (44% consumed >1 serving/week of oily fish) (Table 2). More than half of donors reported the daily use of face cream, hand cream, shower cream, deodorant, and toothpaste and the frequent use of shampoo (≥3 times/week) and perfume (≥once a day) (Table 3).

The median protein content of milk samples was 1.10 g/100 mL (range = 0.20–6.80 g/100 mL), their median fat content was 3.70 g/100 mL (range = 1.16–8.30 g/100 mL), median lactose content was 7.36 g/100 mL (range = 6.54–8.00 g/100 mL), and median energy content was 68 kcal/100 mL (range = 44–110 kcal/100 mL).

Table 4 shows that detection frequencies (DF) of PFAS ranged from 24.4 to 100%, with PFHpA being detected in all samples (median concentration = 19.39 ng/L), followed by PFOA (DF = 84.1%, median = 7.17 ng/L), PFNA (DF = 70.7%, median = 2.59 ng/L), PFHxA (DF = 65.9%, median = 1.58 ng/L), and PFTrDA (DF = 62.2%, median = 1.69 ng/L). Remaining compounds were detected in less than 40% of samples. The median sum of PFAS concentrations was 87.67 ng/L (range = 7.57–1899 ng/L) and was higher for short-chain than for long-chain PFAS (median = 52.69 [range = 2.74–1168] ng/L *vs.* 20.01 [range = 3.06–571.1] ng/L, respectively) and for PFCAs than for PFSAs (median = 74.97 [range = 5.65–1399] ng/L *vs.* 2.45 [range = 0.72–223.2] ng/L,

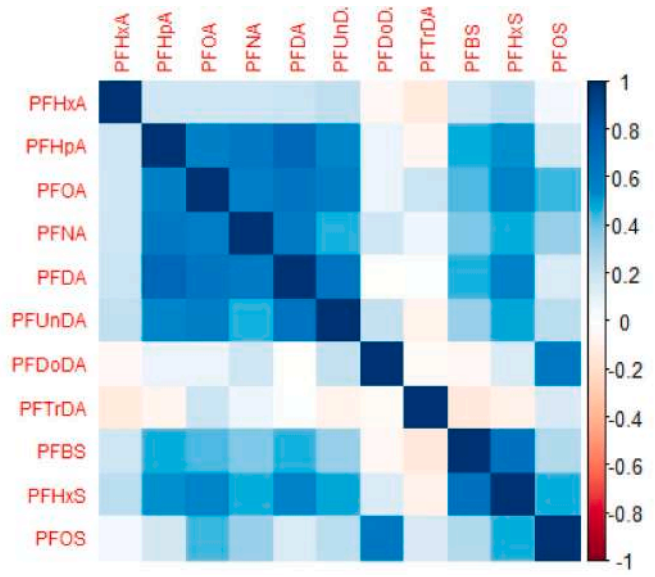

Fig. 1. Correlation heatmap for PFAS concentrations in breast milk.

respectively). At least seven PFAS compounds were detected in the breast milk of 24 donors (29%), 5–6 were detected in 38 (46%), and 2–4 in 20 (24%). Positive correlations were observed between all PFCA compounds except for PFDoDA and PFTrDA, while PFSA concentrations were positively correlated with PFHpA, PFOA, PFNA, and PFDA concentrations (Fig. 1).

Explanatory variables that were associated with PFAS concentrations are exhibited in Table 5 (linear regression models) and Tables S3–S10 (logistic regression models). The R-squared value of models ranged from 14% (∑LC PFAS) to 61% (PFDoDA). Milk samples collected in 2016 or 2017 had lower PFNA, PFDoDA, and ∑LC PFAS but higher PFHxA and ∑SC PFAS concentrations in comparisons to 2015. Multiparous donors had significantly higher concentrations of PFHpA in their milk, while lifetime duration of breastfeeding was associated with higher concentrations of PFOA, PFDA, ∑5 PFAS, ∑PFCAs, ∑LC PFAS, and ∑PFAS. Weight change from before pregnancy (gain or loss) was associated with higher PFDA, PFOS, ∑4 PFAS, and ∑5 PFAS concentrations, and residing in an urban area with higher PFHpA, ∑5 PFAS, and ∑PFAS concentrations.

With regard to food intake, red meat was associated with higher concentrations of ∑5 PFAS; oily fish, milk, and cold meat with higher PFHxA; yoghurt with higher PFDoDA and ∑PFSAs; cheese with higher PFTrDA; butter with higher PFBS; pulses with higher ∑4 PFAS; chocolate with higher PFHpA; and fried food with higher PFOS concentrations. The PCPs most frequently related to increased PFAS concentrations were hand cream, whose use was related to higher PFNA, PFDA, PFBS, PFHxS, PFOS, ∑4 PFAS, ∑SC PFAS, ∑PFCAs, and ∑PFAS; followed by face treatment, associated with higher PFHpA, PFNA, ∑5 PFAS, and ∑PFCAs; and lipstick use, associated with higher PFOA, PFUnDA and PFDoDA. Face cream and body lotion were associated with higher PFUnDA; foundation makeup with higher ∑4 PFAS; eyeliner with higher PFHxA and PFHxS; eye shadow with higher PFOA and PFDA; hair dye with higher PFOA and PFOS; shampoo with higher PFOA; hair mask with higher PFHxS; deodorant with higher ∑PFSAs; and perfume with higher ∑SC PFAS concentrations.

On the other hand, certain factors such as a higher intake of cheese, eggs, cereals, and fish, and a more frequent use of deodorants were associated with a decrease in the milk concentrations of some individual PFAS or total PFAS (Table 5 and Tables S3–S10).

4. Discussion

The concentrations of eleven PFAS were measured in milk samples from 82 donors to a human milk bank in Spain in 2015–2018. More than two-thirds of milk samples had detectable concentrations of PFHpA (100%), PFOA (84%), and PFNA (71%), and almost one-third showed the presence of at least seven PFAS; also, concentrations of short-chain PFAS were higher than of long-chain PFAS, especially in more recently collected samples. These results suggest that breast milk may be an important pathway of PFAS exposure for breastfed infants. Given that donated milk is used for premature newborns with low or very low birth weight (<1500 g) in NICUs, it appears crucial to monitor concentrations of environmental chemicals such as PFAS in human milk banks. Despite the small sample size, these findings suggest that PFAS concentrations in human milk are influenced by various lifestyle factors, such as intake of

Table 4
Concentrations (ng/L) of PFAS in donor breast milk (n = 82).

Compound		LOD	DF (%)	Median	P75	P95	Max.
PFHxA	Perfluorohexanoic acid	0.73	65.9	1.58	26.15	152.3	322.4
PFHpA	Perfluoroheptanoic acid	0.79	100	19.39	55.71	232.3	743.9
PFOA	Perfluorooctanoic acid	0.86	84.1	7.17	23.86	55.12	251.8
PFNA	Perfluorononanoic acid	0.69	70.7	2.59	10.69	25.48	136.5
PFDA	Perfluorodecanoic acid	0.72	24.4	<0.72	1.57	23.01	210.3
PFUnDA	Perfluoroundecanoic acid	0.74	39.0	<0.74	1.60	3.29	14.01
PFDoDA	Perfluorododecanoic acid	0.77	35.4	<0.77	1.66	1.66	131.8
PFTrDA	Perfluorotridecanoic acid	0.78	62.2	1.69	1.69	8.84	13.34
PFBS	Perfluorobutane sulfonic acid	0.80	35.4	<0.80	1.73	66.35	195.0
PFHxS	Perfluorohexane sulfonic acid	0.66	24.4	<0.66	0.74	16.01	45.45
PFOS	Perfluorooctane sulfonic acid	0.86	34.1	<0.86	6.26	26.01	64.75
Sum of PFAS		**P5**	**P25**	**Median**	**P75**	**P95**	**Max.**
∑4 PFAS[a]	PFOA + PFOS + PFNA + PFHxS[b]	<2.04	5.51	14.66	39.69	104.7	437.8
∑5 PFAS[a]	PFOA + PFOS + PFNA + PFHxS + PFHpA[c]	2.19	5.88	53.31	103.9	280.5	1284
∑LC PFAS[a]	PFOA + PFOS + PFNA + PFDA+ PFUnDA + PFDoDA + PFTrDA[d]	3.81	7.32	20.01	47.03	155.1	571.1
∑SC PFAS[a]	PFHpA + PFHxA + PFBS + PFHxS[e]	<2.74	13.75	52.69	125.1	398.4	1168
∑PFSA[a]	PFBS + PFHxS + PFOS	<0.72	<0.72	2.45	8.09	136.6	223.2
∑PFCA[a]	PFHxA + PFHpA + PFOA + PFNA+ PFDA + PFUnDA + PFDoDA + PFTrDA	7.11	30.16	74.97	141.8	450.1	1399
∑PFAS[a]	Sum of all 11 PFAS	11.46	44.58	87.67	208.2	475.4	1899

LOD: Limit of detection; DF: Detection frequency; P75, P95: 75th and 95th percentiles.
LC: long-chain PFAS; SC: short-chain PFAS; PFSAs: Perfluoroalkyl sulfonic acids; PFCAs: Perfluoroalkyl carboxylic acids.
[a] Weighted molar sum of PFAS concentrations (sum of molar concentrations of PFAS based on molecular weight; [b]Most abundant PFAS in human serum (EFSA, 2020); [c]Most abundant PFAS in human serum including PFHpA (Cousins et al., 2020); [d]Long-chain PFAS; [e]Short-chain PFAS.

Table 5
Significant explanatory variables for breast milk concentrations of most prevalent PFAS and summed concentrations of PFAS groups (n = 77).

Predictors	PFHpA	PFOA	PFNA	∑4PFAS	∑5PFAS	∑PFSAs	∑PFCAs	∑Long-chain PFAS	∑Short-chain PFAS	∑PFAS
Year of sample collection (ref: 2015)										
2016			**0.23 (0.10–0.53)**					**0.39 (0.21–0.73)**	**2.91 (1.36–6.17)**	
2017			0.65 (0.27–1.53)					0.64 (0.34–1.18)	**2.75 (1.22–6.22)**	
2018			0.32 (0.09–1.05)					0.55 (0.22–1.38)	0.73 (0.24–2.23)	
Multiparous *vs.* primiparous	2.12 (0.96–4.72)									
Total breastfeeding (ref: <1 month)										
1–10 months		**2.75 (1.31–5.79)**			**2.99 (1.72–5.21)**		**2.27 (1.26–4.11)**	**1.80 (1.01–3.19)**		**2.61 (1.49–4.96)**
>10 months		1.11 (0.51–2.42)			1.01 (0.54–1.89)		0.90 (0.48–1.67)	1.04 (0.58–1.89)		1.31 (0.75–2.29)
BMI (kg/m^2)	0.91 (0.82–1.01)									
Weight change from pre-conception										
Weight gain				**2.22 (1.02–4.81)**	1.66 (0.89–3.09)					
Weight loss				**3.03 (1.58–5.78)**	**1.71 (1.01–2.89)**					
Area of residence (ref: rural)										
Sub-urban	1.20 (0.44–3.30)				1.32 (0.72–2.42)				**0.48 (0.23–0.99)**	0.62 (0.34–1.12)
Urban	**4.04 (1.51–10.8)**				**3.08 (1.77–5.36)**				1.68 (0.80–3.55)	**1.82 (1.06–3.11)**
Ex-smoker									**0.44 (0.22–0.87)**	
Coffee intake: 1 *vs.* <1 cup/day				**0.44 (0.21–0.90)**						
Lean fish intake (ref: <1 sv/week)										
1 sv/week									**0.29 (0.13–0.63)**	**0.46 (0.26–0.84)**
>1 sv/week									0.48 (0.21–1.12)	0.64 (0.34–1.19)
Oily fish intake (ref: <1 sv/week)										
1 sv/week										**0.56 (0.34–0.93)**
>1 sv/week										0.99 (0.52–1.56)
Yoghurt intake: ≥1 *vs.* <1 sv/day						**2.09 (1.02–4.29)**				
Cheese intake (ref: rarely/never)										
>2 sv/week	**0.30 (0.11–0.76)**				**0.46 (0.26–0.81)**	0.46 (0.20–1.08)				**0.51 (0.29–0.86)**
≥1 sv/day	**0.32 (0.10–0.99)**				**0.51 (0.27–0.95)**	0.40 (0.15–1.05)				**0.50 (0.27–0.93)**
Red meat intake (ref: ≤1 sv/week)										
2 sv/week					0.94 (0.52–1.69)					
>2 sv/week					**1.85 (1.07–3.21)**					
Pulses intake (ref: 1 sv/week)										
2 sv/week				1.33 (0.60–2.93)						
>2 sv/week				**2.53 (1.17–5.45)**						

(*continued on next page*)

Table 5 (*continued*)

Predictors	PFHpA	PFOA	PFNA	∑4PFAS	∑5PFAS	∑PFSAs	∑PFCAs	∑Long-chain PFAS	∑Short-chain PFAS	∑PFAS
Egg intake (ref: 1 sv/week)										
2 s/week	**0.30 (0.10–0.92)**									
>2 sv/week	0.46 (0.15–1.38)									
Chocolate intake (ref: never)										
<1 sv/day	3.27 (0.93–11.5)									
≥1 sv/day	2.98 (0.74–12.0)									
Cereal intake (ref: never)										
<1 sv/day				**0.38 (0.21–0.70)**						**0.53 (0.32–0.89)**
≥1 sv/day				**0.36 (0.16–0.80)**						0.65 (0.35–1.22)
Face treatment (ref: never)										
<once a month	1.99 (0.98–2.38)		2.12 (0.85–5.26)		1.65 (0.89–3.06)		1.57 (0.78–3.14)			
once a month	**3.13 (1.26–7.80)**		**4.26 (1.26–14.42)**		**4.02 (1.71–9.47)**		**3.59 (1.38–9.36)**			
Body lotion use (ref: rarely/never)										
<once a day							0.95 (0.48–1.89)			
≥once a day							**0.53 (0.29–0.98)**			
Hand cream use (ref: <once a day)										
once a day			2.09 (0.95–4.61)	**2.05 (1.07–3.92)**			1.77 (0.95–3.29)		**2.03 (1.00–4.19)**	**2.07 (1.23–3.49)**
>once a day			1.46 (0.60–3.56)	1.74 (0.86–3.52)			1.62 (0.79–3.33)		1.30 (0.58–2.91)	1.20 (0.69–2.07)
Foundation makeup use (ref: never)										
<once a day				1.55 (0.80–2.97)						
≥once a day				1.96 (0.98–3.95)						
Lip protector use: ever *vs.* never				**0.46 (0.27–0.80)**						
Lipstick use (ref: rarely/never)										
<once a day		**3.42 (1.50–7.84)**								
≥once a day		0.47 (0.18–1.25)								
Eye shadow use (ref: rarely/never)										
<once a day		0.60 (0.25–1.44)								
≥once a day		**6.39 (2.32–17.6)**								
Hair dye use (ref: never)										
<once a month		1.03 (0.46–2.32)								
once a month		**2.24 (1.01–4.98)**								
Shampoo use: ≥ *vs.* <3 times/ week		**2.01 (1.01–4.00)**								

(*continued on next page*)

Table 5 (*continued*)

Predictors	PFHpA	PFOA	PFNA	∑4PFAS	∑5PFAS	∑PFSAs	∑PFCAs	∑Long-chain PFAS	∑Short-chain PFAS	∑PFAS
Shower cream use: ≥ *vs.* <once a day					**0.37 (0.16–0.89)**					
Deodorant use (ref: <once a day)										
once a day		**0.24 (0.10–0.65)**			**0.40 (0.19–0.86)**	1.17 (0.36–3.86)				
>once a day		0.85 (0.26–2.77)			0.81 (0.35–1.87)	**4.30 (1.13–16.3)**				
Perfume use (ref: rarely/never)										
<once a day									**3.35 (1.25–8.95)**	
≥once a day									1.81 (0.75–4.39)	
R^2	24%	35%	21%	26%	43%	16%	15%	14%	40%	34%

PFSAs: Perfluoroalkyl sulfonic acids; PFCAs: Perfluoroalkyl carboxylic acids.
Associations are reported as exponentiated regression coefficients (exp[β]) with 95% confidence intervals (CI).
Bold: p-value<0.05.

certain animal food items and the use of PCPs. Some of these factors were previously reported, as discussed below.

4.1. PFAS concentrations in milk

There has been increasing research into the presence of PFAS in human breast milk over the past decade (Hu et al., 2021; Macheka-Tendenguwo et al., 2018; Supplementary material, Table S11). Studies have indicated a wide variation in the geographical distribution of PFAS concentrations and profiles, revealing a global decline in the concentration of some PFAS congeners, especially in countries where their production and utilization have been restricted, such as the USA and Germany (Bjerregaard-Oelsen et al., 2016; Černá et al., 2020; Macheka-Tendenguwo et al., 2018). In this regard, the Stockholm Convention (UNEP, 2016) and EU regulations (Commission Regulations [EU] 2017/1000; 207/2011) have contributed to a gradual decline in levels of PFOS and PFOA. In the present study, PFAS concentrations in the donor milk samples collected in 2015–2018 were generally several times lower than in samples collected between 2012 and 2015 in hospitals or primary health care centers in other Spanish regions (Beser et al., 2019; Lorenzo et al., 2016; Motas Guzmán et al., 2016). In the most recent Spanish study by Beser et al. (2019), PFOA, PFOS, and PFNA concentrations in milk samples collected in 2015 were higher than in the present samples, while they did not detect any of the remaining nine PFAS analyzed (Table S11). Likewise, concentrations of PFOS, PFOA, PFNA, and PFHxS in breast milk samples gathered in Catalonia in 2007–2008 were higher in comparison to the present findings (Kärrman et al., 2010; Llorca et al., 2010), although the presence of other PFAS such as PFDA and PFUnDA was not detected (Kärrman et al., 2010). The lower concentrations of PFAS found in donor milk samples from Granada *versus* other Spanish regions may be attributable to the lower level of economic development in the South of Spain, as indicated by the results of a Spanish biomonitoring study of PFAS concentrations in serum samples from adults in 2009–2010 (Bartolomé et al., 2017).

PFAS concentrations in the present samples are generally comparable or in the lower range of those observed in breast milk from other countries, although the majority of previous studies only measured the most abundant PFAS, *i.e.,* PFOS, PFOA, PFNA, PFDA, and PFHxS (Macheka-Tendenguwo et al., 2018; Table S11). A recent Chinese study of the same eleven PFAS as in the present study reported a higher detection frequency of PFOA, PFOS, and PFDA but a much lower detection frequency of the remaining PFAS in breast milk samples (n = 174) than in the present samples (Jin et al., 2020); for instance, PFHpA was not detected in any sample but was found in all of the present samples. In the same way, Lee et al. (2018) reported higher concentrations of PFOA, PFOS, and PFHxS but lower concentrations of the remaining PFAS in milk from 293 Korean mothers in comparison to the present donors. Overall, the present results suggest a decline in breast milk concentrations of PFOS (detected in only one out of three donors), a continued exposure to PFOA, and widespread exposure to short-chain PFAS such as PFHpA and PFHxA, whose concentrations were higher than previously reported in breast milk (Macheka-Tendenguwo et al., 2018; Table S11).

Our findings are in line with studies indicating the predominance of short-chain *versus* long-chain PFAS in breast milk (Fujii et al., 2012; Kang et al., 2016; Kim et al., 2011; Lorenzo et al., 2016). Short-chain PFAS are more soluble and have a lower molecular weight, facilitating their passage through the mammary epithelial membrane and their contamination of breast milk. In addition, the widespread and growing use of alternative short-chain PFAS over the last years would have increased human exposure (Kang et al., 2016; Lorenzo et al., 2016). It has also been suggested that the transfer of sulphonates (PFSAs) to human milk is easier than that of carboxylates (PFCAs) (Roosens et al., 2010); however, the latter were more abundant than the former in the present study.

4.2. Determinants of PFAS concentrations in breast milk

PFAS concentrations were not associated with the age of milk donors. The relationship of breast milk PFAS with age is not clear, with some studies describing higher PFAS concentrations with increasing age (Lee et al., 2018) and others showing no such association (Antignac et al., 2013; Llorca et al., 2010; Motas Guzmán et al., 2016; Nyberg et al., 2018). The BMI of donors was not related to breast milk PFAS concentrations in the present study, except for a suggestive inverse association with PFHpA. Previous reports have been contradictory, showing both positive and negative associations (Berg et al., 2014; Brantsaeter et al., 2013; Cariou et al., 2015; Jensen et al., 2015; Lee et al., 2018; Lorenzo et al., 2016). On the other hand, weight change from before pre-conception appeared to influence the increase the concentrations of PFDA, PFOS, ∑4 PFAS, and ∑5 PFAS. This finding is not easy to explain, given that weight change may be an indicator of changes in diet or lifestyle that could lead to increased PFAS exposure, as previously suggested (Lee et al., 2018).

Lifetime breastfeeding was associated with higher PFDA, PFOA, ∑PFCAs, ∑long-chain PFAS, and total PFAS concentrations, while multiparous status was associated with lower PFBS but higher PFHpA concentrations. In contrast, various studies reported lower PFAS concentrations in the milk of multiparous mothers in comparison to those who were breastfeeding for the first time (Awad et al., 2020; Barbarossa et al., 2013; Croes et al., 2012; Motas Guzmán et al., 2016; Thomsen et al., 2010), suggesting a greater transfer (either placental or via breastfeeding) of PFAS to the first newborn. Thus, Thomsen et al. (2010) reported a reduction rate of 7.7 and 3.1% per month in breast milk concentrations for PFOA and PFOS, respectively, while Mondal et al. (2014) estimated that breastfeeding was associated with monthly decrease of 1–3% in maternal serum concentrations of PFOA, PFOS, PFHxS, and PFNA and of 1–8% in breast milk concentrations of PFOA and PFOS. However, is still not well established that breast milk concentrations of PFAS decrease over the lactation period. In line with our results, a Korean study found higher PFOS, PFOA, PFNA, and total PFAS concentrations in breast milk collected at 30 *versus* 6 days after the delivery, which were attributed to changes in the dietary and lifestyle patterns of mothers throughout the lactational period (Lee et al., 2018). It has also been proposed that the interval between pregnancies may have an impact on the body burden of PFAS, with a longer interval being associated with breast milk concentrations that may be as high as observed for the first breastfeeding episode (Whitworth et al., 2012). Nevertheless, the associations with breastfeeding duration observed in this study remain poorly understood.

Some previous studies observed higher PFAS concentrations in the breast milk of women residing in urban or semi-urban *versus* rural areas (Abdallah et al., 2020; Liu et al., 2010; Tao et al., 2008a, b). In the same line, urban donors in this study showed higher concentrations of PFHpA, ∑5 PFAS, and total PFAS concentrations, while their education and occupation did not appear to influence PFAS concentrations.

Dietary intake has been identified as a substantial source of PFAS exposure (Domingo and Nadal, 2017). The intake of fish and seafood has been associated with higher internal concentrations of PFAS in several studies (Berg et al., 2014; Rylander et al., 2010; Thépaut et al., 2021; Tyrrell et al., 2013), including reports of adult serum and breast milk samples (Bartolomé et al., 2017; Motas Guzmán et al., 2016). In addition, research on the presence of PFAS in food marketed in Spain found that fish and shellfish were the most contaminated groups, showing the highest concentrations of PFOS, PFOA, PFHpA, and PFHxS (Domingo et al., 2012a, b). However, no positive relationship was found between fish/seafood intake and PFAS concentrationss in the present study, although meat consumption was associated with increased total PFAS concentrations, consistent with observations of a higher PFAS content in foods of animal *versus* non-animal origin (Tittlemier et al., 2007). The intake of other food items did not show a clear trend towards an increase in PFAS exposure. Notably, the consumption of fried food was associated with higher PFOS concentrations. This may in part be explained by a greater use of non-stick cookware or PFOS-contaminated oil for frying or by a higher intake of fried processed food contaminated with PFOS. However, no data are available to support these propositions.

Higher concentrations of several PFAS, including long- and short-chain compounds, were found in the milk from women who more frequently used various PCPs, suggesting that PCPs might be a potential source of PFAS exposure. Few data are available on the presence of PFAS in PCPs; however, nine PFAS, including PFOA and PFNA, were detected in foundation, nail polish, and sunscreen products sold in Japan (Fujii et al., 2013), and twenty-five PFAS, most frequently PFHpA and PFHxA, in foundation and cosmetic powder products sold in Sweden (Schultes et al., 2018). In the present study, the use of foundation was positively associated with the sum of PFOA, PFOS, PFNA, and PFHxS concentrations, and the use of skin care and hair products, cosmetics, perfume, and deodorant was associated with higher concentrations of long-chain and short-chain PFAS, PFCAs, and PFSAs. These results support a previous study of 264 Korean women that found the utilization of cosmetics and skin care products to be associated with breast milk concentrations of PFHpA and PFOS, respectively (Kang et al., 2016). In the same line, recent biomonitoring study of adults in Belgium and Norway reported associations between the use of cosmetics (*e.g.*, sunscreen, mouthwash, and lip balm) and serum concentrations of PFAS (Colles et al., 2020; Thépaut et al., 2021). Dermal exposure to PFAS has been considered negligible in comparison to exposure from diet, drinking water, and ingestion of house dust (Trudel et al., 2008; Vestergren et al., 2008). However, exposure assessment studies have not considered the potential contribution of PCPs to dermal uptake due to the lack of adequate dosage data. Dermal permeability studies have also shown that the skin may be a relevant route of PFAS exposure under certain conditions, underscoring the need to re-assess the potential contribution of dermal exposure (Franco et al., 2012).

4.3. Implications for newborn exposure and health

Literature reports suggest that breast milk is an important pathway for the exposure of breastfed infants to PFAS, while also acts as a route for PFAS progressive elimination from the mother's body. In general, PFAS concentrations in the present milk samples were lower than described in these studies; however, it should be taken into account that: 1) exposure may start during the fetal period via placental transfer, meaning that the infant would already have a body burden of PFAS at birth; 2) although epidemiological evidence on the effects of postnatal exposure to PFAS, particularly short-chain PFAS, remains limited, potential effects include thyroid hormone imbalances, altered postnatal growth, and a decreased antibody response to vaccines (Abraham et al., 2020; Grandjean, 2018; Jin et al., 2020; Lopez-Espinosa et al., 2012); 3) there is a lack of knowledge on the toxicological properties of many PFAS in current use and on the combined adverse effects of this complex group of synthetic chemicals; and 4) most importantly, milk donated to the human milk bank is given to highly vulnerable preterm infants in NICUs, for whom the acceptable level of risk should be zero. Interestingly, based on findings of an association between plasma PFAS concentrations and antibodies against diphtheria and tetanus in one-year-olds (Abraham et al., 2020), the EFSA estimated that critical levels in breast milk would be 60 ng/L for PFOA and PFNA, 73 ng/L for PFHxS and PFOS, and 133 ng/L for the sum of the 4 PFAS (EFSA, 2020). These values are comparable to the upper concentrations observed in the present study. Moreover, a recent study reported that the exposure of preterm infants to PFAS through human breast milk might exceed reference values for older and healthier infants (Aceti et al., 2021).

4.4. Strengths and limitations

The main limitation of this study is the small sample size, which reduced the capacity to detect possible determinants of PFAS exposure,

particularly for compounds with a low detection frequency and therefore modeled as binary variables. Nevertheless, similar or even smaller sample sizes were used by most published studies on PFAS in breast milk (Macheka-Tendenguwo et al., 2018; Table S11). In addition, extrapolation of the study findings to lactating women in general is limited, because milk donors tend to be more educated and have higher incomes in comparison to non-donor lactating women (Osbaldiston and Mingle, 2007). Indeed, most donors in this study had a university education and were non-manual workers. Moreover, data were not available to establish whether the socio-demographic profile differed between participating and non-participating donors. However, neither education nor occupation was associated with milk PFAS concentrations. A further limitation was the use of a questionnaire not specifically designed for an exhaustive investigation of sources of PFAS exposure, and the lack of more detailed data on dietary patterns, occupations, and other potential sources of exposure prevented the identification of additional exposure pathways. Further, the considerable number of explanatory factors assessed may have led to some spurious statistically significant associations. It is also possible that bias may have resulted from the misreporting of dietary intakes and other factors. Nevertheless, misclassification is unlikely to be driven by exposure levels. Finally, the wide time frame for sample collection (ranging from 20 days to 9 months since delivery) may hamper comparisons with other studies on PFAS breast milk concentrations, given that internal PFAS exposure may vary over the lactation period due to toxicokinetics and/or lifestyle changes. In fact, our research group is currently investigating time-dependent variations in breast milk PFAS concentrations over the lactation period.

The main strength of this study is the assessment of pooled milk samples (over a maximum of 4 weeks) rather than spot samples. It is well established that lactational transfer of PFAS occurs by binding to milk protein. Protein levels decrease linearly in human milk over the first year of lactation, particularly over the first 6 weeks post-partum (Ballard and Morrow, 2013). Hence, PFAS assessments in spot breast milk samples may increase the risk of exposure misclassification in comparison to the assessment of pooled samples. Moreover, some of the eleven PFAS measured (*e.g.,* PFUnDA, PFDoDA, and PFTrDA) have been less well studied in breast milk samples and human biomonitoring studies. To our knowledge, this is the first report on the presence of PFAS in breast milk samples supplied by donor mothers to a human milk bank. The results suggest that requirements for donor selection may not be sufficient to minimize the exposure of breastfed infants to environmental chemicals.

5. Conclusions

This study of the concentrations of eleven PFAS in donor breast milk demonstrated the wide presence of these compounds in milk samples, especially short-chain PFAS such as PFHpA and PFHxA. PFOA and PFNA showed lower concentrations than observed in previous studies but were still detected in a large proportion of samples, whereas the findings for PFOS suggest a decrease in exposure levels. The data also suggest that certain lifestyle patterns, such as the use of PCPs, may have an influence on the presence of PFAS in breast milk; however, these data should be interpreted with caution given the limited sample size. Further studies are required to elucidate the main factors contributing to the increase in PFAS concentrations in breast milk and to determine changes in exposure levels over the lactation period This issue is especially urgent in relation to the supply of human milk to preterm infants and the need to limit their exposure to harmful chemicals.

Declaration of competing interest

The authors declare no actual or potential competing financial interests.

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Appendix A. Supplementary data

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Detection of SARS-CoV-2 RNA on contact surfaces within shared sanitation facilities

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ABSTRACT

Contamination of contact surfaces with SARS-CoV-2 has been reported as a potential route for the transmission of COVID-19. This could be a major issue in developing countries where access to basic sanitation is poor, leading to the sharing of toilet facilities. In this study, we report SARS-CoV-2 contamination of key contact surfaces in shared toilets and the probabilistic risks of COVID-19 infections based on detection and quantification of the nucleic acid on the surfaces. We observed that 54–69% of the contact surfaces were contaminated, with SARS-CoV-2 loads ranging from 28.1 to 132.7 gene copies per cm^2. Toilet seats had the highest contamination, which could be attributed to shedding of the virus in feces and urine. We observed a significant reduction in viral loads on the contaminated surfaces after cleaning, showing the potential of effective cleaning on the reduction of contamination. The pattern of contamination indicates that the most contaminated surfaces are those that are either commonly touched by users of the shared toilets or easily contaminated with feces and urine. These surfaces were the toilet seats, cistern handles and tap handles. The likelihood (probability) of infection with COVID-19 on these surfaces was highest on the toilet seat (1.76×10^{-4}(1.58×10^{-6})) for one time use of the toilet. These findings highlight the potential risks for COVID-19 infections in the event that intact infectious viral particles are deposited on these contact surfaces. Therefore, this study shows that shared toilet facilities in densely populated areas could lead to an increase in risks of COVID-19 infections. This calls for the implementation of risk reduction measures, such as regular washing of hands with soap, strict adherence to wearing face masks, and effective and regular cleaning of shared facilities.

1. Introduction

The current COVID-19 pandemic has claimed over 3.9 million lives and infected another 184 million globally, as at 7th July 2021 (WHO, 2021). The primary mode of transmission of the SARS-CoV-2 virus, the causative agent for COVID-19, is through respiratory droplets (Chan et al., 2020; Cai et al., 2020; Bahl et al., 2020; Morawska and Milton, 2020). This has led to the implementation of mitigation measures, such as social distancing and the use of face masks (Liu and Zhang, 2020; WHO, 2020; Howard et al., 2020; Dalton et al., 2020; Viner et al., 2020). Additionally, transmission of the virus through contaminated contact surfaces has been postulated (Qu et al., 2020; Zoran et al., 2020; Jones, 2020). These are of concern due to the stability/survival of this virus on surfaces such as plastic, steel, wood and aluminium (Van Doremalen et al., 2020; Pastorino et al., 2020). Their survival on contact surfaces is dependent on the material and environmental conditions, for instance, it is reported to persist on plastics for 3–4 days at 65% relative humidity (RH) and 21–23 °C (Van Doremalen et al., 2020), aluminium for 2–3 h at 19 °C−21 °C temperature range (Pastorino et al., 2020), stainless steel for four days and on glass for two days (Chin et al., 2020), all at room temperature. However, Goldman (2020) posited that most of these studies reporting on the survival of SARS-CoV-2 or surrogate viruses on fomites exaggerate the potential risks due to the use of unrealistic viral titre. Despite in-depth information on the potential transmission routes of the virus, there is a lack of data on the role of shared sanitation facilities as a possible route of transmission. Although this has been studied within hospital settings (Ye et al., 2020; Ong et al., 2020), the risks posed by shared sanitation facilities outside of the hospital

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environment has been neglected.

The reported shedding of viral particles in feces and urine, by both symptomatic and asymptomatic individuals, highlights the increased risks from the use of shared sanitation. The World Health Organization (WHO) reports that between 2 and 27% of COVID-19 patients have diarrhoea (WHO, 2020b), which may result in the shedding of this virus in feces. SARS-CoV-2 viral loads of 1.7×10^6–4.1×10^7 gc/mL have been reported by Han et al. (2020), and 6.3×10^6–1.26×10^8 gc/g of stool by Lescure et al. (2020). Additionally, although not common, the detection of SARS-CoV-2 concentrations of 3.2×10^2 gc/ml (Peng et al., 2020) and 6.1×10^5 gc/ml (Yoon et al., 2020) have been reported in urine. These results show that in circumstances where fecal and urine contamination of surfaces could occur, such as shared sanitation facilities, the risks of COVID-19 infections could be high. This is especially important in slums or informal settlements in developing countries such as South Africa, where a lack of basic sanitation facilities is a significant concern. The World Bank reported that living in cramped conditions within cities has a significant contribution to a high risk of infections with COVID-19 (WBG, 2020).

The risks associated with shared sanitation could be due to the contamination of contact surfaces by infected individuals either via deposition of aerosols or faecal matter contaminations. Additionally, several studies have shown strong evidence in support of the indoor airborne transmission of viruses, especially in crowded and poorly ventilated areas (Nishiura et al., 2020; Coleman et al., 2018; Knibbs et al., 2012), such as shared toilets. For instance, SARS-CoV-2 is reported to survive in aerosols for up to 3 h (Kumar et al., 2020), meaning the sharing of toilet facilities could be a major risk factor.

Therefore, by detecting and quantifying the concentration of SARS-CoV-2 on key contact surfaces within these shared sanitation facilities, the risks of infection could be estimated. The quantitative microbial risks assessment (QMRA) approach has been encouraged as a tool to assess risks associated with bioaerosols, drinking water, reclaimed water and irrigation water (Carducci et al., 2016; Petterson and Ashbolt, 2016; Girardi et al., 2019; Gularte et al., 2019; Ezzat, 2020). This approach has been used in estimation of the risks for COVID-19 infections for wastewater treatment workers (Zaneti et al., 2020; Dada & Gyawali, 2020), exposure in a market setting (Zhang et al., 2020) and most recently via contact surfaces (Pitol and Julian, 2021). According to Haas et al. (2014) the QMRA approach involves a sequence of four interrelated steps: a) hazard identification; b) exposure assessment; c) dose-response assessment and d) risk characterization. This is the first study using QMRA for assessing risks from the use of shared sanitation facilities outside the clinical setting, focusing on contact surfaces despite the widespread understanding that sanitation facilities may facilitate its spread. This could therefore provide background information on the contamination of such surfaces and could be used in developing risk reduction measures aimed at reducing the potential spread of COVID-19 (and possibly other similar outbreaks) via the use of shared toilet facilities.

2. Methodology

2.1. Study area and sampling

Two peri-urban informal settlements located within the eThekwini Municipality (Durban) of South Africa were selected for this study. These two settlements are located approximately 1.5 km apart, with an approximate total population of 16 500. This study was done at a time the reported active clinical cases were low in South Africa, with about 600 000 active cases of COVID-19 in South Africa, specifically the KwaZulu-Natal province had over 100 000 active cases.

A total of eight (8) shared toilets, referred to as community ablution blocks (CABs), were investigated, four in each settlement. It is worth noting that the CABs are categorized into males and females, however, this study focused on the difference in contamination within the vaiorus CABs irrespective of gender. The contact surfaces selected included the following: cistern handle, toilet seat, floor surface in front of the toilet, internal pull latch of cubicle door and tap in handwash basin (Fig. 1). These were selected based on recommendations made in previous studies (Park et al., 2017; Bohnert et al., 2016; Mpotane et al., 2013). A total of 68 swab samples were taken. Sampling was done twice (two weeks apart) in September 2020. On each sampling event, samples were taken in the morning before the toilets are cleaned and approximately 30 min after cleaning by trained caretakers. Cleaning was done with antiseptic detergents and water. The swab samples were taken according to the methodology proposed by Park et al. (2017). Briefly, the swab was moistened with PCR grade nuclease free water moved across the sampling area horizontally, vertically and diagonally. An area of approximately, 50 cm^2 was swabbed for the toilet seat and toilet floors, 20 cm^2 for the cistern handle and internal latch and 30 cm^2 for the tap handle. The swab area was determined based on the available area of these contact surfaces. Swabs were placed in a 400 µL PCR-grade nuclease free water and transported to the laboratory on ice. The personnel carrying out the sampling were fully clothed in personal protective equipment (face masks, shields, lab coats, gloves and face shields).

2.2. Molecular detection of SARS-CoV-2

Upon arrival at the laboratory, each tube containing the swab was vortexed for 10 s and the swab carefully removed from the tube, pressing gently against the side of the tube to remove excess water. The swab was then discarded and disposed of as biohazard waste. Two approaches were used in the detection of the viral RNA in the samples. This include direct quantification without the RNA extraction step, using 5 µL of the initial sample as a template for the molecular analysis. The second approach involved extraction of RNA from the swab samples using the extraction kit followed by quantification of the RNA copy numbers as described below.

2.3. RNA extraction

Nucleic acid (RNA) was extracted directly from 140 µl of swab solution using the QiAmp Viral RNA MiniKit (Qiagen, Hilden, Germany), according to manufacturer's instructions. RNA was eluted in 80 µl of sterile nuclease free water and then quantified using the Implen Nanophotometer® NP 80 (Implen GmbH, Munich, Germany). The quality of the extracted RNA was determined based on the Nanophotometer® NP 80 results prior to amplification. The extracted RNA was then stored at −80 °C for further analysis. The second detection and quantification approach did not require RNA extraction, therefore the swab samples were vortexed vigourously and these samples were used for droplet

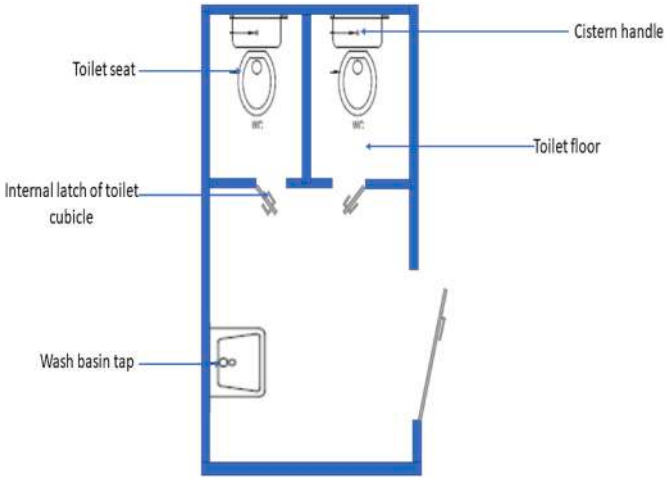

Fig. 1. Key contact surface areas within the internal surfaces of CABs that were considered in this study.

digital Polymerase Chain Reaction (ddPCR) amplification using the protocol described below (Section 2.4).

2.4. Viral detection and quantification using droplet digital PCR

RNA, which was stored for not more than 24 h at −80 °C, was thawed at room temperature and quantitified using the Implen Nanophotometer® NP 80 (Implen GmbH, Munich, Germany). All RNA samples were then diluted and standardized to 1 ng using sterile nuclease free water. Additoinally, direct detection without RNA extraction was also done to determine the suitability of this approach in detection and quantification of viral loads on the surfaces. For the detection of SARS-CoV-2, the 2019-nCoV CDC ddPCR Triplex Probe Assay (Biorad, USA), which simultaneously targets the N1 (FAM labelled) and N2 (FAM and HEX labelled) region of the SARS-CoV-2 genome was used. The assay also targets the human RPP30 (HEX labelled) gene for use as an internal control. Amplification was achieved using the One-Step RT-ddPCR Advanced Kit for Probes Supermix (Biorad, USA), which contains reverse transcriptase and 300 mM Dithiothreitol (DTT). Each ddPCR reaction mix contained 5.5 µl supermix, 2.2 µl reverse transcriptase, 1.1 µl of 300 mM DTT, 1.1 µl of 20X 2019-nCoV CDC ddPCR Triplex Probe Assay, 6.6 µl of sterile DNase free water and 5 µl of the standardized RNA template to get a final volume of 22 µl. All sample plates contained positive, negative and no template control wells. The SARS-CoV-2 positive control (Exact Diagnostics) contained synthetic RNA transcripts of 5 gene targets (E, N, ORF1ab, RdRP and S) while the negative control (Exact Diagnostics) contained human genomic DNA and RNA spiked into a synthetic matrix. Sterile nuclease-free water was used in place of RNA for the no template control. Sample plates were sealed and vortexed for 20 s. Thereafter, droplet generation was carried out using the QXDx Automated Droplet Generator (Biorad, USA), and the plates were then heat sealed with a pierceable foil. A C1000 Touch Thermal Cycler (Biorad, USA) was then used to perform PCR under the following conditions: Reverse transcription at 50 °C for 1 h, enzyme activation at 95 °C for 10 min, 40 cycles of denaturation at 94 °C for 30 s and annealing at 55 °C for 60 s. This was followed by enzyme deactivation at 98 °C for 10 min and droplet stabilization at 4 °C for 30 min with a ramp rate of 2 °C/second. The sealed droplet plate was then transferred to the QX200 Droplet Reader (Biorad, USA). The distribution of positive and negative droplets in each well was read using the QuantaSoft 1.7 software (Biorad, USA) while data analysis was carried out using the QuantaSoft Analysis Pro 1.0 software (Biorad, USA). The results were interpreted as follows: a sample is considered positive if it has any or both of the SARS-CoV-2 markers even in the absence of the RPP30 gene. Similarly, a sample is considered negative if it does not contain any of the SARS-CoV-2 markers even if it contains RPP30. Presence of the RPP30 gene is not mandatory for the presence of SARS-CoV-2. A sample run was considered invalid if there are positives in the negative and no template control wells.

2.5. Probability of COVID-19 infection from the use of shared sanitation: A case of the community ablution blocks

The four interrelated steps used in assessing the potential risks of COVID-19 infections are described below:

***Hazard Identification*:** The SARS-CoV-2 virus is the hazard of choice for this assessment. The concentration of this virus determined based on the extracted RNA was used for the risk assessment.

***Exposure assessment*:** Contact surfaces are recognized as important routes for the spread of infectious diseases, mainly through surface-hand interactions. These surfaces sometimes referred to as fomites, have been associated with different outbreaks in cruise ships, restaurants, nursing homes, schools, daycare centres and gyms (Bures et al., 2000; Aitken and Jeffries, 2001; Barker et al., 2004; Boone and Gerba, 2005). Therefore, the main exposure scenario considered in this study is hand contamination as a result of contact with the surfaces monitored. To assess the dose of the SARS-CoV-2 virus ingested via this route Fig. 2 presents the process flow.

Dose−Response Model: The dose-response relation adopted for this study is the exponential model expressed as;

$$p(d) = 1 - \exp\left(-\frac{d}{k}\right) \quad (1)$$

Where p(d) is the infection risk at a dose of d in units of PFU and k is a pathogen dependent parameter, referred to as the infectivity constant. The k was taken as 4.1×10^2 PFU for SARS-CoV. The dose response model and k were determined based on data for the infection of transgenic mice susceptible to SARS-CoV (Watanabe et al., 2010). These are adopted for the SARS-CoV-2 because SARS-CoV-2 and SARS-CoV have the same cell receptor (angiotensin-converting enzyme 2 (ACE2)) and a similar cellular tropism (Chu et al., 2020; Hoffmann et al., 2020). These dose-response parameters have been used in assessing the risks of COVID-19 infections for workers in wastewater treatment plants (Zaneti et al., 2020).

The dose d was based on the concentration of the viral RNA detected by the ddPCR analysis. This accounted for the fraction of the viral particles that are transferred from the contact surfaces to the mouth/lips or eyes. A two-step process was used to calculate the dose;

1. The efficiency of viral transfer from the contact surface to the hand was accounted for by assuming that 2 cm^2 of the surface will be touched with a transfer efficiency as presented in Table 1.
2. The potential of transfer of the viral particle on the hands to the mouth/lips or eyes.

Table 1 presents the information used to ascertain the concentration of the SARS-CoV-2 virus transferred from the contact surface to the hands and subsequently from the hands to the mouth/lips or eyes.The dose (d) also took into account the ratio of genome copies to viable SARS-CoV-2 viral particles. For this study we assumed a uniform distribution ratio between 1:100 to 1:1000 for genome copy to viable SARS-CoV-2 viral particle (Pitol and Julian, 2021). Additionally, we

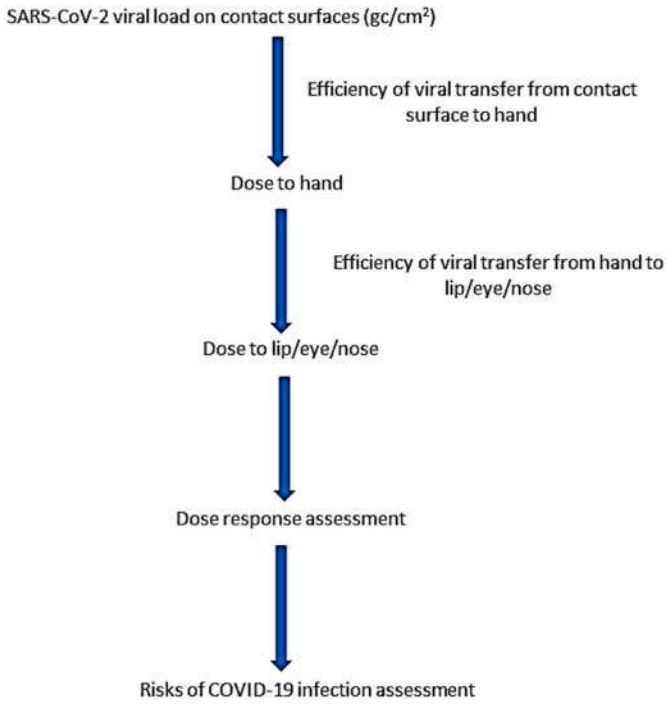

Fig. 2. Scenario for assessing the exposure and possible risks associated with contamination of the contact surfaces (Adapted from Ryan et al., 2014).

Table 1
Transfer efficiencies for determination of dose of SARS-CoV-2 transferred from contact surfaces to mouth/lips or eyes.

Parameter	Input value	Reference
Viral transfer from contact surface to hands	Uniform distribution (0.33; 0.68)	Ryan et al. (2014)
Viral transfer from hands to mouth/lips or eyes	Median value of 0.34	

factored the prevalence of contamination of the various contact surfaces into the risk assessment. This accounts for the likelihood that the contact surfaces will be contaminated at the time when a user comes into contact.

***Risk characterization*:** The outcome of the previous steps were combined to determine the risks of infection for users of the shared toilet facilities. Risk of infection from multiple exposures within a day were assessed by assuming that inhabitants use the toilet facilities between two to three times daily. Therefore the number of times of exposure per day was assumed to be uniformly distributed between 2 and 3. This was used in assessing the daily risks as well as yearly risks based on exposures for everyday in the year. This was determined due to the fact that these shared toilet facilities are the only source of sanitation access in the study area. The risks from multiple exposures was therefore determined using the following formula:

$$p(n) = 1 - (1 - p(d))^n \quad (2)$$

Where *p(n)* is the risks of infection after *n* times of exposure; and *p(d)* is the risk of infection from a single exposure. To determine the annual risks of infection, *p(d)* refers to the daily risks of infection.

2.6. Sensitivity analysis of QMRA inputs

To determine the impact of the various inputs in the QMRA analysis, the following parameters were considered, concentration of the SARS-CoV-2 (gc/cm^2), gene copy to infective viral particle ratio, the transfer efficiency of the viral particles from the surface to the hand and the number of times of exposure within a day. These parameters were varied from their minimum to maximum values. For the purpose of the sensitivity analysis only the risk of infection from exposure to the uncleaned toilet seats was considered. To determine the impact of these parameters, the calculated median infection risks were averaged and used to calculate the factor sensitivity coefficients (*FSi*), using the equation:

$$FSi = P_{i,x}/P_{baseline} \quad (3)$$

Where $P_{i,x}$ is the calculated averaged median risks per parameter, after varying the input values *x*, and $P_{baseline}$ is the baseline median infection risks.

2.7. Statistical analysis

Descriptive statistics to represent the mean and standard deviation were performed with Excel (Microsoft Corporation, USA). Comparison of viral load between the different contact surfaces was performed using the Kruskal-Wallis Test, comparison between two data categories (such as comparing viral load on cleaned and uncleaned surfaces) was done using the Mann Whitney Test. Comparative statistical analysis were all performed with GraphPad Prism Version 7 (GraphPad Software, CA, USA).

3. Results

3.1. Prevalence of contamination using extracted RNA

The chance/likelihood of contamination on the contact surfaces varied over the two sampling events. The highest prevalence of contamination of 68.8 (±20.6) % was observed for the tap handle, followed by the toilet floor with the internal latch giving the lowest prevalence of contamination (54.1 (±16.2) %) among the studied contact surfaces (Fig. 3). Despite the observed difference, there was no statistically significant difference in the prevalence (p value ≥ 0.05). This information on likelihood of contamination was used in estimating the probable risk of infection due to contact with these surfaces.

3.2. Concentration of SARS-CoV-2 on contact surfaces before and after cleaning based on extracted RNA

Per cm^2 swabbed, the mean concentration of SARS-CoV-2 was highest on the toilet seats (132.9(±39.8) gc/cm^2), followed by the cistern handle (69.1(±21.6) gc/cm^2) and internal latch (60.1(±14.5) gc/cm^2). The differences in the concentration between the different contact surfaces were statistically significant (p value ≤ 0.05).

Cleaning reduced the concentration of SARS-CoV-2 RNA on these contact surfaces, with significant (p value ≤ 0.05) reduction on the toilet seat, cistern handle, internal latch and toilet floors. For instance, after cleaning, the mean viral load on the toilet seats was reduced to 2.1 (±0.21) gc/cm^2 from the initial 132.9(±39.8) gc/cm^2 (Fig. 4). However, there was no significant reduction observed on the tap handles after cleaning (p value ≥ 0.05), as shown in Fig. 4.

3.3. Comparison of direct quantification vs quantification via extracted RNA

Detection of the SARS-CoV-2 on the swab without the initial RNA extraction step presented higher prevalence compared with the prevalence observed using the extracted RNA. For instance, via direct sample analysis, the highest prevalence was observed for cistern handle (83.3 (±29.2) %) with a corresponding prevalence of 59.3(±17.8) % when the viral RNA was extracted first before analysis. Similar trends were observed, where prevalence was consistently lower when the RNA was extracted. The only exception were swab samples from the floor, where prevalence via analysis of extracted RNA was higher (59.7(±19.3) %) compared to direct detection (50(±17.5) %) (Fig. 5A).

There was a similar trend in the viral load difference when these two approaches (direct quantification and quantification via extracted RNA) were used. For instance, via direct quantification without RNA extraction, 244.9(±85.7) g/cm^2 was recorded on the toilet seats, however when the RNA was extracted, the concentrations was reduced to 132.7 (±39.8) gc/cm^2. These differences are statistically significant (p value ≤ 0.05), indicating consistently lower concentrations when the RNA was extracted from the samples prior to analysis. However, as observed with the prevalence, the only exceptions were the floor and cistern handle

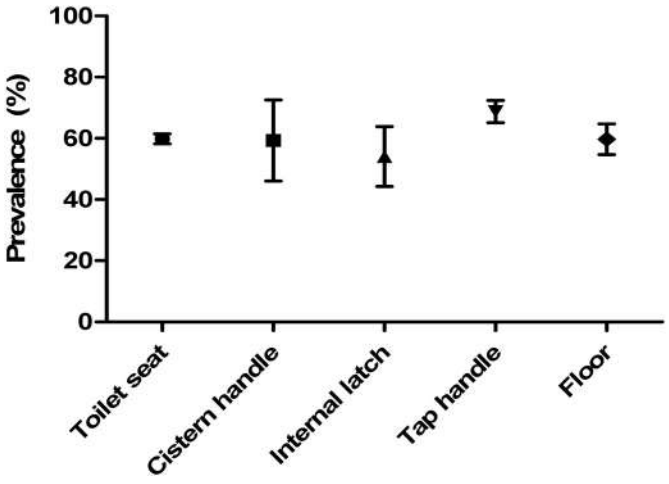

Fig. 3. Percentage of contact surfaces contaminated with SARS-CoV-2 (n = 16).

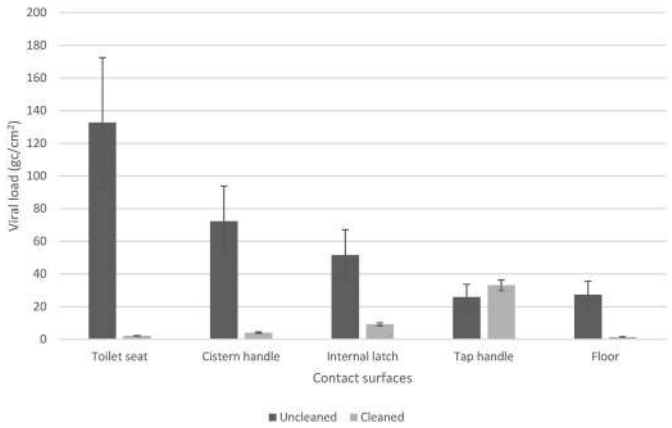

Fig. 4. Concentration of SARS-CoV-2 on key contact surfaces in the shared toilets (n = 16). *Error bars representing standard deviation.

swab samples (Fig. 5B). The RPP30 gene was present in all extracted and unextracted RNA samples regardless of whether or not they contained any of the SARS-CoV-2 genetic markers. Presence of the RPP30 gene is indicative of sufficient cellular material and proper nucleic acid extraction.

3.4. Probability of infection with COVID-19 from use of the shared toilets

The probability of infection with COVID-19 as a result of exposure to the SARS-CoV-2 virus particles on the contact surfaces varied considerably, driven mainly by the difference in the viral loads described above and the prevalence/likelihood of contamination of these surfaces. The magnitude of the risks after single exposure was similar for contact with almost all the surfaces (10^{-5}), however the highest median risks were observed for contact with the uncleaned toilet seats. It was estimated that approximately two people out of every 10 000 people using the toilet who touch the toilet seat could potentially be infected with COVID-19 (1.76×10^{-4} ($\pm 1.58 \times 10^{-6}$) per person). These estimates were made based on a single exposure event. However, considering that these toilet facilities are the only source of sanitation services within the communities studied, providing both access to potable water and sanitation, multiple exposures within a day were considered. Use of the toilet facilities twice or three times in a day was observed to increase the risks of infections with COVID-19. For instance, multiple contacts with the toilet seat within a day (daily risks) resulted in an increase in the median risks from 1.76×10^{-4} ($\pm 1.58 \times 10^{-6}$) per person for a single exposure to 4.33×10^{-4} (4.03×10^{-6}) per person for daily risks (multiple exposures in a day). This means that for every 10 000 people who use the toilet facility between two or three times in a day, about four of them may be infected. Similar significantly increased risks (p value ≤ 0.05) were observed for all the other contact surfaces (Table 2). We further observed an increase in the risks of infection with COVID-19 when exposure over the course of a year (yearly risk) is considered (Table 2), relying on the fact that these shared sanitation facilities are the only source of sanitation in the studied areas.

The risks of infection were reduced, considering exposure after the toilets have been cleaned, although not statistically significant for most of the surfaces (Table 2). Most notable reductions were exposure via contact with the toilet seat, internal latch and toilet floor. For instance, the probability of infection reduced from about two people out of 10 000 exposed people potentially been infected to about two people out of one million being infected (2.34×10^{-6} ($\pm 2.09 \times 10^{-8}$)). Similar significant reduction in probable risks were recorded after cleaning for contact with the other contact surfaces mentioned previously (p value ≤ 0.05), except the tap handle and internal latch (Table 2). As observed for multiple exposures to the uncleaned surfaces, multiple exposures to the cleaned surfaces could also increase the risks of infection, as reported in Table 2.

3.5. Parameter sensitivity in the infection risk calculation

The sensitivity analysis for the various input parameters based on their minimum and maximum input ranges showed that these values had an impact on the risk estimates calculated. However, their impact varied depending on the parameter. The gene copies of SARS-CoV-2 measured on the various contact surfaces was determined to have the highest impact on the risks estimates, with an *FSi* of 2.88 (Fig. 6). Among the four parameters chosen for the sensitivity analysis, varying the number of times of exposure within a day between twice or three times had the least impact on the risk estimates, with an *FSi* of 1.01. These results therefore, shows that the concentration of the viral particles measured could be the main parameter affecting the risks estimates.

4. Discussion

Contact surface contamination within the toilet facilities was widespread (Fig. 3), with a high prevalence of contamination on the tap handles, the floor of these toilets and the internal latch of the toilet cubicles. Several studies have reported similar findings in relation to the most contaminated surfaces in toilet facilities (McGinnis et al., 2019; Abiose, 2019; Verani et al., 2014; Sabra, 2013; De Alwis et al., 2012; Flores et al., 2011; Fankem et al., 2006). Notably, Fankem et al. (2006)

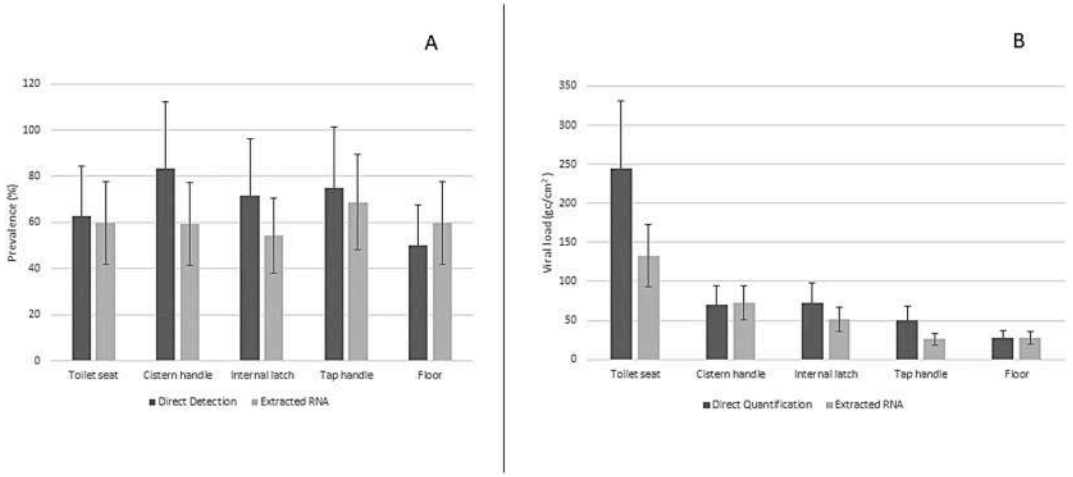

Fig. 5. Difference in the detection and quantification of SARS-CoV-2 via direct analysis and RNA extraction (n = 16): (A) comparison on prevalence and (B) viral loads. *Error bars representing standard deviation.

Table 2
Median risks (±90% CI) of infection with COVID-19 due to contact with surfaces within shared toilets.

Exposure frequency	Toilet seat		Cistern handle		Internal Latch		Tap handle		Floor	
	Uncleaned	Cleaned	Uncleaned	Cleaned	Uncleaned	Cleaned	Uncleaned	Cleaned	Uncleaned	Cleaned
One-time risk	1.76×10^{-4} ($\pm 1.58 \times 10^{-6}$)	2.34×10^{-6} ($\pm 2.09 \times 10^{-8}$)	9.16×10^{-5} ($\pm 8.20 \times 10^{-7}$)	9.00×10^{-6} ($\pm 8.07 \times 10^{-8}$)	6.10×10^{-5} ($\pm 5.47 \times 10^{-7}$)	2.03×10^{-5} ($\pm 1.82 \times 10^{-7}$)	3.95×10^{-5} ($\pm 3.54 \times 10^{-7}$)	3.67×10^{-5} ($\pm 3.29 \times 10^{-7}$)	3.79×10^{-5} ($\pm 3.39 \times 10^{-7}$)	3.13×10^{-6} ($\pm 2.80 \times 10^{-8}$)
Daily risk	4.33×10^{-4} ($\pm 4.03 \times 10^{-6}$)	5.73×10^{-6} ($\pm 5.33 \times 10^{-8}$)	2.24×10^{-4} ($\pm 2.09 \times 10^{-6}$)	2.21×10^{-5} ($\pm 2.06 \times 10^{-7}$)	1.49×10^{-4} ($\pm 1.58 \times 10^{-6}$)	4.98×10^{-5} ($\pm 4.63 \times 10^{-7}$)	9.69×10^{-5} ($\pm 9.02 \times 10^{-7}$)	8.99×10^{-5} ($\pm 8.37 \times 10^{-7}$)	9.29×10^{-5} ($\pm 8.65 \times 10^{-7}$)	7.67×10^{-6} ($\pm 7.14 \times 10^{-8}$)
Annual risks	6.03×10^{-2} ($\pm 5.22 \times 10^{-4}$)	8.22×10^{-4} ($\pm 7.41 \times 10^{-6}$)	3.17×10^{-2} ($\pm 2.80 \times 10^{-4}$)	3.16×10^{-3} ($\pm 2.84 \times 10^{-5}$)	2.12×10^{-2} ($\pm 1.89 \times 10^{-4}$)	7.12×10^{-3} ($\pm 6.39 \times 10^{-5}$)	1.38×10^{-2} ($\pm 1.23 \times 10^{-4}$)	1.28×10^{-2} ($\pm 1.15 \times 10^{-4}$)	1.32×10^{-2} ($\pm 1.18 \times 10^{-4}$)	1.10×10^{-3} ($\pm 9.91 \times 10^{-6}$)

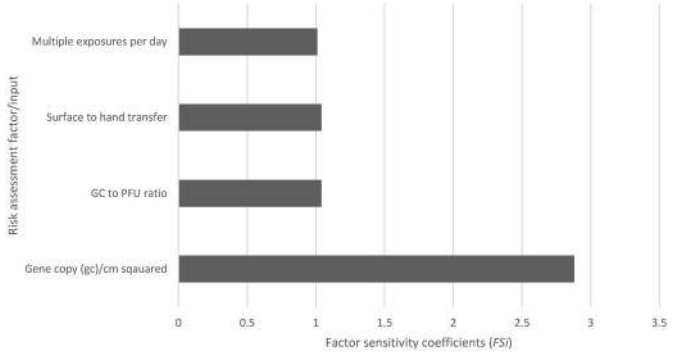

Fig. 6. Sensitivity ranking of the infection risks calculation input parameters for exposure to the uncleaned toilet seat.

observed that the most contaminated surfaces in public toilets found in airports, bus terminals, and universities were the sanitary napkin dispensers, toilet seats, sinks, and floors. However, in those studies, the frequency of contamination on these surfaces was much lower (3–21%) compared to the frequency observed in our study. Our prevalence of contamination was in accordance with the observations reported by Sabra (2013), where 91.3% toilet handles, 73% of toilet doors, 53% of toilet sink and 50% of tap handles were reportedly contaminated with bacteria. It must be noted that these findings were observed for bacterial contamination; therefore, the difference could further be due to the difference in organisms. When using a human adenovirus virus (HAdV), Verani et al. (2014) found 135 out of 172 surfaces within toilet facilities in a health care setting to be contaminated. Contamination of contact surfaces outside the sanitation setting has also been reported in hospitals (Chia et al., 2020; Ryu et al., 2020; Peyrony et al., 2020; Lei et al., 2020), home settings (Xie et al., 2020a,b; Fernández-de-Mera et al., 2020; Döhla et al., 2020) and public spaces (Fernández-de-Mera et al., 2020).

Contamination of the contact surfaces could be as a result of direct contact with feces or urine, unclean hands or even through cough or sneeze. For instance, the high frequency of contamination on the cistern handle, the tap handle and internal latch could be as a result of this direct contact with uncleaned hands. Contamination of the toilet seat and the toilet floor could also be from contaminated fecal matter and urine. The frequency of contact has been proposed as the most critical factor in the direct contamination of contact surfaces within public toilets (Fankem et al., 2006). The higher frequency of contact could therefore be responsible for the high prevalence of contamination on these contact surfaces. In addition to the frequency of use, the contamination of these contact surfaces could be an indication of hygiene. De Alwis et al. (2012) reported a high bacterial contamination on door handles used by males, whereby 50% of the users of these toilets did not wash their hands with soap. The contamination of toilet floors has been attributed to a high frequency of contact with the bottom of shoes (Flores et al., 2011). This could potentially be a significant source of contamination for other contact surfaces, such as cistern handles. A study by Flores et al. (2011) observed that the bacterial community on toilet floors was similar to those found on toilet flush/cistern handles. They attributed this to the use of foot in operating these cistern/flush handles by some of the users. This is a common practice in shared sanitation facilities.

Contamination of the toilet seat and the floors could be via indirect contact. For instance, flushing of toilets could be a significant source of contamination. Flushing results in the generation of droplets and aerosols that could be deposited on theses surfaces (Flores et al., 2011). Using modelling approaches, Li et al. (2020) postulated that massive upward transport of viral particles is observed with over 40–60% of the particles potentially deposited on the toilet seat. Contamination of the toilet seat up to 24 flushes after initial shedding in feces and urine could still occur, although the concentrations could reduce with each flush (Johnson et al., 2017). Using bacterial indicators, Johnson et al. (2017) observed $3\log_{10}$ reduction after the first flush, 1–2 $\log_{10}$ after the second and thereafter less than 1 $\log_{10}$ reduction with each flush. Therefore, SARS-CoV-2 viral particles shed in feces and urine could be deposited on the toilet seat during flushing, this could potentially be the main source of the contamination of the toilet seats. Additionally, contamination of the floor could be due to accidental urination on the floor, which could be a common phenomenon in the male toilets, although this study did not specifically measure the difference in contamination within the male and female toilets.

We observed that direct detection and quantification of SARS-CoV-2 in swab solutions gave higher prevalence of contamination and viral load (Fig. 5). The lower numbers recorded for analysis done using the RNA extraction approach, could be attributed to losses during the RNA extraction process. The higher frequency of contamination and viral load on the floor swabs determined via the RNA extraction approach as compared with the direct estimation approach could be due to the elimination of PCR inhibitors during RNA extraction as compared to other surfaces. It is worth noting that the toilet floor was constantly soiled, as a result without RNA extraction, several PCR inhibitors inherent in soil could be transferred to the amplification stage resulting in interferences. Therefore, although direct quantification of SARS-CoV-2 on contact surfaces without RNA extraction is possible and gives higher concentrations, we do not recommend it for surfaces with high solid contents, such as floors. However, direct quantification is an important approach to consider for the estimation of risks from contact with contaminated surfaces with less solids.

The difference in concentration of SARS-CoV-2 observed in this study (Fig. 4) could also be attributed to the same factors responsible for the frequency of contamination, which are fecal matter contamination, unclean hands and cough or sneeze. However, the viral load on the toilet seats per cm^2 were significantly higher (p value ≤ 0.05) than any of the

other contact surfaces. This could be attributed to the phenomenon of droplet and aerosol generation during flushing. Shedding of SARS-CoV-2 in feces and urine of both symptomatic and asymptomatic patients is well reported (Jones et al., 2020; Amirian, 2020; Bowser, 2020; Pan et al., 2020; Xie et al., 2020a,b; Peng et al., 2020; Yoon et al., 2020), therefore higher viral load on the toilet seats is to be expected. The concentrations on the other contact surfaces points towards direct contamination via uncleaned hands. Hand transmission of COVID-19 is one of the main routes of transmission, leading to hand washing as a major intervention to reduce infections (Gupta and Lipner, 2020; Lin et al., 2020; Beiu et al., 2020). The toilets are cleaned once a day, which resulted in a significant reduction of viral load on almost all the contact surfaces, except for the tap handle (Fig. 4). The viral loads detected on the internal latch and tap handle indicates that cleaning does not usually focus on these surfaces, despite a high contact frequency. The findings, therefore, show that cleaning of shared sanitation facilities should consider surfaces with high contact frequency and small crevices, such as the toilet seat, tap handle and internal latch.

The viral contamination of key contact surfaces within shared toilets could potentially result in COVID-19 infections. The estimated risks show that the highest probability of infection from a one-time use of the toilets is the contact with the toilet seat (Table 2). A manageable risk of 1.17×10^{-3} has been recommended by Zhang et al. (2020), meaning 1 person out of a thousand being infected is acceptable. In contrast Zaneti et al. (2020) derived a tolerable risk of infection for SARS-CoV-2 to be 5.5×10^{-4} per person per year (pppy), setting a very high tolerable/acceptable risk figure. Considering one-time exposures, the risks estimates from our study are lower than these recommended tolerable/acceptable risks figures. However, with multiple exposure within a day or over a year, the risks of infection with COVID-19 within our study area were higher than these tolerable or acceptable risks estimates published (Table 2). Comparatively, the risks estimated from this study are lower compared to the risks published by Zaneti et al. (2020) for workers in wastewater treatment plants (2.6×10^{-3} to 1.3×10^{-2}) per exposure. Furthermore, Pitol and Julian (2021) reported median risks of 1.6×10^{-4} to 5.6×10^{-9} when they modelled the risks of infection with COVID-19 based on surface contamination, similar to our findings. The application of QMRA to measure the potential risks of infection via surfaces, therefore shows that this may not be a significant route of infection. This could be due to the conversion ratio of the gc/cm^2 to PFU/cm^{2}of 1:100 and 1:1000 of gc/cm^2 to PFU/cm^2 which was used both in our study and the study by Pitol and Julian (2021). Reports have shown that SARS-CoV-2 viral particles shed in feces may still be infectious (Zhang et al., 2020; Wang et al., 2020; Xiao et al., 2020), however this is inconclusive due to the varying reports on their survival in the environment. It is also important to consider that the potential risk can be high due to the frequent use of these facilities by the communities. The contact time is very short due to a high population that rely on these facilities and the SARS-CoV-2 virus is reported to survive on surfaces from a few hours (Chin et al., 2020), to four days (Chin et al., 2020; Van Doremalen et al., 2020).

Cleaning could potentially reduce the risks of infection, however, in our study, we observed that despite the significant reduction in viral load after cleaning on almost all the surfaces, the potential of infections with COVID-19 was still high. Tuladhar et al.,(2012) found residual bacterial and viral contamination on surfaces after cleaning, which means the detection of the SARS-CoV-2 on the contact surfaces after cleaning could be residual viral particles. Therefore, the estimated risks on the contact surfaces after cleaning could be much lower. However, to ensure maximum protection for users of these shared toilets and other facilities with similar characteristics, other risks reduction interventions should be considered.

5. Limitation of the study

The risk or probability of infection with COVID-19 was based on the assumption of a worst-case scenario where a gene copy is considered an infectious viral particle. By using the ratio of genome copies to viable SARS-CoV-2 viral particles of 1:100 to 1:1000 (Pitol and Julian, 2021), this was addressed. However, the risk assessment based on SARS-CoV-2 viral RNA concentration could potentially result in over estimation of the associated risks, because the detection and quantification of viral RNA and inactivated viruses may still yield positive results.

6. Conclusions

We established in this study that key contact surfaces within shared toilets investigated in this study were contaminated with SARS-CoV-2, with the highest prevalence of contamination on the floor, tap and cistern handles. This shows areas of high hand contact had the highest possibility of being contaminated, indicating that uncleaned hands may be the main source of contamination. However, based on viral load per cm^2, the most contaminated surface is the toilet seat, the shedding of SARS-CoV-2 virus in feces and urine could be the main reason for this high concentration. We also showed that the presence and quantity of SARS-CoV-2 on contact surfaces could be determined directly without an RNA extraction step using ddPCR, which can potentially reduce the cost associated with such analysis. However, this is not recommended for surfaces with high solid contents, such as floors. Cleaned contact surfaces had significantly lower viral load compared to the uncleaned surfaces except for the tap handle, this shows that the potential risks of infection with COVID-19 due to contact with these surfaces could be reduced with effective and regular cleaning.

7. Recommendation/risk reduction interventions

The calculated risks of infections associated with the use of the shared toilets call for the introduction of additional measures to protect public health, especially in developing countries where large proportion of the population may rely on shared toilet facilities. Some of these risk reduction measures are:

1. **Frequent and effective cleaning:** Cleaning of the shared toiles is currently done once a day, due to the high contamination found on the key contact surfaces we recommend that cleaning be carried out at least twice. For instance, Tuladhar et al. (2012) observed that a second wipe of a contaminated surface with chlorine resulted in an extra 1–3 $\log_{10}$ reduction in concentration of various pathogens including influenza virus.
2. **Close of water closet lid during flushing:** The viral concentration on the toilet seats was the highest, this could be attributed to the shedding of SARS-CoV-2 in feces and urine. These could have been dispersed unto the toilet seat and possibly the floor during flushing. Therefore, by closing the water closet lid, the spread of the droplets or aerosols generated could be reduced, therefore limiting exposure.
3. **Hand washing with soap:** To reduce the possibility of transmission and contamination of the contact surfaces, frequent washing of hands with soap, as recommended, should be encouraged. This provide a two-way protection, firstly limits contamination of contact surfaces and secondly, reduces the possibility of infection from contaminated hands.
4. **Face masks:** Aerosols are easily generated during flushing and these may remain suspended for a while, therefore the use of face masks could provide an additional layer of protection.

Author contributions statement

All authors were involved in the conceptualization of the manuscript, data collection was performed by I.D.Amoah, L. Pillay, N. Deepnarian, O. Awolusi, K. Pillay and P. Ramlal. Writing of the original draft manuscript was done by I.D.Amoah, L. Pillay, N. Deepnarian, O. Awolusi and K. Pillay under the supervision of S. Kumari and F. Bux. Initial

reviewing and editing of the manuscript was done by I.D.Amoah, S. Kumari and F. Bux. Final revision of the and approval was done by all authors.

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Effect of operational strategies on microbial water quality in small scale intermittent water supply systems: The case of Moamba, Mozambique

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ABSTRACT

Intermittent drinking water supply affects the health of over 300 million people globally. In Mozambique, it is largely practiced in cities and small towns. This results in frequent microbial contamination of the supplied drinking water posing a health risk to consumers. In Moamba, a small town in Southern Mozambique with 2,500 water connections, the impact of changes in operational strategies, namely increased chlorine dosage, increased supply duration and first-flush, on the microbial water quality was studied to determine best practices. To that aim, water quality monitoring was enhanced to provide sufficient data on the microbial contamination from 452 samples under the different strategies. The water at the outlet of the water treatment plant during all strategies was free of *E. coli* complying to the national standards. However, *E. coli* could be detected at household level. By increasing the chlorine dosage, the number of samples that showed *E. coli* absence increased at the two sampling locations in the distribution network: in Cimento from 72% to 83% and in Matadouro from 52% to 86%. Modifying the number and duration of supply cycles showed a different impact on the water quality at both locations in the distribution network. A positive effect was shown in Cimento, where the mean concentrations decreased slightly from 0.54 to 0.23 CFU/100 mL and 16.7 to 7.3 CFU/100 mL for *E. coli* and total coliforms respectively. The percentage of samples positive for bacteria was, however, similar. In contrast, a negative effect was shown in Matadouro where the percentage of positive samples increased and the mean bacterial concentrations increased slightly: *E. coli* from 0.9 to 1.5 CFU/100 mL and total coliforms 17.6 to 23.0 CFU/100 mL. Enhanced water quality monitoring improved operational strategies safeguarding the microbial water quality. The *E. coli* contamination of the drinking water at household level could point at recontamination in the distribution or unsafe hygienic practices at household level. Presence of faecal contamination at household level indicates potential presence of pathogens posing a health risk to consumers. Increasing chlorine dosage ensured good microbiological drinking water quality but changing the number of supply cycles had no such effect.

1. Introduction

Safe drinking water is acknowledged as a basic human right (UN, 2010) and the Sustainable Development Goal (SDG) 6, target 6.1, aims to achieve "a universal and equitable access to safe and affordable drinking water for all by 2030" (UN, 2016). It is widely known that drinking unsafe water may cause exposure to pathogens, which can result in waterborne diseases, such as cholera, gastroenteritis or hepatitis E (Howard and Bartram, 2003). However, inadequate water, sanitation and hygiene still caused 829,000 diarrhoeal deaths worldwide in 2016, which corresponds to about 60% of total diarrhoeal-related mortality rates (Prüss-Ustün et al., 2019). Progress on SDG 6 is monitored using indicator 6.1.1, which is the percentage of population using "safely managed" water supplies, i.e. whether water sources are improved, accessible on premises, available when needed (for more than 12 h per day), and free from microbial contamination.

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According to the WHO/UNICEF Joint Monitoring Programme (JMP), 29% of the world population does not have access to safely managed drinking water (WHO and UNICEF, 2017). In Mozambique, diarrheal diseases play an important role in deaths and disability, and are strongly associated with precipitation (Horn et al., 2018). Several studies describe the prevalence of infections with waterborne pathogens, such as *Vibrio cholerae, Cryptosporidium* and rotavirus in Mozambique (Casmo et al., 2018; Deus et al., 2018; Semá Baltazar et al., 2017).

Over 300 million people globally rely on intermittent water supply (IWS), piped water delivered for less than 24 h per day (Kumpel and Nelson, 2016). Numerous countries in Africa, Asia and Latin America practice IWS as a normal operational strategy because water supply companies are not able to supply water continuously and sustain a positive operating pressure within the distribution network. This is also due to high levels of leakage in distribution networks (Agathokleous and Christodoulou, 2016; Galaitsi et al., 2016; Klingel, 2012). Various studies noted that IWS is multi-faceted and co-produced by lack of water resources, infrastructure deficits and the ever increasing non-revenue water (Galaitsi et al., 2016; Kumpel and Nelson, 2016). IWS can lead to the risk of waterborne diseases due to microbial contamination through ingress of pathogens in non- or low pressurized pipes through cracks or fittings, release of microbial biofilms formed under stagnant conditions during re-pressurization, recontamination during household storage, use of unsafe alternative water sources, or limited water availability for hygiene practices (Coelho et al., 2003; Kumpel and Nelson, 2016). Cases of waterborne illnesses due to IWS continue to be documented and Bivins et al. (2017) suggested that, globally, IWS may account for 17.2 million infections causing 4.5 million cases of diarrhoea and 1560 deaths each year. Which feature of IWS increases the growth of opportunistic pathogens still needs to be investigated (Bautista-de los Santos et al., 2019). When the drinking water supply is turned on after a period without supply, drinking water may contain elevated turbidity, and high concentrations of indicator bacteria can be flushed out of the pipes (Kumpel and Nelson, 2014). Pathogens may also enter the drinking water and upon consumption may cause infections (Skraber et al., 2005).

Despite the high prevalence of IWS in the world, the literature published to date on water quality in IWS systems is limited to a few studies in large urban areas (Kumpel and Nelson, 2016), refugee camps (Alazzeh et al., 2019) and one small town in Central America (Erickson et al., 2017). In particular, small towns in sub-Saharan Africa are experiencing an increase in water demand due to population growth, while the development and appropriate management of water infrastructure and services is lagging behind (Matsinhe et al., 2008). This may lead to water shortage resulting in an increase in IWS in these towns. These towns are not only heterogeneous among themselves, but are diverse within the administrative boundaries as they often have both urban and rural areas, has implications for infrastructure planning and resource allocation (Marks et al., 2020). In Mozambique, water supply is intermittent due to old transport and distribution networks, high levels of leakage, limited hydraulic capacity and increased city demand and population growth. As of 2015, small towns represent 15% of the total Mozambican population, and this share is projected to increase to 18% (about 6.5 million people) by 2030 (World Bank, 2018). The majority of the cities in Mozambique experience intermittent supply with variable water supply duration (Gumbo et al., 2003). Therefore, the aim of this study was to evaluate how different operational strategies at full scale can improve drinking water quality in an IWS system in a small town of Mozambique. We studied the impact of increased disinfectant dosage, increased supply duration and first-flush. To the authors' knowledge this is the first study to investigate the effect of operational strategies on drinking water quality in small scale IWS systems in sub-Saharan Africa. The results will be of interest for practitioners and researchers that focus on small water systems, particularly in low-resources settings.

2. Material and methods

2.1. Study area

Moamba district is located in Mozambique, in the southern part of the Maputo province and has an area of 4,628 km^2. The district consists of four towns and has a population of 83,876 inhabitants (Instituto Nacional de Estatistica, 2018). Vila de Moamba, one of the four towns of the province, has a population of 24,650 inhabitants and 83% of the population is supplied with piped drinking water.

The Water Treatment Plant (WTP) of Moamba has a capacity of 3,000 m^3/day. The source for the production of drinking water is the Incomáti river; water is abstracted 3.5 km from the WTP. After infiltration the water is pumped into a buffer tank (80 m^3), which is connected to the WTP with a pipeline. At the WTP, the river water is subjected to:

- coagulation-flocculation based on dosing of aluminium sulphate
- rapid sand filtration by six pressure filters with a capacity of 40 m^3/h each
- disinfection by dosing chlorine solution with a calculated dose of 1.8 mg CL_2/L

The WTP is operational in two shifts: from 6:00–12:00 (morning cycle) and 15:00–19:00 (afternoon cycle). The disinfected water is stored in a 500 m^3 reservoir and 150 m^3 water tower before distribution into the network. The water supply system of the WTP covers the areas of the District of Moamba and the Administrative Post of Pessene (14 km from Moamba). The distribution network has a total length of 45 km with approximately 3,336 connections. The distribution network is made of class 9 PVC with diameters ranging from 50 mm to 250 mm. The treated water is intermittently supplied to Moamba from approximately 6:00–10:00 (morning cycle) and 15:00–18:00 (afternoon cycle), whereas Pessene receives drinking water from 10:00–15:00 and 18:00–19:00.

2.2. Experimental design

2.2.1. Chlorine dosing

To assess the effect of chlorine dosing on drinking water quality, different dosages of granular high test hypochlorite ($Ca(OCl)_2$) with 65% of active chlorine were applied. A chlorine solution was prepared by diluting $Ca(OCl)_2$ in a 200 L tank and then dosed via an injector chlorinator for 48 h. The tank was fitted with a stirrer and a positive displacement diaphragm dosing pump (Grundfos DMX 14-10, Denmark). The chlorine solution was added to the filtered water to achieve a calculated dosage of 1.8 and 2.2 mg Cl_2/L, respectively. The dosing rate of the injector chlorinator was kept constant throughout the experiments. Samples were taken every hour during supply. All experiments were performed in duplicate.

During the different dosing experiments, the concentration of the chlorine dosing suspension was adjusted to achieve the desired chlorine dosage in the different experiments.

2.2.2. Daily supply cycles

During standard operations of the WTP, water is supplied to Moamba for approximately 7 to 9 h in two daily cycles. In between those two cycles the WTP continues operating and water is supplied to the village of Pessene located about 14 km from Moamba. To investigate an effect of supply duration, water was supplied continuously for 10 h and 12 h (one cycle) to Moamba only and compared with normal operation (two cycles).

2.2.3. First flush

To assess the water quality during restart of the drinking water supply after an idle time of not supplying (first flush), samples were taken every 10 min during at least 50 min at two locations in the distribution network. The first flush was studied during standard operations with two supply cycles per day, resulting in a first flush in the morning and one in the afternoon. The effect of the first flush was examined by pairwise comparison of the results of t = 0 and t = 10 min.

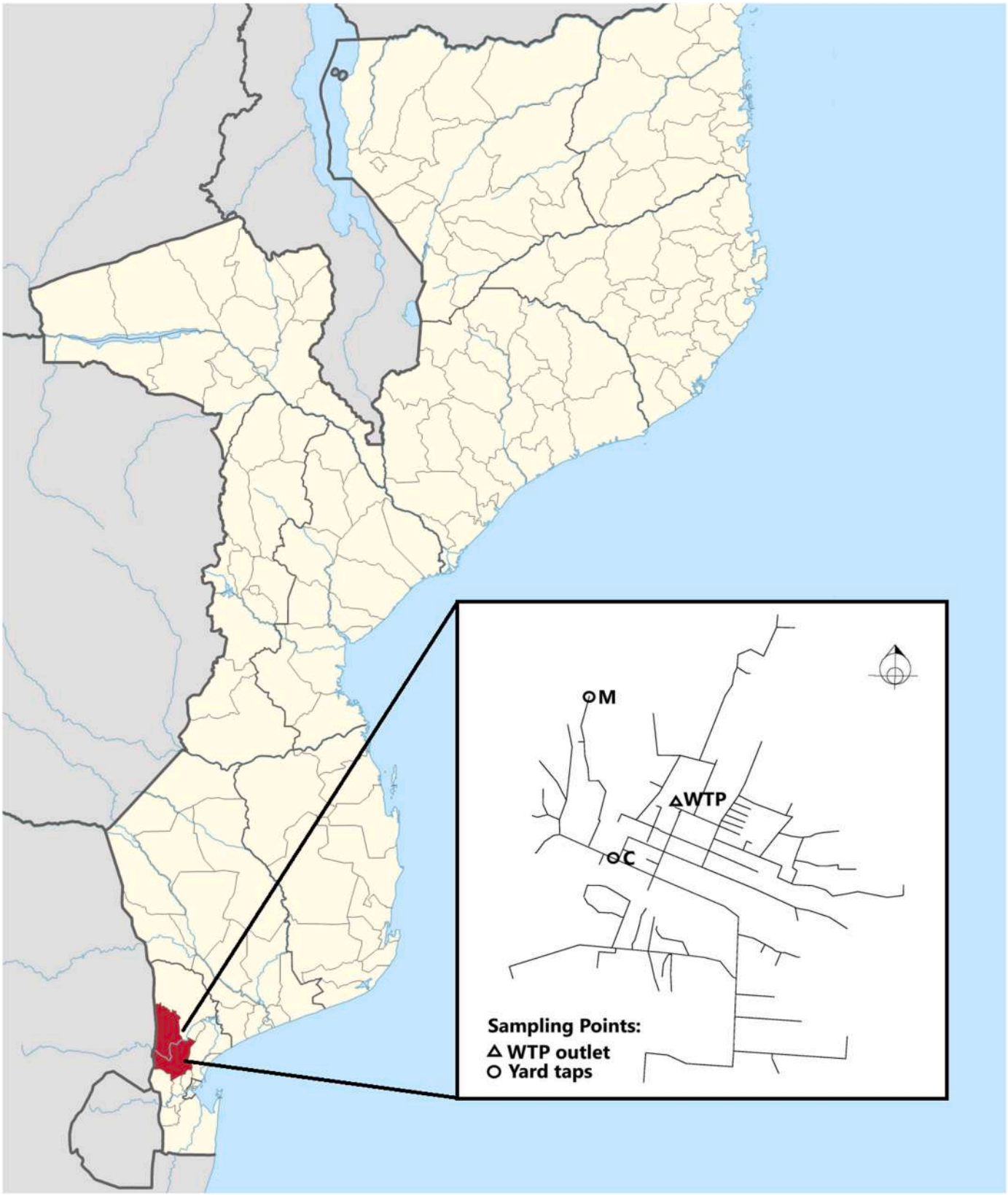

Fig. 1. Distribution network of Moamba and location of the WTP and sampling points in Matadouro (M) and Cimento (C).

2.3. Selection of sampling points

Three sampling points were selected: one at the outlet of the WTP and two household yard taps in different neighbourhoods, namely Bairro Cimento and Bairro Matadouro. The two neighbourhoods were selected based on the distance from the WTP (800 and 2,200 m, respectively) and spatial patterns of the neighbourhoods (Matadouro is a densely populated neighbourhood with lack of formal spatial planning whereas Bairro Cimento is a less dense and planned neighbourhood). The sampling points are shown in Fig. 1.

2.4. Sampling

The experiments were conducted between November 2017 and October 2018. During these experiments, samples were taken every 10 min for the first hour after starting the supply cycle and then hourly from the following locations: outlet WTP, Cimento household yard tap (C) and Matadouro household yard tap (M). The tap at the sampling points was cleaned with a clean tissue soaked with ethanol 70% and flamed before samples were taken. Samples for microbiological analyses were collected in 100 mL sterile whirl-pak thio-bags® containing sodium thiosulfate for neutralizing the residual chlorine and directly put in a cooling box for transport. The samples were stored at most 24 h prior to microbiological analyses. Samples for physico-chemical analyses were collected in 75 mL plastic cups and directly analysed in the field. In total, 717 samples were collected in this study (Table 1).

2.5. Methods

2.5.1. Physico-chemical analyses

Water temperature and pH (PT115 pH meter, Palintest, United Kingdom), free and total chlorine (PTH7100, Palintest, United Kingdom), conductivity (PT157, Palintest, United Kingdom), and turbidity (PTH092, Palintest, United Kingdom) were measured on site for 655 samples.

2.5.2. Microbiological analyses

In 452 samples enumeration of total coliforms and *Escherichia coli* was based on ISO 9308-1 (ISO, 2014), using the membrane filtration method and incubation on chromocult agar nutrient pad sets (Sartorius Stedim Biotech, Germany) for 24 h at 37 °C in a portable incubator (Aquagenx, United States), according to the manufacturer's instructions. For each sample, 100 mL was tested in duplicate. Enumeration of all dark blue to violet colonies provided the presumptive amount of *E. coli* in the filtered water volume. Salmon red colonies were coliform bacteria colonies other than *E. coli* as indicated in the suppliers' documentation.

2.5.3. Statistical analyses

Concentrations of *E. coli* and total coliforms, free chlorine concentrations and turbidity were logarithmically (base 10) transformed. Time of sampling was registered in minutes from starting drinking water supply. Water supply was from 7:00 and lasted 9, 10 or 11 h. Or, water supply was stopped after 5 h, and started again 4 h later for a duration of 4 h. Multivariate linear regression analyses of the relationship of the concentrations of *E. coli* and total coliforms respectively with the total distance from the WTP (m), time of sampling, free chlorine concentration, temperature (°C), pH, turbidity (NTU) and conductivity (micro-Siemens/cm) were conducted using R (version 3.5.2 (2018-12-20) - "Eggshell Igloo") and lm (Chambers, 1992; Wilkinson and Rogers, 1973). The model with the lowest Akaike information criterion was selected using the step-function (parameter k = 3.84). For graphical presentation of the data package ggplot2 was used (Wickham, 2016). Relations for *E. coli* and total coliforms were analysed separately, but also for the joint bacteria concentration, whereby the factor bacteria with values "*E. coli*" and "Total coliforms" was included. Similarly, a relation between both bacteria groups was analysed, as well as effects of the environmental factors, such as temperature, pH and conductivity, on free chlorine concentration and turbidity.

Table 1
Number of samples taken per experiment.

Supply duration	Calculated chlorine dosing concentration (mg Cl_2/L)		Total number of samples
	1.8	2.2	
Standard operations	275	230	505
10 h	56	54	110
12 h	64	38	102
Total number of samples	*395*	*322*	*717*

3. Results

3.1. Effect of increased chlorine dosing

Under standard operations (two supply cycles per day), increasing the calculated chlorine dosage from 1.8 to 2.2 mg Cl_2/L resulted in an increase of the mean concentration of chlorine at the outlet of the WTP by 30% from 0.79 mg/L to 1.03 mg/L, in Cimento by 77% from 0.52 mg/L to 0.92 mg/L, and in Matadouro by 75% from 0.36 mg/L to 0.63 mg/L (Fig. 2). The concentration of free chlorine observed at the same sampling points using a higher chlorine dosing concentration complied with the national Mozambican standard of 0.2–0.5 mg/L (MISAU, 2004) and the number of compliant samples at the WTP outlet increased from 90% to 100%, in Cimento from 81% to 100% and in Matadouro from 70% to 82%. The bacterial load at the outlet of the WTP showed absence of *E. coli* in all samples, and the mean concentration of coliforms was 6.5 CFU/100 mL. The concentrations of *E. coli* and total coliforms increased from the outlet of the WTP, through Cimento to Matadouro, but the difference in mean concentration with the two chlorine dosages was minimal (Fig. 2). The main difference is shown by the number of samples that showed *E. coli* absence: in Cimento it increased from 72% to 83% and in Matadouro it increased from 52% to 86% (see Supplementary Table 1). The increased chlorine dosing had an effect on the compliance with the national Mozambican standard of 0.2–0.5 mg/L (MISAU, 2004). The number of non-compliant samples containing <0.2 mg/L free chlorine at the WTP outlet decreased from 10% to 0%, in Cimento from 19% to 0% and in Matadouro from 30% to 18%. However, by increasing the chlorine dosing the number of non-compliant samples containing >0.5 mg/L free chlorine increased at the WTP from 68% to 100%, in Cimento from 45% to 75% and in Matadouro from 22% to 49%.

The free chlorine concentration under standard operations is highly significantly dependent on chlorine dose, distance from WTP and temperature (Supplementary table 2a). The residual concentration of free chlorine increased with dose (1.8–2.2 mg/L) and pH (7.2–9.2), and decreased with distance (0–2,200m) and temperature (4.2–37 °C).

Increased *E. coli* and total coliforms concentrations at higher distances from the WTP were observed (Fig. 2), this effect was statistically insignificant, probably because *E. coli* concentrations were low and the majority of data (77%, n = 232) consisted of non-detects. For more statistical power, *E. coli* and total coliform concentrations were jointly statistically analysed with bacteria as a factor. In this combined analysis, none of the conditions were found to have a significant effect (see Supplementary Table 2b). The turbidity, conductivity, pH and temperature under standard operations are shown in Supplementary Table 3.

3.2. Effect of varying daily supply cycles

Similar results were obtained for the different levels of chlorine when varying the number of daily supply cycles and the overall supply duration with a decrease in the concentration of residual chlorine over the

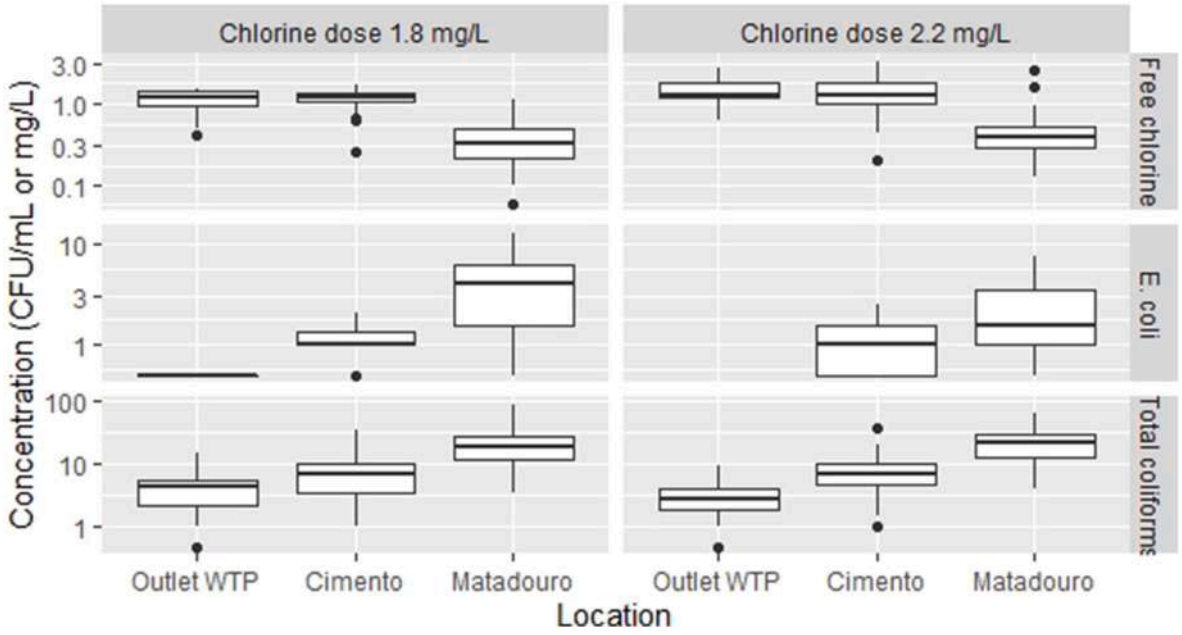

Fig. 2. ox-Whiskerplots of free chlorine, E. coli and total coliforms concentrations according to location. Each grid represents the concentration of the free residual chlorine, E. coli or total coliforms achieved with chlorine doses of 1.8 and 2.2 mg Cl_2/L. The box represents the median and quartiles, the whiskers show the 95%-interval and dots are outliers.

distance from the WTP and higher concentrations of residual chlorine by using higher dosing concentrations. For supply during one or two daily cycles, the percentage of samples positive for microbial contamination with a higher mean concentration of total coliforms and *E. coli* in Matadouro (most distant point from the WTP) than in Cimento (Table 2). Specifically, the number of samples positive for *E. coli* increased with distance from 22% in Cimento to 30% in Matadouro for two daily supply cycles and from 20% in Cimento to 42% in Matadouro for one cycle. Comparing standard operation and modified operation, no clear differences could be identified for bacterial or physico-chemical contamination. In Cimento, the percentage of samples positive for *E. coli* and total coliforms was similar while supplying one or two cycles, whereas the mean concentrations decreased with one cycle: *E. coli* decreased from 0.54 to 0.23 CFU/100 mL and total coliforms from 16.7 to 7.3 CFU/100 mL. At Matadouro the percentage of positive samples and mean concentrations of *E. coli* and total coliforms slightly increased when one supply cycle was applied. The percentage of positive samples increased from 29% to 42% for *E. coli* and from 92% to 100% for total coliforms, respectively. The mean concentration for *E. coli* and total coliforms increased from 0.9 CFU/100 mL to 1.5 CFU/100 mL and from 17.6 CFU/100 mL to 23.0 CFU/100 mL, respectively. These results show that the effect of modifying the operations can differ by location in the same distribution network.

An increase in the median and average residual concentration of free chlorine were observed at Cimento, but not at Matadouro, when changing the supply from two cycles to one cycle. Dosing experiments with 1.8 mg Cl_2/L showed a median concentration of 0.44 mg Cl_2/L and an average concentration of 0.52 mg Cl_2/L using 2 cycles, while supplying with one cycle median and average concentrations were 1.16 and 1.13 mg Cl_2/L, respectively. In Matadouro the mean and average concentration were similar, 0.32 and 0.39 mg Cl_2/L for one supply cycle versus 0.24 and 0.36 mg Cl_2/L for two supply cycles. Similar results were obtained with dosing experiments of 2.2 mg Cl_2/L. No significant change was observed for different supply durations in *E. coli* and total coliform concentrations. The bacterial concentration on $\log_{10}$ scale was highly significantly dependent on the distance, time and conductivity, see Supplementary Table 2c. Bacterial concentrations increased with distance, but decreased with increasing time and pH. Free chlorine, temperature and conductivity did not play a role according to this model. The turbidity, conductivity, pH and temperature under modified operations are shown in Supplementary Table 3.

3.3. *Effect of first flush*

In order to ascertain the effect of first flush, samples were collected every 10 min after re-starting the water supply to Moamba for the first 50 min, both in the morning and afternoon cycles. Fig. 3 shows the results of the concentration *E. coli* and total coliforms measured in the neighbourhood of Cimento (closer to the WTP) and Matadouro (further away from the WTP). The concentration of *E. coli* and total coliforms did not show a considerable increase at the beginning of the supply cycle, during the first 50 min. The mean concentration of total coliforms

Table 2
E. coli and total coliforms mean concentrations for different water supply durations.

Parameter		Standard operation Water supply during 11 h (2 cycles)		Modified operation Water supply during 10 and 12 h (1 cycle)	
		Cimento	**Matadouro**	**Cimento**	**Matadouro**
E. coli	Number of samples	105	103	76	78
	Mean concentration CFU/100 mL (min – max)	0.54 (0–11)	0.9 (0–15.5)	0.23 (0–2.5)	1.5 (0–12.5)
	Number of samples with >1 CFU/100 mL (%)	23 (22%)	31 (30%)	15 (20%)	32 (42%)
Total coliforms	Number of samples	105	103	76	78
	Mean concentration CFU/100 mL (min – max)	16.7 (0–100)	17.6 (0–75)	7.3 (0–36.5)	23.0 (0–89)
	Number of samples with >1 CFU/100 mL (%)	84 (79%)	95 (92%)	62 (80%)	78 (100%)

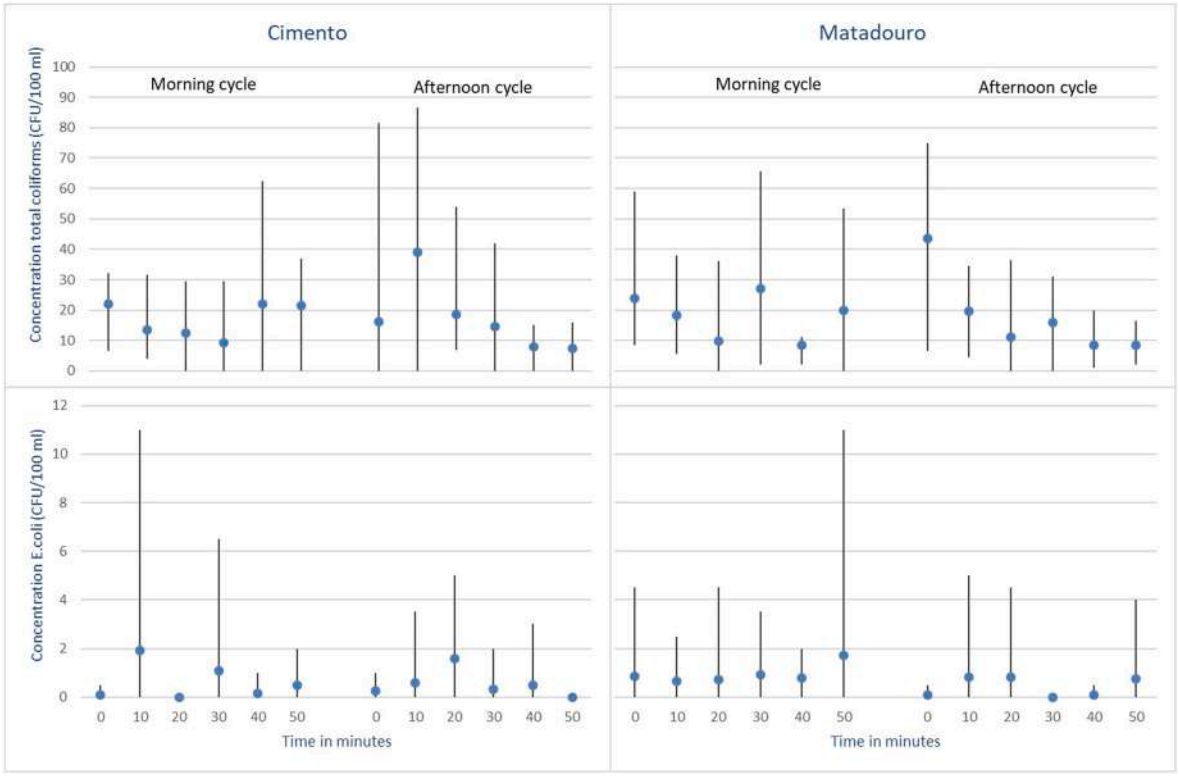

Fig. 3. Bacteriological results of the first flush after starting the distribution of drinking water during the morning and afternoon cycle. Total coliform and E.coli concentrations for Cimento and Matadouro are presented as a function of the time since the beginning of the supply cycle.

fluctuates at both locations in the morning and afternoon cycle. The mean concentration for *E. coli* in Matadouro slightly increased: in the morning cycle from 0.9 CFU/100 mL at t = 0–1.8 CFU/100 mL at t > 50 min and in the afternoon cycle 0.1 at t = 0–1.8 CFU/100 mL at t > 50. No clear correlation was found comparing every pair of measurements at 10 min intervals up to 50 min. The clearest first flush effect was expected directly after re-starting the water supply, especially by comparing t = 0 and t = 10 min. Comparing the bacterial results at t = 0 and t = 10 pairwise, showed that the concentration of coliform bacteria in Matadouro varied between a decrease of 0.47 CFU/100 mL to an increase of 0.26 CFU/100 mL, and in Cimento between a decrease of 0.20 CFU/100 mL and an increase of 0.30 CFU/100 mL. The bacterial concentration was dependent on the free chlorine concentration, but a statistical effect of time (t = 0 versus t = 10 min) was not found.

Turbidity and residual concentration of free chlorine did not show a clear increase or decrease at either of the locations. The deviation of the residual concentration of free chlorine between t = 0 and t = 10 min varied between −0.04 and 0.47 mgCl_2/L for Cimento and −0.17 and 0.43 mgCl_2/L for Matadouro. In 61% and 52% of the samples taken from Cimento and Matadouro respectively, the deviation was less than 0.1 mg Cl_2/L. The deviation in turbidity between t = 0 and t = 10 min varied between −9.4 and 1.8 NTU for Cimento and −7.0 and 2.6 NTU for Matadouro. Turbidity fluctuated between 2.2 and 27.1 NTU in Cimento and 1.2–23.5 NTU in Matadouro during all first flush experiments from t = 0 to t = 50 min, but also in this case no clear increasing or decreasing trend was identified.

4. Discussion

The aim of this study was to understand the effect of increased disinfectant dosage, number and duration of supply cycles, and first-flush on drinking water quality in an IWS system in a small town in Mozambique. When considering the indicator of faecal contamination, *E. coli*, no contamination was detected in treated water leaving the WTP, nevertheless *E. coli* was detected at the point of delivery at household level. Recontamination of the treated drinking water in the distribution could have occurred from ingress in the pipes. If faecal contamination entered the distribution system, some pathogens could have persisted even though faecal indicators were inactivated, which poses a health risk (LeChevallier et al., 2004). Similar results were obtained in large urban centres in, among others, Pakistan, India and Uganda where the water distribution systems were not capable of maintaining high water quality from the water treatment facilities to the end-user (Hashmi et al., 2008; Matsinhe et al., 2014). Based on literature, Bivins et al. (2017) showed that the available evidence suggests large variability in the prevalence of faecal contamination in IWS networks with the proportion of samples positive for *E. coli* ranging from 2% to 32%. In our study the prevalence of *E. coli* was 28% at the sampling point closer to the WTP, but as high as 48% at the furthest sampling point. For research purposes, we recommend detection of waterborne pathogens in the distribution network, such as adenovirus, rotavirus, *Cryptosporidium* and *Vibrio cholerae*, which cause infections in Mozambique (Casmo et al., 2018; Deus et al., 2018; Liu et al., 2016; Semá Baltazar et al., 2017). This information supports the need or improvement of control measures, such as chlorination, and the health risk to consumers.

An increased chlorine dose of 2.2 mg Cl_2/L improved the residual chlorine level in the distribution network by a minimum of 0.2 mg/L, thereby complying with international guidelines (WHO, 2017b) and national standards (MISAU, 2004). The residual concentration of free chlorine decreased with the distance, which is similar to other studies (Egbe and Bassey, 2016; Karikari and Ampofo, 2013; Sakomoto et al., 2020). The percentage of samples with levels of residual chlorine lower than 0.2 mg/L, 19% in Cimento and 30% in Matadouro, was much lower compared to the percentage of samples with *E. coli*, 28% in Cimento and 48% in Matadouro, and total coliforms, 81% in Cimento and 89% in Matadouro. Similar results were obtained in other studies where drinking water samples contained coliforms or *E. coli* even though the concentration of residual free chlorine was above 0.2 mg/L

(Erickson et al., 2017; Sakomoto et al., 2020). Although the number of samples complying with the national Mozambican standard of >0.2 mg/L residual chlorine increased by increasing the chlorine dose, the number of samples with concentration >0.5 mg/L and therefore not complying with the national standards (MISAU, 2004) also increased. In this study, the percentage of samples at the WTP outlet with a residual concentration of free chlorine higher than 0.5 mg/L increased from 68% to 100%, by increasing the chlorine dose from 1.8 mg Cl_2/L to 2.2 mg Cl_2/L. Of all samples from yard taps containing bacteria, 56% contained coliform bacteria even though the residual concentration of free chlorine was higher than 0.5 mg Cl_2/L. Analogously, in water samples from a WTP outlet to the tap in Ethiopia, coliforms could be detected even though containing 0.5 mg Cl_2/L free chlorine (Duressa et al., 2019). By increasing chlorine dosage, the number of samples positive for *E. coli* in the distribution network decreased (see Supplementary Table 1), in line with other studies in which a weak inverse correlation was observed between free chlorine levels and faecal coliforms (Karikari and Ampofo, 2013). If the range of residual chlorine at the WTP outlet is between 0.2 and 0.5 mg/L the concentrations at the tap very distant from the WTP may be less <0.2 mg/L. To ensure higher levels of free chlorine further in the distribution network, booster chlorination might be an option as suggested in a study in Uganda (Sakomoto et al., 2020). However, when faecal contamination of a drinking-water supply is detected, the World Health Organization recommends that the concentration of free chlorine should be increased to greater than 0.5 mg/l throughout the system as a minimum immediate response (WHO, 2017b). As *E. coli* concentrations were low and the majority of data (77%, n = 232) consisted of non-detects. For more statistical power, *E. coli* and total coliform concentrations were jointly statistically analysed with bacteria as a factor. However, no clear inverse relation was shown between increasing chlorine dosing and levels of bacteria.

In the case of Moamba, water is supplied in multiple daily cycles (Silva-Novoa Sanchez et al., 2019). In another study on IWS with multiple daily cycles in rural Nepal, consumers' perception of the level of service in terms of water quality worsens as the duration of supply decreases (Guragai et al., 2017). However, there is no evidence that the duration and number of supply cycles correlate to the water quality. In this study we increased the supply duration to up to 12 h per day, the minimum threshold used by the WHO/UNICEF JMP to track the 'available when needed' factor of target 6.1 of SDG 6. However, no association between increased availability and lower number of daily cycles (one as opposed to two) and microbial water quality was observed. In fact, the effect of modifying the operations in Moamba differed per location within the same distribution network: the bacterial concentration decreased close to the WTP outlet, and increased further in the distribution network. The residual concentrations of free chlorine at the tap closer to the WTP outlet were higher supplying one cycle compared to two cycles, but further in the distribution network the concentrations were similar. In general, microbial growth and public health implications depend on the duration of the stagnation periods, the composition of the microbial community, and disinfectants in IWS (Bautista-de los Santos et al., 2019). Microbial growth due to overnight stagnation has also been reported in continuous water supply (Lautenschlager et al., 2010; Lipphaus et al., 2014). However, our findings have not yet been followed up by further studies to investigate the causes of differentiated water quality outcomes at these specific locations. Research on the composition of the microbial community as described by Bautista-de los Santos et al. (2019) or microbial source tracking can clarify these differences or to identify possible contamination sources (Liu et al., 2018).

In this study, the effect of first flush on the microbiological water quality is not significant, although the bacterial concentrations are slightly higher at t = 0 min and t = 10 min, after starting the operation, compared with other time points. This is similar to the findings of Alabdula'aly and Khan (2017), who showed that stagnation in the distribution network affects the water quality, but not to a degree that would warrant collective actions. In contrast to our findings, other studies showed an effect of first-flush on the drinking water quality. Kumpel and Nelson (2013) showed more contamination during the first flush after the supply re-started and during periods of low pressure. In another study, the water quality was degraded during some first-flush events and after pipe breaks and repairs (Erickson et al., 2017). In the same study, higher concentrations of heterotrophic plate count and spore-forming bacteria were found during many first flush events, even when total coliform and *E. coli* were not detected (Erickson et al., 2017). Stagnation of water in the piping system caused by pressure deficits and intermittent feeding of the system entails that pathogens may enter and grow in the water distribution network (Andey and Kelkar, 2007; Jensen et al., 2002; Lee and Schwab, 2005). This hazard increases at high temperatures by running pipes close to the surface (Klingel, 2012). In this study, the water stagnated at most 14 h, and no significant difference was found between first flush events that occurred after different stagnation times. Future research is needed to better understand the importance of the effect of first-flush on pathogens.

In addition to these risks inherent to IWS, distribution systems in low and middle-income countries often have additional vulnerabilities which may degrade the water quality. Some examples are frequent pipe breaks (Lee and Schwab, 2005), poor quality control of treated water entering the distribution network (Besner et al., 2002; Lee and Schwab, 2005), and unhygienic repair practices (Besner et al., 2002). In general, for coping with the contamination ingress due to backflow through leaky joints, air valves, perforations in IWS, the WHO (2017b) recommends implementing the following control measures, where feasible: maintain positive pressure, provide continuous supply; maintain minimum chlorine residuals in the distribution network and, if necessary, install secondary/booster chlorination; implement a leak detection and repair programme; implement a pipe and fittings replacement programme; and develop design and construction specifications and standards. Climate change affects safe drinking water supply as it is expected to alter the frequency and severity of extreme weather events (WHO, 2017a). As large areas of the country are exposed to cyclones, droughts and flooding, Mozambique is vulnerable for climate change (Arndt et al., 2011). Assuming climate change alters precipitation patterns and subsequently the number of wet days diarrheal cases might increase in Mozambique (Horn et al., 2018). Therefore adaptation of the drinking water supply to climate change is required (WHO, 2017a). Implementation of a systematic risk assessment and risk management approach, such as climate-resilient Water Safety Plans, might support better understanding of possible health risks and how these can be managed, including climate change aspects (WHO, 2017a).

5. Study limitations

The results of this study are subject to a few limitations. First, experiments on the effect of supply duration at the full scale were impossible in Pessene in order not to alter the supply pattern, and water supply with even longer duration was not possible due to the existing work shifts of the utility operators. Second, only two chlorine dosages were included in this publication due to limited skills of the operator working in one of the shifts that arbitrarily decided to bypass the chlorine dosing tank and to add chlorine directly in the reservoir, making it impossible to control chlorine concentration. This episode highlighted once again the issue of limited technical capacities locally available in small towns (Tutusaus et al., 2018). Finally, in this study, only negative controls were used for microbial analyses to exclude false positive results. No positive controls were used to exclude false negatives.

6. Conclusion

The main conclusions of this study are:

- Residual concentration of free chlorine increased with dose and pH, and decreased with distance and temperature.
- No faecal contamination was detected in treated water leaving the WTP, but was assumed to enter in the distribution system. The presence of faecal contamination is indicative of the potential presence of pathogens posing a health risk for consumers.
- Increased chlorine dosage can improve compliance with microbiological water quality standards.
- The presence of chlorine resistant pathogens can still pose a risk for human health.
- The mean concentration of *E. coli* in the two sampling points in the distribution network was nearly unchanged.
- Changing the number and duration of water supply cycles showed a positive impact on microbial water quality in the sampling point closest to the WTP and negative impact in the sampling point furthest from the WTP. Thus, modifying the operations can have different impacts on the different locations in the same distribution network.
- Contrary to published literature, the effect of first flush on the microbiological water quality was not statistically significant in this study.

Declaration of competing interest

The authors declare no conflict of interest.

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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.2021.113794.

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Effects of long-term air pollution exposure on ankle-brachial index and cardio-ankle vascular index: A longitudinal cohort study using data from the Electricity Generating Authority of Thailand study

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ABSTRACT

Background: Ankle-brachial index (ABI) and cardio-ankle vascular index (CAVI) are surrogate measures of atherosclerosis based on the functional performance of vessels, and are highly related to cardiovascular events. However, only a few longitudinal studies have been conducted on their associations with long-term air pollution exposure.

Objective: This study aimed to examine whether long-term air pollution exposure is associated with ABI and CAVI in workers of the Electricity Generating Authority of Thailand (EGAT) in the Bangkok Metropolitan Region (BMR).

Methods: This longitudinal study included 1261 participants (age range, 57–76 years as of 2007) of the EGAT study (2007–2017). ABI and CAVI were measured in 2007, 2012, and 2017. Annual mean concentrations of particulate matter ≤10 μm in diameter (PM_{10}), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), ozone (O_3), and carbon monoxide (CO) were estimated by ordinary kriging using data from 22 background and 7 traffic monitoring stations in BMR between 2002 and 2017. Linear mixed-effects models were used to assess associations between air pollution (expressed as 1-year, 3-year, and 5-year average concentration) and ABI and CAVI (expressed as percent changes per interquartile range (IQR) increase in PM_{10}, O_3, NO_2, SO_2, and CO). We also applied the mixed-effect ordinal logistic models to calculate odds ratios (ORs) of having high or moderate CAVI per an IQR increase in air pollution.

Results: After controlling for potential confounders, 1-year average CO was negatively associated with ABI, but not significantly (−0.48%, 95% CI: −1.03, 0.07). Three-year average NO_2 was positively associated with CAVI (6.67%, 95% CI: 0.21, 13.1). In contrast, 1-year average PM_{10} was inversely associated with CAVI although the association was not significant. Although not significantly, 1-year average NO_2 and CO were positively associated with prevalence of high or moderate CAVI.

Conclusions: Although not statistically significant, long-term NO_2 and CO exposure was associated with ABI and CAVI in the participants of the EGAT study.

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1. Introduction

Atherosclerosis is the primary cause of ischemic heart disease and stroke. It is a chronic process in which lipids and fibrous plaque accumulate in the arteries and, coupled with inflammatory processes, cause lumen occlusion and plaque rupture (Libby and Theroux, 2005; Lusis, 2000; Ross Russell, 1993). Künzli et al. (2011) demonstrated that measures of atherosclerosis can serve as health outcomes in air pollution-related epidemiological research, especially those derived from morphological characteristics of the arterial wall (e.g., carotid intima-media thickness, coronary artery calcification) and functional performance of vessels (e.g., ankle-brachial index (ABI), arterial stiffness).

ABI is a biomarker of the degree of subclinical peripheral atherosclerosis and is measured as a ratio of systolic blood pressure at the ankle to that at the brachial artery in the arm (Heald et al., 2006; Norgren et al., 2007). The diagnosis of peripheral artery disease is indicated by ABI <0.9. Additionally, a high ABI (>1.3) suggests arterial stiffness in the lower extremities, which indicates vessel incompressibility (Aboyans et al., 2012). An increased risk of cardiovascular diseases (CVD) in relation to both low and high ABI has been suggested (Ankle Brachial Index Collaboration, 2008; Heald et al., 2006; Resnick et al., 2004).

There is evidence for relationships between ABI and exposure to air pollutants in North American and Western European countries, albeit inconsistent. A higher prevalence of low and high ABI was associated with long-term exposure to particulate matter ≤2.5 μm in diameter ($PM_{2.5}$), particulate matter ≤10 μm in diameter (PM_{10}), and nitrogen dioxide (NO_2) (Zhang et al., 2018). Long-term exposure to traffic-related NO_2 was also positively associated with high ABI, but not low ABI (Rivera et al., 2013). On the other hand, some studies found no associations between ABI and $PM_{2.5}$ and PM_{10} (Hoffmann et al., 2009; Roux et al., 2008). To the best of our knowledge, no study has been conducted in Asian countries, where air quality is relatively poor.

Cardio-ankle vascular index (CAVI) is a new non-invasive parameter of arterial stiffness which can be measured by electrocardiogram, phonocardiogram, and pulse wave velocity (PWV). It is a measure of overall stiffness of the artery from the aorta to the ankle. Since CAVI is calculated from heart-ankle PWV, it is theoretically independent of blood pressure at the time of measurement (Asmar, 2017; Miyoshi and Ito, 2016; Shirai et al., 2011; Sun, 2013; Yambe et al., 2004) and may thus serve as a better predictor of arterial stiffness (Takaki et al., 2007). Previous studies have also suggested CAVI to be a useful long-term predictor of CVD risk (Mizuguchi et al., 2007; Nakamura et al., 2008; Yingchoncharoen et al., 2012). High CAVI was associated with coronary artery disease (CAD), cerebral artery disease, and chronic kidney disease (Asmar, 2017). However, limited studies exist on CAVI in relation to air pollutants, with only two studies from Taiwan suggesting a possible positive association between CAVI and personal short-term exposure to particulate matter 1.0–2.5 μm in diameter ($PM_{1\text{-}2.5}$), ozone (O_3), and $PM_{1\text{-}2.5}$ components (Wu et al., 2010, 2012). No study has investigated the association between CAVI and long-term air pollution exposure.

The main sources of air pollutants in Bangkok are the city's suffocating traffic and agricultural burning (Chuersuwan et al., 2008). Periodically, the concentrations of PM_{10}, $PM_{2.5}$, and other gaseous pollutants such as O_3, NO_2, sulfur dioxide (SO_2), and carbon monoxide (CO) have intermittently been measured to be over the yearly, or 24-h average values indicated in WHO air quality guidelines (WHO Regional Office for Europe, 2006). Although many epidemiological studies in Thailand have demonstrated significant associations between air pollution and CVD, those studies considered only short-term effects, and long-term effects of air pollution have seldom been examined (Paoin et al., 2021). Therefore, the present study aimed to examine the association between long-term air pollution exposure and ABI and CAVI in a large retrospective cohort study, called the Electricity Generating Authority of Thailand (EGAT) study, which was conducted in workers of EGAT on the chronic disease and incidence of CVD (Vathesatogkit et al., 2012) in the Bangkok Metropolitan Region (BMR).

2. Methods

2.1. Study design and participants

Details of the EGAT study have been described previously (Vathesatogkit et al., 2012). Briefly, the EGAT cohort study consisted of three cohorts (i.e., EGAT1, EGAT2, and EGAT3). EGAT1 and EGAT3 cohorts located at the EGAT's headquarters in BMR, while EGAT2 was located at three separate hydro-electric dams at Western and Northern Thailand. The current study included the data from EGAT1 cohort from 2007 to 2017. This cohort was conducted in 1985 (followed up in 1997, 2002, 2007, 2012, and 2017) among 3499 randomly enrolled workers for CVD risk factors in nutrition and toxicology (Vathesatogkit et al., 2012). The EGAT study was approved by the Ethics Committee of Ramathibodi Hospital.

For the present longitudinal study, we extracted data of 1839 participants (age range, 57–76 years as of 2007) who lived in BMR in 2007 (Figure S1). The following criteria were used: 1) participants who were followed up 2 or 3 times during the period spanning 2007 to 2017; 2) participants who lived in Bangkok, Nonthaburi, Samut Prakarn, or Pathum Thani. We excluded 578 participants who were lost to follow-up or had died or moved out of the study area since 2007. We also excluded 46 participants with ABI <0.9 (32 participants) or >1.3 (14 participants) for the CAVI study because of possible peripheral arterial disease and calcification of ankle arteries (Marumo et al., 2018; Sato et al., 2016), respectively. These participants may give a falsely low CAVI score (Shirai et al., 2006).

ABI and CAVI were measured with a VaSera CAVI instrument (Fukuda Denshi Co., Ltd., Tokyo, Japan) using previously described methods (Shirai et al., 2006). Each measurement was performed for both the right and left ankles, yielding two sets of ABI and CAVI measurements for each participant. For both ABI and CAVI, the average of the left- and right-side measurements was obtained (Yingchoncharoen et al., 2012).

2.2. Data collection and physical examination

Physical examination data and sociodemographic characteristics such as sex, age, blood pressure, heart rate, weight, height, waist and hip circumference, body mass index (BMI), waist/hip ratio, smoking status, alcohol drinking, regular exercise, education level, income, prevalence of diseases (e.g., hypertension, diabetes, and hypercholesterolemia), treatment status, and medication use were retrieved from EGAT1 cohort data (Vathesatogkit et al., 2012).

2.3. Exposure assessment

Hourly air pollution data, including PM_{10}, SO_2, NO_2, CO, and O_3 levels, were extracted from the database of the Pollution Control Department (PCD), a governmental organization responsible for monitoring air pollution in Thailand.

We used ordinary kriging (Geniaux et al., 2017; Leem et al., 2006; Liu et al., 1996) to estimate daily average exposure to PM_{10}, SO_2, NO_2, and CO. For O_3, daily maximum 8-hr average was estimated, as described elsewhere (Paoin et al., 2021). In brief, concentrations of air pollutants measured at 22 background and 7 traffic monitoring sites in BMR from 2002 to 2017 were used to generate a long-term annual average from all discontinuous site-specific measurements. The number of monitoring sites which used to generate ordinary kriging models were varied during study period (Table S1). Grids of 100 × 100 m were generated for the prediction. For each air pollutant, the average concentration was estimated at the sub-district level based on concentrations in grids closest to the centroid of each sub-district. The assignment was based on the sub-district address of each participant (sub-district

levels).

One, three, and five-year average concentrations preceding the measurement of ABI and CAVI were used as indices for long-term exposure to air pollution, as the period of follow-up was 5 years in the present study.

2.4. Covariates

A wide range of covariates were selected from previous reports (Hoffmann et al., 2009; Rivera et al., 2013; Roux et al., 2008; Wu et al., 2010; Zhang et al., 2018). Data including age (years), sex (male/female), BMI (kg/m^2), smoking status (never-smoker/former-smoker/current-smoker), alcohol drinking (non-user/former-user/occasional-user/current-user), regular exercise (<3 times per week/at least 3 times per week), education level (0th-8th grade/9th-12th grade/>12th grade), income (<10,000 Thai baht/10,000–20,000 Thai baht/20,000–50,000 Thai baht/>50,000 Thai baht), hypertension (yes/no), diabetes (yes/no), hypercholesterolemia (yes/no), treatment of hypertension (yes/no), and treatment of diabetes (yes/no).

2.5. Statistical analysis

Linear mixed-effects models were used to analyze associations between long-term air pollution exposure (PM_{10}, O_3, NO_2, SO_2, and CO) and ABI and CAVI. Within-participant variation was treated as a random effect. Time-varying covariates (i.e., age, BMI, hypertension, diabetes, hypercholesterolemia, treatment of hypertension and diabetes, regular exercise, smoking status, alcohol drinking, income) were treated as constant during each 5-year follow-up period (2002–2007, 2007–2012, and 2012–2017). For example, covariates in year 2002 were carried forward for the period of 2002–2007, while covariates in year 2007 were carried forward for the period of 2007–2012. These covariates remained almost constant during all 5-year follow-up periods.

For each air pollutant, three predefined adjustment models were constructed, as follows: the basic model included only age and sex as covariates (Model I); the second model (Model II) included age, sex, BMI, regular exercise, smoking status, alcohol drinking, education level and income as covariates; and the third model (Model III; main model) included covariates from Model II plus hypertension, diabetes, hypercholesterolemia, treatment of hypertension and diabetes as covariates.

The main models were also stratified by different exposure windows using 1-year, 3-year, and 5-year average of air pollution concentrations before examination only for non-movers during 2007–2017. We applied two-pollutant models for each pollutant to evaluate the robustness of the one-pollutant models if more than one pollutant had a significant effect on an outcome. To assess the robustness of the results, we examined the associations using both right- and left-side ABI and CAVI, as well as the maximum and minimum right- and left-side ABI and CAVI. We also restricted analysis in the group of participants with normal ABI (ABI = 0.9–1.3). In addition, the main models were also stratified by income group, including high income (>50,000 Thai baht per month) and low to middle income (≤50,000 Thai baht per month); education levels, including high education (>12th Grade) and low education (≤12th Grade); overweight (BMI ≥ 25 or BMI < 25 kg/m^2); disease prevalence (yes or no), including hypertension, diabetes, and hypercholesterolemia at the baseline in 2007. We estimated percent changes in ABI and CAVI per interquartile range (IQR) increase in PM_{10}, O_3, NO_2, SO_2, and CO, with 95% confidence intervals (CIs).

We also tested for statistical differences between the effect estimates among different subgroups by calculating the 95% CI as shown below:

$$Q_1 - Q_2 \pm 1.96\sqrt{(SE_1)^2 + (SE_2)^2}$$

Where Q_1 and Q_2 are the estimates of two categories, and SE_1 and SE_2 are their respective standard error (Schenker and Gentleman, 2001).

We also applied mixed-effect ordinal logistic models to assess the odds ratios (ORs) per an IQR increase in air pollution on prevalence of high CAVI using the category of normal or a mild risk of atherosclerosis (CAVI<8 (low CAVI), n = 472 CAVI measurement), borderline or a moderate risk of atherosclerosis (8 ≤ CAVI < 9 (moderate CAVI), n = 884 CAVI measurement), and a high risk of atherosclerosis (CAVI ≥ 9 (high CAVI), n = 1464 CAVI measurement) as used by previous studies (Gómez-Marcos et al., 2015; Park et al., 2018; Saiki et al., 2020) per an IQR increase in air pollution. The higher value of the measures between right- and left-sided CAVI values was used for the analysis of having high or moderate CAVI versus low CAVI. All models were adjusted using the variables from the main model (Model III). All statistical analyses were performed using R statistical project version 3.6.1. P < 0.05 was considered statistically significant.

3. Results

Approximately 70% of participants in the EGAT1 cohort were male (age range, 57–76 years as of 2007) (Table 1). In 2007, almost 96% of participants lived in Bangkok and Nonthaburi, almost 40% had a high

Table 1
Basic characteristics of study participants at baseline (in 2007).

Variables	ABI study (N = 1261)	CAVI study (N = 1215)
Sex, n (%)		
Male	901 (71.5)	867 (71.4)
Female	360 (28.5)	348 (28.6)
Age, years		
Mean ± SD	63.6 ± 4.5	63.5 ± 4.5
Range	57–76	57–76
Body mass index, kg/m^2		
Mean ± SD	24.9 ± 3.5	24.8 ± 3.4
Smoking status, n (%)		
Never smoker	721 (57.2)	696 (57.3)
Former smoker	420 (33.3)	406 (33.4)
Current smoker	113 (9.0)	106 (8.7)
Alcohol drinking, n (%)		
Non-user	469 (37.2)	450 (37.0)
Former-user	167 (13.2)	161 (13.3)
Occasional-user (<1 day/week)	379 (30.1)	367 (30.2)
Current-user	234 (18.6)	225 (18.5)
Regular exercise, n (%)		
<3 times/week	941 (74.6)	904 (74.4)
At least 3 times/week	311 (24.7)	302 (24.9)
Education level, n (%)		
0 – 8th Grade	236 (18.7)	227 (18.7)
9th – 12th Grade	400 (31.7)	388 (31.9)
>12th Grade	610 (48.4)	585 (48.1)
Income (monthly), n (%)		
<10,000 Baht	138 (10.9)	134 (11.0)
10,000–20,000 Baht	150 (11.9)	145 (11.9)
20,000–50,000 Baht	338 (26.8)	329 (27.1)
>50,000 Baht	484 (38.4)	464 (38.2)
Prevalence of diseases, n (%)		
Hypertension	684 (54.2)	652 (53.7)
Diabetes	217 (17.2)	205 (16.9)
Hypercholesterolemia	593 (47.0)	569 (46.8)
Treatment status, n (%)		
Hypertension	471 (37.4)	448 (36.9)
Diabetes	201 (15.9)	191 (15.7)
City of residence, n (%)		
Bangkok	615 (48.8)	590 (48.6)
Nonthaburi	593 (47.0)	576 (47.4)
Samut Prakarn	23 (1.8)	22 (1.8)
Phathum Thani	30 (2.4)	27 (2.2)
ABI		
Median ± IQR	1.1 ± 0.07	1.1 ± 0.09
CAVI		
Median ± IQR		8.48 ± 1.35

Abbreviations: ABI, ankle brachial index; CAVI, cardio ankle vascular index; SD, standard deviation; IQR, interquartile range.

income (>50,000 baht/month) and high education level (>12th Grade), all were retired (age >55 years), around 50% had hypertension and hypercholesterolemia, and over 15% had diabetes.

Each participant was followed-up every 5 years in 2007 (1839 participants), 2012 (1189 participants) and 2017 (876 participants). The characteristics of study participants at second follow-up in 2012 were shown in Appendix (Table S2). We included 1261 participants with 2933 ABI measurement for ABI analysis, and 1215 participants with 2820 CAVI measurement for CAVI analysis. Median (range) ABI and CAVI were 1.1 (0.53–1.45) and 8.87 (1.12–14.6), respectively. All ABI and CAVI values, including average values and left- and right-side values, followed a normal distribution. During 10 years of follow-up, there were 46 participants who had abnormal ABI, including 32 participants with ABI <0.9 and 14 participants with ABI >1.3. Additionally, more than a half of CAVI measurements (1464 of 2820 CAVI measurements) had CAVI ≥ 9 which referred to a high risk of atherosclerosis.

Our model showed good model performance from 2002 to 2017; leave-one-out cross validation R^2 values were 0.99 for PM_{10}, O_3, NO_2, and SO_2, and 0.98 for CO. The model predictions also showed low bias values, whereby the cross-validation slopes (predicted vs. observed) were 0.99 for PM_{10}, O_3, NO_2, and SO_2, and 0.98 for CO.

Table 2 shows summary statistics of air pollutant exposure levels with mean, standard deviation (SD), range, and IQR for all participants (1-year average air pollution levels) from 2002 to 2017. The statistics were calculated based on the 1-year average assigned to participants (districts with more participants had more weight). In this study, the average concentration of PM_{10} was 43.3 μg/m^3, which is close to the annual PM_{10} standard in Thailand (50 μg/m^3). The 1-year average PM_{10} (standard deviation) concentrations were 46.1 (9.0), 34.3 (6.0), and 51.1 (5.5) μg/m^3 in 2007, 2012, and 2017, respectively. Annual concentrations of PM_{10} in each sub-district in 2002, 2007, 2012, and 2017 were shown in Appendix (Figure S2). NO_2 and SO_2 concentrations were lower than the respective annual standards in Thailand. There is no annual standard established for O_3 and CO in Thailand. Table 3 shows the positive correlation between the air pollutants.

Table 4 presents percent changes in ABI and CAVI per IQR increase in 1-year average levels of PM_{10}, O_3, NO_2, SO_2, and CO estimated in Models I, II, and III. PM_{10}, SO_2, and CO were negatively associated with ABI in Models I and II. In the main model (i.e., Model III), higher PM_{10}, SO_2, and CO were associated with lower ABI, although the marginally negative association was only for CO [-0.48% (95%CI: −1.03, 0.07) per IQR increment]. We observed a null association between O_3 and NO_2 with ABI in all models.

NO_2 was significantly associated with higher CAVI in Models I and II. After adjusting for history of diseases and treatment status, the result of NO_2 and CAVI became insignificant in Model III [5.73% (95%CI: −1.31, 12.8) per IQR increment]. PM_{10} was inversely associated with CAVI [-5.05% (95%CI: −10.7, 0.59) per IQR increment], although the association was not significant in Model III.

During 10 years of follow-up (2007–2017), there were 85 (for ABI study) and 81 (for CAVI study) participants who moved to other addresses but they still lived in our study area. For the non-movers, associations of 3-year and 5-year average air pollutants with ABI and CAVI were similar to those of 1-year average air pollutants (Table S3).

Table 2
Summary statistics of exposure levels for all participants (1-year average air pollution levels) during the study period.

Environmental variable	Mean ± SD	Range	IQR
PM_{10} (μg/m^3)	43.3 ± 10.0	23.6–111.2	11.9
O_3 (ppb)	31.3 ± 4.1	17.0–40.1	5.3
NO_2 (ppb)	19.4 ± 4.5	7.1–30.2	5.0
SO_2 (ppb)	3.6 ± 1.4	1.0–11.1	2.1
CO (ppm)	0.7 ± 0.1	0.3–1.6	0.2

Abbreviations: SD, standard deviation; IQR, interquartile range; ppb, part per billion; ppm, part per million.

Table 3
Correlation coefficients between air pollutants during the study period.

	PM_{10}	O_3	NO_2	SO_2	CO
O_3	0.32				
NO_2	0.44	0.28			
SO_2	0.14	0.04	0.14		
CO	0.33	0.16	0.49	0.12	

Table 4
Estimated percent change (95% CI) in ABI and CAVI per IQR increase in 1-year average levels of pollutants.

	PM_{10} (μg/m^3)	O_3 (ppb)	NO_2 (ppb)	SO_2 (ppb)	CO (ppm)
ABI					
Model I	−0.53 (−0.83, −0.24)*	0.05 (−0.35, 0.46)	0.1 (−0.2, 0.41)	−0.73 (−1.2, −0.27)*	−0.59 (−1.01, −0.17)*
Model II	−0.47 (−0.85, −0.1)*	0.16 (−0.34, 0.65)	−0.17 (−0.61, 0.27)	−0.66 (−1.2, −0.11)*	−0.77 (−1.24, −0.3)*
Model III	−0.25 (−0.66, 0.17)	−0.32 (−0.89, 0.26)	−0.17 (−0.69, 0.36)	−0.29 (−0.92, 0.34)	−0.48 (−1.03, 0.07)
CAVI					
Model I	−7.38 (−11.5, −3.24)*	−1.22 (−7.03, 4.59)	5.25 (0.89, 9.61)*	−3.87 (−10.5, 2.75)	2.17 (−3.91, 8.25)
Model II	−6.57 (−11.9, −1.24)*	−1.67 (−8.82, 5.47)	6.62 (0.37, 12.9)*	−1.58 (−9.44, 6.28)	2.08 (−4.7, 8.86)
Model III	−5.05 (−10.7, 0.59)	−0.97 (−8.94, 7.01)	5.73 (−1.31, 12.8)	0.5 (−8.08, 9.08)	3.56 (−3.96, 11.1)

Coefficients are expressed as percent change per IQR (11.9 μg/m^3 for PM_{10}, 5.3 ppb for O_3, 5.0 ppb for NO_2, 2.1 ppb for SO_2 and 0.2 ppm for CO). Significance indicated by *P < 0.05. Model I: adjusted for age and sex; Model II: further adjusted for BMI, smoking status, alcohol drinking, regular exercise, education level and income. Model III (main model): further adjusted for hypertension, diabetes, hypercholesterolemia, and treatment of hypertension and diabetes.

Although not statistically significant, 5-year average CO showed a marginally inverse association with ABI [-0.51% (95%CI: −1.13, 0.12) per 0.2 ppm]. CAVI was positively associated with 3-year [6.67% (95% CI: 0.21, 13.1) per 3.4 ppb] and 5-year average NO_2 [5.92% (95%CI: −0.45, 12.3) per 3.3 ppb].

In the sensitivity analyses (Table S4), the results for right- and left-side ABI and CAVI, as well as maximum and minimum right- and left-side ABI and CAVI, followed the same patterns as those observed for average ABI and CAVI. The results of the sub-analysis in the group of participants with normal ABI were not substantially different from those observed in all participants (Table S5).

The associations of PM_{10} with ABI and CAVI were significantly different among participants with different income levels (p-value of 0.02 for ABI and 0.08 for CAVI). Specifically, stronger negative association was observed in low-middle income compared to high income participants (Table S6 and Table S7). In addition, the association between PM_{10} and ABI was significantly different among participants with and without hypercholesterolemia (p-value = 0.03), where stronger negative estimate was observed in participants without hypercholesterolemia (Table S6). We found stronger inverse associations between CO and ABI in participants with high income and education level, and participants without diabetes (Table S6). Besides, NO_2 was significantly stronger associated with higher CAVI in participants with hypertension (p-value = 0.02), and also participants without hypercholesterolemia (p-value = 0.08) (Table S7).

Table 5 presents the ORs of having high or moderate CAVI versus low CAVI per IQR increase in 1-year average levels of PM_{10}, O_3, NO_2, SO_2, and CO estimated in the main model (Model III). Although NO_2 and CO

Table 5
The odd ratios (95%CI) of having high or moderate CAVI versus low CAVI for IQR increase in 1-year average levels of pollutants.

	High CAVI
PM_{10}	0.95 (0.83, 1.07)
O_3	0.97 (0.83, 1.14)
NO_2	1.08 (0.92, 1.26)
SO_2	1.06 (0.88, 1.27)
CO	1.15 (0.98, 1.34)

Coefficients are expressed as ORs per IQR (11.9 μg/m^3 for PM_{10}, 5.3 ppb for O_3, 5.0 ppb for NO_2, 2.1 ppb for SO_2 and 0.2 ppm for CO). Significance indicated by: * P-value < 0.05. All models were 3rd model.

were positively associated with prevalence of high or moderate CAVI, the associations were not significant. We observed no clear association between prevalence of high or moderate CAVI with PM_{10}, O_3, and SO_2.

4. Discussion

In this longitudinal study, we assessed the associations between long-term air pollution exposure and ABI and CAVI in workers of EGAT in BMR, Thailand. We found that higher 1-year average CO exposure was associated with lower ABI, but not significantly. We also observed a positive association between 3-year average NO_2 and CAVI. In contrast, 1-year average PM_{10} showed a marginally inverse association with CAVI. Although not significantly, 1-year average NO_2 and CO were positively associated with prevalence of high or moderate CAVI.

In a cross-sectional study, long-term exposure to $PM_{2.5}$, PM_{10}, and NO_2 was associated with the prevalence of abnormal ABI (ABI <0.9 or >1.3) (Zhang et al., 2018). Another cross-sectional study reported that long-term exposure to traffic-related NO_2 was positively related to a higher prevalence of high ABI (>1.3), but not low ABI (<0.9) (Rivera et al., 2013). In contrast, some recent studies found no clear associations of $PM_{2.5}$ and PM_{10} with ABI (Hoffmann et al., 2009; Roux et al., 2008). In the present study, ABI was negatively associated with long-term exposure to CO, but the association was not significant.

The mean or median of ABI levels of our study and previous studies were around 1.1 (Hoffmann et al., 2009; Rivera et al., 2013; Roux et al., 2008; Zhang et al., 2018). The average PM_{10} (43.3 μg/m^3) and NO_2 (19.4 ppb) levels in our study were much higher than PM_{10} (Roux et al., 2008; Zhang et al., 2018) and NO_2 (Rivera et al., 2013; Zhang et al., 2018) levels in previous studies, respectively.

Based on evidence from animal studies, mechanisms underlying the development of atherosclerosis due to air pollution exposure have been suggested to involve vascular damage through systemic inflammation and oxidative stress (Araujo et al., 2008; Araujo and Nel, 2009; Chen et al., 2010; Chen and Nadziejko, 2005; Niwa et al., 2007; Simkhovich et al., 2008; Sun et al., 2005; Suwa et al., 2002), autonomic imbalance, and endothelial dysfunction (Brook and Rajagopalan, 2010; Rodríguez-Mañas et al., 2009).

To our knowledge, this is the first longitudinal cohort study to examine the association between long-term air pollution exposure and CAVI. We observed a positive association between CAVI and long-term exposure to NO_2 in workers of EGAT, but no associations were observed for other pollutants. Decreased bioavailability of nitric oxide may cause altered vascular autonomic control and endothelial dysfunction, leading to changes in arterial stiffness (Tanaka and Safar, 2005; Unosson et al., 2013).

In previous cross-sectional studies, the time period of exposure to air pollution ranged from 1 year (Hoffmann et al., 2009; Zhang et al., 2018) to 10 (Rivera et al., 2013) or 20 (Roux et al., 2008) years. Two of these studies described associations between ABI and 1-year average PM and NO_2 (Zhang et al., 2018) and 10-year average NO_2 (Rivera et al., 2013), suggesting that the time period of air pollution exposure that affects ABI and CAVI is long (i.e., from 1 year up to 10 years). Furthermore, a previous pilot study detected changes in human carotid atherosclerosis using high-resolution magnetic resonance imaging in individual participants at both 16 and 24 months (Adams et al., 2004). Therefore, the progression of atherosclerosis may be observed more clearly in longer time frames. Although not significantly, we observed associations between long-term NO_2 and CO exposure and ABI and CAVI, i.e., indicators of the progression of atherosclerosis based on the functional performance of vessels.

The information set shows essential data, such as the disease prevalence, BMI, educational attainment, income, exercise habits, alcohol intake, and smoking status. Subsequently, these variables were included in the model. Ordinary kriging was developed to assess the spatial representativeness of monitoring stations and improve the exposure of air pollution estimation accuracy.

This study has several limitations. First, EGAT participants had a higher salary, education level, and socioeconomic status than the general Thai population (Vathesatogkit et al., 2012). Furthermore, this study only involved individuals of older ages, who might be more susceptible to air pollution effects. Second, traffic variables and land use data were excluded from the ordinary kriging system according to the limitation of Thailand's available information. Nevertheless, air pollution data from traffic monitoring stations were used for the assessments of traffic-related air pollution exposure (e.g., NO_2 and CO). Moreover, the limitation of ordinary kriging method is that when the limited number of monitoring stations were used for model, bias might be arisen especially in the area with one or none of monitoring station close by. However, our study area has 29 monitoring stations (22 ambient stations and 7 traffic stations) in 7762 km^2 as shown in Figure 2 of our previous study (Paoin et al., 2021). Therefore, we believe that the current approach is justifiable for this analysis. Lastly, the date of movement for each participant was not available if some participants moved to other addresses but they still lived in our study area. We assumed that they did not move during 1-year prior the ABI and CAVI measurement. However, we added sensitivity analyses using 1-year, 3-year, and 5-year average air pollution concentrations prior the examination for only non-movers.

5. Conclusions

Long-term exposure to NO_2 and CO was associated with ABI and CAVI in participants of the EGAT study, but the associations were not significant. Further longitudinal studies on air pollutants, especially $PM_{2.5}$, and their associations with CAVI and ABI will be needed to understand and elucidate potential underlying mechanisms. Besides, future studies should focus on the variations in exposure levels based on emission sources, mostly driven by the geographical variabilities.

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Ethics and consent

Approval for the study was obtained from the Ethics Committees of Ramathibodi Hospital and the Graduate School of Engineering, Kyoto University.

Declaration of competing interest

The authors declare no conflicts of interest.

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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.2021.113790.

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First nationwide exposure profile of major persistent organic pollutants among Korean adults and their determinants: Korean National Environmental Health Survey Cycle 3 (2015–2017)

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ABSTRACT

Since 2009, Korea has measured the exposure levels of major environmental chemicals and heavy metals among representative adult populations through the Korean National Environmental Health Survey (KoNEHS). However, exposure to persistent organic pollutants (POPs) has never been assessed. This study reports the serum concentrations of twenty-four POPs and their influencing factors for Korean adults (n = 1295) who participated in the KoNEHS Cycle 3 (2015–2017). The POPs included seven organochlorine pesticides (OCPs), eleven polychlorinated biphenyls (PCBs), and six polybrominated diphenyl ethers (PBDEs). Among them, three OCPs (i.e., hexachlorobenzene (HCB), *p,p′*-dichlorodiphenyltrichloroethane (*p,p′*-DDT), and *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE)) and five PCBs (i.e., PCB52, PCB118, PCB138, PCB153, and PCB180) were detected in over 60% of the samples. PBDEs were not detected at a detection frequency of 60% or above. The most frequently detected POPs were *p,p′*-DDE (99.8%, geometric mean of 128.47 ng/g lipid), followed by PCB180 (98.8%, 8.49 ng/g lipid), PCB153 (98.8%, 13.14 ng/g lipid), HCB (96.2%, 67.08 ng/g lipid), PCB138 (95.2%, 8.84 ng/g lipid), PCB118 (89.6%, 2.66 ng/g lipid), *p,p′*-DDT (80.5%, 6.68 ng/g lipid), and PCB52 (71.2%, 1.57 ng/g lipid). The concentrations of most POPs were lower than or similar to concentrations reported in national-scale biomonitoring surveys. The only exception was HCB, whose concentration was up to seven-fold higher than the concentration reported by the Canadian Health Measures Survey. Excluding HCB and PCB52, most POPs showed increasing serum levels among older adults, adults with higher body mass index, adults living in coastal areas, and more frequent fish consumption. Relatively higher POP concentrations were observed in menopausal women. This study provides the first data on POP exposure levels among the representative adult population in Korea, and the results highlight the need to integrate POPs in the national biomonitoring program.

1. Introduction

Persistent organic pollutants (POPs) are not easily dissipated in the natural environment and are prone to long-range environmental transport (Wania and Mackay, 1996). They are highly lipophilic chemicals that tend to bio-accumulate and bio-magnify in food chains (Jones and De Voogt, 1999). Owing to the demonstrated or potential adverse effects of POPs such as reproductive disorders, endocrine system disorders, and carcinogenicity, most countries have banned their use since the early 1970s (Arrebola et al., 2018; Luo et al., 2017). These POPs include toxic substances, such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) (WHO, 2003). In addition to the initial list of twelve legacy POPs, new POPs were recognized by the Stockholm Convention on POPs in 2009, including eight polybrominated diphenyl ethers (PBDEs) (Lohmann et al., 2007; Sharkey et al., 2020; UNEP, 2009). PBDEs were previously extensively used as organic flame retardants and are structurally and toxicologically similar to PCBs (Meeker et al., 2009; Rahman et al., 2001; We et al., 2011).

Following the prohibition or regulation of the production and use of POPs, their concentrations in environmental media, biota and humans have slowly but gradually decreased; however, they are still widely detected in ecosystems and human tissues owing to their persistence and bio-accumulative characteristics. Moreover, such POPs are still released from some products that were manufactured prior to the introduction of associated regulations, and they circulate in the environment through treatment processes, leading to continuous human exposure (Černá et al., 2008; Panseri et al., 2019). In Korea, similar to in many other

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Abbreviations

BMI	Body mass index
CHMS	Canadian Health Measures Survey
HCB	hexachlorobenzene
KoNEHS	Korean National Environmental Health Survey
LOD	limit of detection
OCPs	organochlorine pesticides
PBDEs	polybrominated diphenyl ethers
PCBs	polychlorinated biphenyls
POPs	persistent organic pollutants
p,p′-DDE	*p,p′*-dichlorodiphenyldichloroethylene
p,p′-DDT	*p,p′*-dichlorodiphenyltrichloroethane
PCB52	2,2′,5,5′-tetrachlorobiphenyl
PCB118	2,3′,4,4′,5-pentachlorobiphenyl
PCB138	2,2′,3,4,4′,5′-hexachlorobiphenyl
PCB153	2,2′,4,4′,5,5′-hexachlorobipheny
PCB180	2,2′,3,4,4′,5,5′-heptachlorobiphenyl

countries, various POP components have been reported in the environment and in humans (Choo et al., 2020; Han et al., 2016; Lee et al., 2015; Lim et al., 2019).

Ingestion, mostly through food consumption, has been identified as a major route of exposure to POPs in the general population (Bräuner et al., 2011; Vaccher et al., 2020). Once absorbed, POPs accumulate in the adipose tissues of organisms, and persist in the body for decades (Arrebola et al., 2014; Yu et al., 2011). In addition, accumulated POPs can be transferred from mother to fetus through the blood or to breastfed infants through breast milk. However, in Korea, POP biomonitoring in the general population is generally limited to the populations with limited sample sizes (n < 500) or specific groups such as pregnant women or infants. For example, OCP and PCB levels were measured in maternal and cord blood serum from the Children's Health and Environmental Chemicals of Korea (CHECK) cohort (Choi et al., 2018). The CHECK cohort examined pregnant women (n = 148) recruited between February 2011 and December 2011 and their newborns. Several POPs were also reported in serum (n = 401) from participants who received health examinations in Korean Cancer Prevention Study-II (KCPS-II) (Moon et al., 2017).

The Stockholm Convention on POPs recommended commitments to the prevention of further harm to human health through the monitoring and reduction of POPs in the environment (UNEP, 2009). Consequently, many countries have conducted biomonitoring surveys of POPs in the general population, to identify reference levels and evaluate temporal trends of human exposure (Coakley et al., 2018; Porta et al., 2008; Sharkey et al., 2020). National biomonitoring surveys are essential for monitoring current exposure and evaluating the effectiveness of regulations governing the manufacture, import, and use of chemicals (Singh et al., 2019). In Korea, according to Article 14 of the Environmental Health Act, the Korean National Environmental Health Survey (KoNEHS), was initiated in 2009, (Choi et al., 2017; Park et al., 2016). KoNEHS aims to periodically estimate the representative values of environmental chemicals in the Korean population, at three-year intervals, and identify the major factors influencing their distribution. However, until Cycle 3, POPs were not included among the list of chemicals to be measured, leaving a big knowledge gap for the group of persistent environmental chemicals.

The aim of the present study was to provide the profile of POP exposure in a representative Korean adult population using an adult subpopulation that participated in the KoNEHS Cycle 3 (2015–2017). The results of this survey will help find POPs of concern among Korean adult population, and improve the design of KoNEHS to incorporate major POPs in future surveys.

2. Methods

2.1. Study population

This study was conducted using archived serum samples of adults who had participated in KoNEHS Cycle 3 (2015–2017). KoNEHS is an ongoing cross-sectional biomonitoring program that explores human exposure levels to major environmental contaminants in the general adult population in Korea, the factors associated with the exposure, and several key clinical indicators. Unlike the first two cycles which were focused on the adult population only, KoNEHS Cycle 3 extended its coverage to include children and adolescents. For adults, the same sampling strategy and field survey processes of KoNEHS Cycle 2 were applied. For this survey, fasting was not requested for the participating subjects. Detailed information on the KoNEHS research design can be found in previous studies (Choi et al., 2017; Park et al., 2016).

Representative subpopulations (n = 1295) were randomly selected among 3787 adult participants (19 years and older) of the KoNEHS Cycle 3, following classification into 8 groups by sex and age (19–39, 40–49, 50–59, 60 years and older), and consideration of the distribution of Korean adults in the 2015 census (Statistics Korea, 2016).

The participants were surveyed using face-to-face interviews, and data that could be linked to exposure to environmental substances, such as demographics, socioeconomic characteristics, transportation habits, indoor environments, and lifestyles, were collected. In addition, dietary habits were investigated using the food frequency questionnaire.

The present study was approved by the Institutional Review Board of the National Institute of Environmental Research (NIER), Korea (NIER-2015-BR-006-01).

2.2. Measurement of serum persistent organic pollutants (POPs)

Seven OCPs, 11 PCBs and 6 PBDEs were selected as target compounds and measured in the serum samples. These chemicals were chosen because of their high bioaccumulation and toxicity potentials. The measured OCPs included hexachlorobenzene (HCB), beta-HCH, gamma-HCH, *o,p′*-DDT, *p,p′*-DDT, *o,p′*-DDE and *p,p′*-DDE. PCBs included PCB52, PCB105, PCB118, PCB126, PCB138, PCB153, PCB157, PCB167, PCB169, PCB180 and 189. PBDEs included PBED28, PBDE47, PBDE99, PBDE100, PBDE153 and PBDE154.

The serum POPs concentrations were measured from serum that had been archived at −70 °C, using high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS, Agilent 6890/JEOL JMS-800D) (CDC, 2006; Moon et al., 2014). After adding an internal standard to the samples, they were stirred for 15–20 s and left to stand for 15 min. Subsequently, 1 mL ultrapure water was added and stirred. The solid phase extraction method was used to analyze 0.5 mL of the sample for POPs. An NH_2 cartridge that can easily distinguish C18 fatty acids was used for the efficient extraction of organic substances while excluding water-soluble substances. Silica gel (1 g) and Florisil (0.5 g) cartridges were used to remove other substances that could interfere with the extracted solvent thereafter, an internal standard was added through a syringe in a nitrogen-enriched atmosphere at ≤35 °C, and HRGC/HRMS analysis was performed.

Quality control (e.g., linearity and slope of the calibration curve, limit of detection [LOD], accuracy, and precision) was performed to ensure the reliability of the analysis and for verification of the analytical method. Quality and accuracy of the analytical method were assessed externally by participating in the German External Quality Assessment Scheme (G-EQUAS). LOD represents the minimum concentration of the substance that can be detected. It was determined by multiplying the standard deviation, which was obtained from seven experiments, with 3.143 (t-value) (α = 0.01; 99% significance level) after spiking the concentration to 2–5 times the expected LOD. The estimated LODs of the OCPs, PCBs, and PBDEs were 0.86–1.37, 0.41–0.76, and 0.35–0.92 ng/g lipid, respectively (Table 2).

Table 1
Demographic, socio-economic, and behavioral characteristics of the adult participants.

	n	%
Total	1295	100
Sex		
Male	637	49.19
Female	658	50.81
Age (years)		
19–29	187	14.44
30–39	275	21.24
40–49	266	20.54
50–59	252	19.46
60–69	206	15.91
≥70	109	8.42
Residence area		
Rural	195	15.06
Urban	1065	82.24
Coastal	35	2.70
Education level		
Middle school or lower	280	21.62
High school	412	31.82
College or higher	603	46.56
Monthly household income (US$/month)		
Low (<1,300)	153	11.81
Middle low (1,300–2,600)	210	16.22
Middle high (2,600–3,600)	276	21.31
High (≥3,600)	648	50.04
Not answered	8	0.62
Smoking status		
Non-smoker[a]	1035	79.92
Smoker	260	20.08
Body mass index (BMI)[b]		
Normal	496	38.30
Overweight	327	25.25
Obese	472	36.45

[a] Non-smoker includes former smokers.

[b] BMI (kg/m^2): Normal (<23.0), Overweight (23.0 ≤ BMI < 25), Obese (≥25).

The serum total lipid were analyzed by colorimetry using an UV spectrophotometer (Libra, Biochrom, UK). Quality control was ensured by analyzing the reference materials (D-Tek LLC, USA). The range of analytical measurement was 0.0003–5000 mg/dL. A lipid-adjusted concentration of POPs (ng/g lipid) was used for the analysis. The final lipid-adjusted concentrations were calculated by dividing the results with the total lipid concentrations.

2.3. Statistical analysis

The concentrations of POPs in the serum of subjects had a skewed distribution; therefore, they were log-transformed before statistical analysis. The results that were below the LOD were substituted with a value of LOD/$2^{1/2}$ (Croghan and Egeghy, 2016). The geometric mean and 95% confidence intervals of the lipid-adjusted concentrations of the POPs were calculated for descriptive statistics. When the values less than the LOD accounted for more than 40% of the total amount, we reported the proportions and the values greater than the LOD (Health Canada, 2015). Spearman correlation analysis was used for analyzing relationships among the POPs concentrations. In addition, the associations of POPs concentrations with each variable were analyzed using t-tests and Analysis of Variance. The following variables were included: sex (dichotomous), age (categorical), residence area (categorical), education level (categorical), smoking status (dichotomous), fish consumption (categorical), body mass index (categorical), and menopause status (dichotomous). Multiple regression analysis was performed to examine the factors associated with POP exposure. The relevant covariates were selected based on previous studies (Arrebola et al., 2018; Černá et al., 2008; Hardell et al., 2010). For the multiple regression analysis, covariates were included in the models, including age (19–29, 30–39, 40–49, 50–59, 60–69, or ≥ 70 years), sex (male or female), BMI (normal, overweight, or obese), and residence area (urban, rural, or coastal area). Factor analysis was performed to explore the association between the determined factors. Two factors with minimum eigenvalues greater than one were selected. Factor loadings greater than 0.4 were considered high. The statistical significance was determined at $p < 0.05$. All the statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, US).

3. Results

3.1. Characteristics of the participants

The general characteristics of the participants are presented in Table 1. The participating adults (n = 1295) were 637 males and 658 females with a mean age of 47 years. The majority of the proportion showed a normal BMI (<23, 38.3%) and resided in urban areas (82.2%).

3.2. Distributions of the concentrations of POPs

Among the 24 POPs (7 OCPs, 11 PCBs and 6 PBDEs) that were analyzed in the collected serum samples, those with detection rates of 60% or higher were three OCPs (HCB, *p,p′*-DDT and *p,p′*-DDE) and five PCBs (PCB52, PCB118, PCB138, PCB153 and PCB180). The most frequently detected POPs included *p,p′*-DDE (99.8%), followed by PCB180 (98.8%), PCB153 (98.8%), HCB (96.2%), PCB138 (95.2%), PCB118 (89.6%), *p,p′*-DDT (80.5%) and PCB52 (71.2%) (Table 2). None of the PBDEs were detected in over 60% of the samples; 2,2′,4,4′-tribromodiphenyl ether (PBDE47) had the highest detection rate (48.6%). Details of the serum concentrations are listed in Table S1.

3.3. Concentrations of organochlorine pesticides

The serum concentrations of several OCPs exhibited differences based on the demographic, socioeconomic, or behavioral characteristics of the participating adult population (Table 3). For HCB, which had a geometric mean of 67.08 ng/g lipid, males showed higher levels than females (73.39 vs. 61.49 ng/g lipid), and individuals aged 40–49 years had the highest concentrations (77.51 ng/g lipid). HCB levels tended to be higher among women before menopause (69.43 vs. 53.46 ng/g lipid). In the case of *p,p′*-DDT (geometric mean, 6.68 ng/g lipid), females had slightly but significantly higher levels (6.82 vs. 6.53 ng/g lipid). The *p,p′*-DDT levels tended to be higher among the older population, those living in coastal area, with lower levels of education, frequent fish consumers, and with relatively high BMI. In addition, women after menopause tended to exhibit higher *p,p′*-DDT concentrations. In the case of *p,p′*-DDE (geometric mean, 128.47 ng/g lipid), the trends based on sex, age, residence, education, fish consumption, BMI, and menopause were similar to those of *p,p′*-DDT.

3.4. Concentrations of polychlorinated biphenyls

Among the PCBs, PCB153 was present at the highest concentrations, with a geometric mean of 13.14 ng/g lipid, followed by PCB138 (8.84 ng/g lipid), and PCB180 (8.49 ng/g lipid) (Table 3). All PCBs concentrations were lower in females than in males, although statistical significance was not observed for certain PCBs like PCB118. The concentrations of all PCBs, excluding PCB52, were significantly higher among older adults, the residents in coastal areas, those with low education levels, frequent fish consumers, and the women after menopause ($p < 0.01$).

Table 2
Distributions of concentrations of measured persistent organic pollutants ($n = 1295$).

Analyte	LOD[a] (ng/g lipid)	Percentage below LOD[b]	GM[a] (ng/g lipid)	Percentile (ng/g lipid)				
				25th	50th	75th	95th	Max
OCP								
HCB	1.24	3.8	67.08	52.39	82.42	126.28	219.15	1026.22
beta-HCH	1.02	43.0	–	<LOD	4.76	9.45	22.03	79.08
gamma-HCH	1.37	91.5	–	<LOD	<LOD	<LOD	4.27	59.76
o,p′-DDT	0.86	99.5	–	<LOD	<LOD	<LOD	<LOD	445.06
p,p′-DDT	1.06	19.5	6.68	4.36	8.97	15.28	31.00	135.19
o,p′-DDE	1.01	100	–	–	–	–	–	–
p,p′-DDE	1.18	0.2	128.47	72.84	121.63	215.83	548.32	2565.63
PCB								
PCB52	0.51	28.8	1.57	<LOD	1.77	4.08	9.72	34.77
PCB105	0.41	46.0	–	<LOD	0.51	1.16	2.70	14.0
PCB118	0.72	10.4	2.66	1.50	2.75	4.94	10.96	44.21
PCB126	0.66	99.1	–	<LOD	<LOD	<LOD	<LOD	32.14
PCB138	0.52	4.8	8.84	5.87	10.13	17.12	35.01	151.73
PCB153	0.67	1.2	13.14	7.56	13.33	23.83	52.45	189.35
PCB157	0.67	91.0	–	<LOD	<LOD	<LOD	0.93	14.23
PCB167	0.62	76.1	–	<LOD	<LOD	<LOD	1.59	6.50
PCB169	0.41	97.5	–	<LOD	<LOD	<LOD	<LOD	16.30
PCB180	0.76	1.2	8.49	4.70	8.58	15.75	37.18	260.51
PCB189	0.47	88.1	–	<LOD	<LOD	<LOD	0.82	26.64
PBDE								
PBDE28	0.35	86.6	–	<LOD	<LOD	<LOD	1.87	13.08
PBDE47	0.59	51.4	–	<LOD	<LOD	1.71	4.80	120.68
PBDE99	0.61	85.9	–	<LOD	<LOD	<LOD	2.28	37.78
PBDE100	0.72	91.7	–	<LOD	<LOD	<LOD	1.07	12.01
PBDE153	0.92	63.6	–	<LOD	<LOD	2.28	7.16	75.31
PBDE154	0.74	95.1	–	<LOD	<LOD	<LOD	<LOD	6.85

[a] LOD: limit of detection; GM: geometric mean.

[b] If >40% of the samples were below the LOD, the percentile distribution is reported but mean was not calculated.

3.5. Factors associated with serum POP levels

The results of the multiple regression analysis, which was conducted with sex, age, residence area, education level, smoking status, and fish consumption frequency as independent variables revealed several demographic, socioeconomic, and behavioral factors influencing the serum POP levels among Korean adults (Table 4). Most substances, were present at lower concentrations in females than in males, tended to increase with an increase in age. In terms of residential area, the concentrations of *p,p′*-DDE, PCB153, and PCB180 were more positively correlated with living in urban or coastal areas than rural areas. The concentrations of all POPs, excluding HCB and PCB52, were also positively correlated with fish consumption more than once a week ($p < 0.05$, $p < 0.01$). The concentrations of *p,p′*-DDE, PCB52, PCB138, PCB153, and PCB180 were positively correlated with levels of education (college education level or above).

Furthermore, the correlation analysis revealed significant correlation between HCB and PCB52 (correlation coefficient = 0.515) (Table S2). Factor analysis also showed that HCB and PCB52 were grouped as a common factor (Factor 2, Table S3). In addition, *p,p′*-DDT, *p,p′*-DDE, PCB118, PCB138, PCB153, and PCB180 were grouped together (Factor 1), and had positive correlations with age, residential area, education level, and fish consumption (less than once per week); however, Factor 2 exhibited positive correlations with education level and fish consumption; however, the correlations were not significant.

4. Discussion

4.1. POP distribution among Korean adult population

This study showed that major POPs are widely present in the serum samples of a representative population of Korean adults, similar to in other countries (CDC, 2009; Health Canada, 2010). The occurrence patterns of most POPs, such as PCBs and PBDEs, among Korean adults, were generally similar to those reported in other national biomonitoring programs, except for HCB. The serum concentrations of HCB (67.08 ng/g lipid) observed among Korean adults were approximately seven-fold higher than those measured in Canada, and approximately twice as high as those in Spain (Table 5). HCB is an organochlorine compound that was used as a fungicide for many years, and shows a broad half-life that can be as high as to 22.9 years (anaerobic biodegradation in soil) (Barber et al., 2005). Even after inclusion in the Stockholm Convention on POPs (Miret et al., 2019), it is still released into the environment as a byproduct of several industrial processes (Starek-Świechowicz et al., 2017). Several studies have investigated various HCB exposure pathways (Barmpas et al., 2020; Bravo et al., 2017; Harmouche-Karaki et al., 2018; Saoudi et al., 2014). The reason for the high HCB levels in serum of Korean adults, however, is not clear, and is subject to the results of future investigations. In Korea, HCB emissions are reported to have increased from 2006 to 2009 owing to incineration processes in non-metallic and ferrous industries (Kim and Yoon, 2014).

Most PCBs assessed in this study were present at concentrations lower than those reported in similar biomonitoring studies in other countries (Table 5). In the present population, PCB153 was present at the highest concentrations among the measured PCBs. In the body, PCB153 and PCB180 have low removal rates because of their prolonged half-lifes in adipose tissue, and extremely slow metabolism (Phillips et al., 1989; We et al., 2010). In general, hexa- and hepta-chlorinated congeners are detected at high concentrations in human serum samples (Glynn et al., 2000), for example, PCB138, PCB153, and PCB180 (Health Canada, 2010; Haines et al., 2017; Porta et al., 2010; Singh et al., 2019; Wattigney et al., 2015).

Apparently, PCB52 concentrations are not significantly correlated

Table 3
Serum concentrations of POPs by demographic, socioeconomic, and behavioral characteristics (ng/g lipid).

	HCB		*p,p′* -DDT		*p,p′*-DDE		PCB52		PCB118		PCB138		PCB153		PCB180	
	GM	(95% CI)	GM	(95% CI)	GM	(95% CI)	GM	(95% CI)	GM	(95% CI)	GM	(95% CI)	GM	(95% CI)	GM	(95% CI)
Total	67.08	(63.07–71.35)	6.68	(6.24–7.14)	128.47	(122.77–134.43)	1.57	(1.47–1.67)	2.66	(2.54–2.80)	8.84	(8.36–9.35)	13.14	(12.51–13.80)	8.49	(8.08–8.92)
Sex																
Male	73.39	(66.46–81.04)*	6.53	(5.86–7.28)	129.43	(121.90–137.42)	2.03	(1.84–2.24)**	2.68	(2.49–2.89)	9.48	(8.63–10.41)*	15.52	(14.48–16.64)**	10.57	(9.81–11.40)**
Female	61.49	(57.10–66.21)	6.82	(6.29–7.39)	127.54	(119.15–136.53)	1.22	(1.13–1.32)	2.64	(2.48–2.82)	8.26	(7.76–8.80)	11.18	(10.46–11.95)	6.86	(6.45–7.30)
Age (years)																
19–29	72.55	(61.09–86.17)**	3.35	(2.80–4.00)**	63.13	(58.80–67.77)**	1.47	(1.24–1.75)**	1.47	(1.34–1.62)**	4.43	(3.84–5.11)**	5.70	(5.09–6.39)**	2.89	(2.64–3.18)**
30–39	60.91	(52.32–70.90)	3.24	(2.78–3.77)	77.16	(72.46–82.16)	1.71	(1.48–1.97)	1.60	(1.47–1.74)	5.74	(5.10–6.47)	8.26	(7.62–8.95)	5.08	(4.73–5.45)
40–49	77.51	(68.47–87.76)	8.96	(7.90–10.15)	114.75	(106.69–123.42)	2.03	(1.76–2.34)	2.04	(1.85–2.25)	7.03	(6.21–7.96)	11.80	(10.81–12.88)	8.43	(7.83–9.08)
50–59	71.00	(61.62–81.81)	8.41	(7.26–9.75)	174.75	(158.44–192.73)	1.57	(1.37–1.80)	4.14	(3.77–4.54)	12.73	(11.53–14.05)	18.75	(17.12–20.54)	12.46	(11.44–13.56)
60–69	52.46	(45.04–61.11)	12.78	(11.49–14.23)	252.62	(228.94–278.74)	1.08	(0.93–1.26)	5.16	(4.71–5.65)	17.33	(15.83–18.97)	25.67	(23.36–28.21)	17.23	(15.61–19.02)
≥70	73.36	(63.77–84.38)	11.35	(9.60–13.44)	283.49	(245.73–327.06)	1.47	(1.20–1.81)	5.24	(4.57–6.01)	18.10	(15.99–20.48)	28.56	(25.21–32.36)	21.62	(18.86–24.79)
Residence area																
Rural	69.24	(59.00–81.26)	7.46	(6.31–8.81)*	131.42	(114.62–150.69)**	1.44	(1.22–1.68)	2.96	(2.63–3.32)**	9.35	(8.25–10.60)**	13.61	(12.10–15.32)**	8.39	(7.39–8.52)**
Urban	66.31	(61.90–71.03)	6.43	(5.97–6.93)	126.09	(120.13–132.35)	1.59	(1.48–1.71)	2.58	(2.44–2.72)	8.56	(8.03–9.12)	12.83	(12.15–13.54)	8.38	(7.93–8.85)
Coastal	79.94	(64.10–99.70)	11.05	(6.69–18.25)	199.64	(156.44–254.76)	1.56	(1.03–2.37)	3.91	(2.79–5.47)	17.33	(12.97–23.15)	22.38	(16.60–30.19)	13.48	(9.39–19.35)
Education level																
≤ Middle school	60.71	(53.38–69.05)	11.36	(10.23–12.62)**	202.97	(185.16–222.50)**	1.21	(1.06–1.37)**	4.71	(4.35–5.10)**	13.96	(12.73–15.29)**	21.09	(19.37–22.95)**	13.99	(12.77–15.32)**
High school	65.12	(58.32–72.70)	7.42	(6.62–8.31)	140.21	(129.06–152.33)	1.60	(1.42–1.79)	2.73	(2.51–2.98)	9.28	(8.41–10.26)	14.12	(12.94–15.41)	9.65	(8.86–10.52)
≥ College	71.70	(65.45–78.55)	4.85	(4.37–5.39)	97.86	(92.32–103.72)	1.74	(1.59–1.91)	2.01	(1.88–2.15)	6.91	(6.35–7.52)	10.04	(9.36–10.76)	6.16	(5.75–6.61)
Smoking status																
Non-smoker	67.09	(62.90–71.57)	6.83	(6.35–7.35)	131.48	(124.78–138.55)*	1.47	(1.37–1.57)**	2.80	(2.65–2.95)**	8.83	(8.30–9.39)	13.03	(12.32–13.77)	8.39	(7.94–8.87)
Smoker	67.02	(56.57–79.40)	6.09	(5.16–7.18)	117.12	(107.36–127.78)	2.03	(1.75–2.35)	2.18	(1.95–2.44)	8.88	(7.78–10.13)	13.59	(12.25–15.07)	8.88	(7.93–9.93)
Fish consumption																
Rarely	65.13	(53.62–79.12)	5.13	(4.26–6.18)**	105.27	(91.80–120.71)**	1.34	(1.10–1.65)	2.22	(1.94–2.55)**	7.30	(6.16–8.64)**	10.93	(9.49–12.59)**	6.46	(5.52–7.56)**
< Once a week	66.19	(59.86–73.19)	5.50	(4.92–6.15)	110.82	(103.12–119.09)	1.59	(1.43–1.76)	2.30	(2.13–2.48)	7.87	(7.21–8.59)	11.26	(10.44–12.15)	7.32	(6.76–7.93)
≥ Once a week	68.20	(62.61–74.29)	8.18	(7.46–8.98)	150.01	(140.90–159.70)	1.60	(1.47–1.75)	3.10	(2.89–3.32)	10.07	(9.29–10.91)	15.36	(14.33–16.46)	10.08	(9.43–10.78)
BMI																
Normal	69.33	(62.77–76.58)	5.41	(4.84–6.04)**	115.11	(106.74–124.13)**	1.60	(1.45–1.78)	2.26	(2.10–2.44)**	8.07	(7.40–8.81)*	11.48	(10.60–12.43)**	7.86	(7.24–8.53)
Overweight	71.30	(63.04–80.64)	7.01	(6.14–8.01)	136.46	(124.97–149.01)	1.55	(1.37–1.76)	2.86	(2.60–3.14)	8.93	(7.94–10.04)	13.93	(12.58–15.42)	9.05	(8.23–9.95)
Obese	62.11	(56.06–68.82)	8.05	(7.24–8.96)	138.27	(128.47–148.83)	1.54	(1.38–1.71)	3.01	(2.77–3.27)	9.66	(8.80–10.60)	14.54	(13.47–15.69)	8.81	(8.10–9.57)
Menopause[a]																
No	69.34	(63.75–75.43)**	4.26	(3.79–4.78)**	81.09	(75.54–87.03)**	1.50	(1.35–1.68)**	1.67	(1.56–1.79)**	5.66	(5.24–6.10)**	7.05	(6.55–7.58)**	4.43	(4.15–4.74)**
Yes	53.46	(47.16–60.59)	11.79	(10.96–12.68)	216.16	(197.20–236.91)	0.95	(0.86–1.06)	4.52	(4.20–4.87)	12.84	(11.87–13.89)	19.14	(17.61–20.81)	11.41	(10.58–12.32)

*$p < 0.05$, **$p < 0.01$.
[a] Women only ($n = 658$).

Table 4
Factors determining serum concentrations of POPs based on multiple regression analysis.

	HCB		*p,p′* -DDT		*p,p′*-DDE		PCB52		PCB118		PCB138		PCB153		PCB180	
	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)
Sex																
Male	ref.															
Female	−0.20	(−0.32, −0.07)**	0.04	(−0.09, 0.16)	−0.05	(−0.12, 0.02)	−0.52	(−0.64, −0.39)**	−0.04	(−0.12, 0.04)	−0.18	(−0.28, −0.08)**	−0.37	(−0.45, −0.30)**	−0.51	(−0.57, −0.44)**
Age (years)																
19–29	ref.															
30–39	−0.15	(−0.36, 0.06)	−0.07	(−0.28, 0.13)	0.20	(0.08, 0.32)**	0.18	(−0.03, 0.39)	0.06	(−0.07, 0.20)	0.27	(0.10, 0.43)**	0.38	(0.25, 0.51)**	0.60	(0.49, 0.71)**
40–49	0.09	(−0.12, 0.30)	0.95	(0.74, 1.15)**	0.60	(0.48, 0.72)**	0.35	(0.14, 0.56)**	0.31	(0.18, 0.45)**	0.47	(0.30, 0.64)**	0.74	(0.61, 0.87)**	1.12	(1.00, 1.23)**
50–59	0.00	(−0.21, 0.22)	0.86	(0.65, 1.08)**	1.03	(0.91, 1.16)**	0.11	(−0.11, 0.32)	1.01	(0.87, 1.15)**	1.07	(0.90, 1.24)**	1.21	(1.08, 1.35)**	1.54	(1.42, 1.65)**
60–69	−0.28	(−0.50, −0.05)*	1.28	(1.05, 1.50)**	1.40	(1.27, 1.53)**	−0.21	(−0.44, 0.01)	1.23	(1.09, 1.38)**	1.40	(1.22, 1.58)**	1.56	(1.42, 1.70)**	1.91	(1.79, 2.03)**
≥70	0.02	(−0.25, 0.29)	1.18	(0.91, 1.44)**	1.52	(1.37, 1.68)**	0.03	(−0.24, 0.30)	1.26	(1.08, 1.43)**	1.42	(1.20, 1.63)**	1.63	(1.46, 1.79)**	2.08	(1.93, 2.22)**
Residence area																
Rural	ref.															
Urban	−0.08	(−0.25, 0.09)	0.05	(−0.12, 0.23)	0.16	(0.06, 0.26)**	0.02	(−0.15, 0.20)	0.06	(−0.06, 0.17)	0.09	(−0.05, 0.23)	0.12	(0.01, 0.23)*	0.20	(0.10, 0.29)**
Coastal	0.14	(−0.26, 0.55)	0.30	(−0.11, 0.70)	0.33	(0.09, 0.56)**	0.07	(−0.34, 0.48)	0.18	(−0.08, 0.45)	0.52	(0.20, 0.84)**	0.38	(0.13, 0.63)**	0.34	(0.12, 0.56)**
Education level																
≤ Middle school	ref.															
High school	0.05	(−0.14, 0.24)	0.06	(−0.14, 0.25)	0.19	(0.08, 0.30)**	0.19	(−0.01, 0.38)	−0.07	(−0.20, 0.06)	0.11	(−0.05, 0.26)	0.13	(0.01, 0.24)*	0.24	(0.14, 0.34)**
≥ College	0.15	(−0.07, 0.36)	−0.05	(−0.27, 0.16)	0.22	(0.09, 0.34)**	0.23	(−0.01, 0.44)*	−0.06	(−0.20, 0.08)	0.17	(0.00, 0.34)*	0.16	(0.03, 0.29)*	0.24	(0.12, 0.35)**
Smoking status																
Non-smoker	ref.															
Smoker	−0.13	(−0.30, 0.04)	0.06	(−0.11, 0.23)	0.04	(−0.06, 0.13)	0.05	(−0.12, 0.22)	−0.14	(−0.25, −0.03)*	0.10	(−0.04, 0.23)	0.04	(−0.06, 0.14)	0.02	(−0.07, 0.11)
Fish consumption																
Rarely	ref.															
< Once a week	0.01	(−0.20, 0.22)	0.07	(−0.14, 0.28)	0.06	(−0.06, 0.18)	0.13	(−0.08, 0.34)	0.04	(−0.10, 0.17)	0.08	(−0.09, 0.24)	0.02	(−0.11, 0.15)	0.12	(0.01, 0.23)*
≥ Once a week	0.04	(−0.17, 0.24)	0.34	(0.13, 0.54)**	0.21	(0.10, 0.33)**	0.13	(−0.07, 0.34)	0.20	(0.06, 0.33)**	0.17	(0.01, 0.33)*	0.15	(0.02, 0.27)*	0.23	(0.12, 0.33)**
BMI																
Normal	ref.															
Overweight	0.01	(−0.15, 0.17)	0.15	(−0.01, 0.31)	0.04	(−0.06, 0.13)	−0.07	(−0.23, 0.09)	0.11	(0.01, 0.22)*	−0.04	(−0.17, 0.08)	0.02	(−0.08, 0.12)	−0.07	(−0.16, 0.01)
Obese	-0.14	(-0.28, 0.01)	0.26	(0.11, 0.40)**	0.01	(-0.07, 0.10)	-0.11	(-0.26, 0.03)	0.13	(0.03, 0.22)**	-0.01	(-0.13, 0.11)	0.00	(-0.09, 0.09)	-0.18	(-0.26, -0.10)**
Menopause[a]																
No	ref.															
Yes	−0.31	(−0.58, −0.05)*	0.14	(−0.10, 0.38)	0.31	(0.12, 0.50)**	−0.36	(−0.63, −0.08)*	0.40	(0.22, 0.57)**	0.19	(0.00, 0.37)*	0.24	(0.06, 0.42)*	0.21	(0.05, 0.37)*

Adjusted for age, sex, BMI, residence area. [a]Women only ($n = 658$). *$p < 0.05$, **$p < 0.01$.

Table 5
Comparison of POPs concentrations among national-scale biomonitoring programs.

Chemical	Country[a]	Year of survey	Age (years)	*n*	GM[b] (ng/g lipid)	(95% CI[c]) (ng/g lipid)
HCB	Korea	2015–2017	≥20	1295	67.08	(63.07–71.35)
	Canada	2007–2009	20–79	1666	9.09	(8.02–10.30)
	US	2003–2004	≥20	1373	15.5	(14.7–16.2)
	Spain	2009–2010	18–65	712	28.50	(25.56–31.38)
	Belgium	2002–2006	50–65	1530	56.3	(54.6–58.0)
	Belgium	2012–2016	50–65	201	13.7	(12.1–15.5)
***p,p′*-DDT**	**Korea**	**2015–2017**	**≥20**	**1295**	**6.68**	**(6.24–7.14)**
	Canada[d]	2007–2009	20–79	1664	–	
	US[d]	2003–2004	≥20	1370	–	
***p,p′*-DDE**	**Korea**	**2015–2017**	**≥20**	**1295**	**128.47**	**(122.77–134.43)**
	Canada	2007–2009	20–79	1666	152.05	(127.03–182.00)
	US	2001–2002	≥20	1540	338	(303–376)
	US	2003–2004	≥20	1368	268	(217–332)
	Spain	2009–2010	18–65	934	158.8	(149.8–168.4)
	Belgium	2002–2006	50–65	1530	418	(394–444)
	Belgium	2012–2016	50–65	201	224	(199–253)
PCB52	**Korea**	**2015–2017**	**≥20**	**1295**	**1.57**	**(1.47–1.67)**
	Canada[d]	2007–2009	20–79	1661	–	
	US	2003–2004	≥20	1300	2.59	(2.36–2.84)
	Czech	2006	18–58	202	5	–
PCB118	**Korea**	**2015–2017**	**≥20**	**1295**	**2.66**	**(2.54–2.80)**
	Canada	2007–2009	20–79	1666	4.43	(3.78–5.20)
	Czech	2006	18–58	202	14	–
PCB138	**Korea**	**2015–2017**	**≥20**	**1295**	**8.84**	**(8.36–9.35)**
	Canada	2007–2009	20–79	1668	10.13	(8.92–11.51)
	US	2003–2004	≥20	1298	17.7[e]	(16.5–19.0)
	Spain	2009–2010	18–65	1880	31.89	–
	French	2006–2007	18–74	386	70.8	(64.4–77.7)
	Czech	2006	18–58	202	186	–
PCB153	**Korea**	**2015–2017**	**≥20**	**1295**	**13.14**	**(12.51–13.80)**
	Canada	2007–2009	20–79	1666	18.31	(15.83–21.16)
	US	2001–2002	≥20	1549	32.6	(29.5–36.1)
	US	2003–2004	≥20	1300	23.7	(22.3–25.1)
	Spain	2009–2010	18–65	1880	43.64	–
	French	2006–2007	18–74	386	113.3	(102.1–125.7)
	Czech	2006	18–58	202	423	–
PCB180	**Korea**	**2015–2017**	**≥20**	**1295**	**8.49**	**(8.08–8.92)**
	Canada	2007–2009	20–79	1666	15.21	(13.52–17.11)
	US	2001–2002	≥20	1547	23.0	(20.8–25.5)
	US	2003–2004	≥20	1298	19.0	(17.9–20.1)
	Spain	2009–2010	18–65	1880	55.97	–
	French	2006–2007	18–74	386	93.7	(83.1–105.5)
	Czech	2006	18–58	202	374	–

[a] Korea : Korean National Environmental Health Survey Cycle 3 (KoNEHS), This study; Canada : Canadian Health Measures Survey (CHMS); US : National Health and Nutrition Examination Survey (NHANES); French : *Etude National Nutrition Sante* (French National Nutrition and Health Survey, ENNS); Spain : BIOAMBIENT.ES (Spanish adult population); Belgium : Flemish Environmental and Health Survey (FLEHS); Czech : CZ-HBM project (Czech Human Biomonitoring).

[b] GM: geometric mean.

[c] CI: confidence interval.

[d] If > 40% of samples were below the LOD, the percentile distribution is reported but mean was not calculated.

[e] PCB135 + PCB158 (2009).

with most factors because it has the shortest half-life, resulting in lower accumulation in the body compared to other substances (Ritter et al., 2011; Rossi et al., 2010).

The serum concentrations of *p,p′*-DDE were similar to those reported in Canada and Spain, and lower than those observed in the US and Belgium (Table 5). The mean concentration of *p,p′*-DDE (128.47 ng/g lipid) was considerably higher than that of *p,p′*-DDT (6.68 ng/g lipid). DDT is metabolized over time to form *p,p′*-DDE, whose half-life is ≥ 8.6 years in serum (Wolff et al., 2000); therefore, it tends to persist longer than the parent compound (Arrebola et al., 2013; WHO, 1979).

PBDEs, which were added by the Stockholm Convention on POPs as new POPs in 2009, are expected to have high bio-persistence and toxicity. Information about their accumulation in the human body has attracted considerable attention from researchers (Kang et al., 2010; Kim et al., 2012; Moon et al., 2012). However, in the present study, the geometric means of the PBDEs could not be determined because the detection rates of all the PBDEs were lower than 60%. PBDE47 was present at a detection rate of 48.6%, which is the highest among the PBDEs, but it was difficult to estimate its accumulation level. Comparable results were reported by the Canadian Health Measures Survey

(CHMS) (Health Canada, 2010) and in pregnant women in the US (Zota et al., 2018). The CHMS survey, for instance, reported PBDE47 detection rate as the highest (75%), whereas the other PBDEs had lower detection rates, which is similar to the findings of the present study (Health Canada, 2010). Similar patterns have been observed in pregnant women in Korea (Choi et al., 2014).

4.2. Factors influencing serum POP levels

Depending on persistence and bio-accumulative potency, most POPs, excluding those with low molecular weight and lipophilicity (e.g., HCB and PCB52) showed serum concentration trends influenced by demographic factors such as age, sex, BMI, and menopause status, and other factors such as education, residence, smoking, and fish consumption (Tables 3 and 4).

4.3. Sex

Most POPs had higher concentrations in males than in females. The concentrations of POPs tended to be higher in menopausal women, except HCB and PCB52 (Tables 3 and 4). Furthermore, most POPs, except *p,p′*-DDT, PCB52, and PCB118, were slightly lower in women with breastfeeding history, but the differences were not statistically significant (Table S1). Excretion mechanisms specific to females, such as menstruation, and breastfeeding, may explain the sex difference (Hardell et al., 2010; Ibarluzea et al., 2011; Jovanović et al., 2019; Louis et al., 2011; Windham et al., 2005). The present observations are comparable to those that reported lower levels of POPs in women.

4.4. Age

In the present study, all substances, excluding HCB and PCB52, were significantly correlated with age, and PCB180 exhibited a particularly high positive correlation with age (Table 4). Age dependent increase in serum POPs levels has been documented in many studies. Among 444 subjects participating the Korean Cancer Prevention Study-II (2004–2011), the serum concentrations of *p,p′*-DDT, *p,p′*-DDE, PCB118, PCB138, PCB153, and PCB180 were positively correlated with age in both males and females ($p < 0.05$) (Moon et al., 2014). Similarly, according to the CHMS, the concentrations of PCBs and *p,p′*-DDE increased with an increase in age (Singh et al., 2019). In Korea, while the first regulation on POPs was implemented in the late 1960s, PCBs were first prohibited through the Electric Utility Act in 1979 (Kim and Yoon, 2014; Wattigney et al., 2015). Therefore, greater exposure that had taken place before 1970s among older Koreans could explain the relatively high serum concentrations (Moon et al., 2014; Kim and Yoon, 2014). Moreover, the age-related increase in POPs (PCBs and *p,p′*-DDE) in humans could be explained by an increase in half-life with age, and a reduction in the elimination rate with an increase in age (Bates et al., 2004; Černá et al., 2008; Hardell et al., 2010; Porta et al., 2012).

4.5. BMI

The concentrations of *p,p′*-DDT, *p,p′*-DDE, PCB118, PCB138, and PCB153 significantly increased with increasing BMI (Table 3). The observed relationship between serum POP levels and BMI is similar to observations reported from several human populations (Arrebola et al., 2014; Karmaus et al., 2009; Qin et al., 2010). The concentrations of POPs were higher in the group with the highest BMI (Hardell et al., 2010). The participants with a BMI of 24.5–35 had the highest concentrations of PCB153 (Wood et al., 2016). The only exception was PCB180 concentrations, which exhibited negative correlations with BMI in the present population (Table 4), which is also similar to the negative association reported previously between PCB180 and BMI (Dirinck et al., 2011). Although PCB serum concentrations could be lower in people with relatively high BMI because they are disproportionally accumulated in lipids (Agudo et al., 2009; Dirinck et al., 2011; Wolff et al., 2005), the reasons for such potentially negative relationships should be investigated further, since other factors such as age could also influence the serum POP levels (Collins et al., 2007; We et al., 2010).

4.6. Residence and fish consumption

Both residence in coastal areas and frequent fish consumption were significantly associated with higher POPs, which may be because of subjects living in coastal areas exhibiting relatively high fish and shellfish intake habits. Among the individuals living in coastal areas, none ate fish rarely, while 31.4% ate fish meals less than once a week, and 68.6% ate fish more than once a week (data not shown). Previous studies have reported that the primary sources of exposure to POPs are seafood, including fish, and dairy products (Lee et al., 2017; Schecter et al., 2010). PCB concentrations in serum as well as in breast milk and adipose tissue have been reported to be relatively high among individuals consuming fish (Qin et al., 2010).

4.7. Smoking

The serum concentrations of *p,p′*-DDE and PCB118 observed in the present study were higher in non-smokers than in smokers, which is similar to the observations reported from previous studies (Černá et al., 2008; Moon et al., 2017). The only exception was PCB52 which was higher among smokers. Other studies have reported that serum concentrations of PCBs are positively correlated with smoking (Deutch et al., 2003; Lackmann et al., 2000), while Apostoli et al. (2005) observed no significant correlation with smoking. Considering inconsistent observations among studies, smoking may not be a direct source of exposure to OCPs and PCBs, and could be associated with other sources of exposure to such compounds.

4.8. Strengths and limitations

This study employs a subset of the representative Korean adult population and provides, for the first time, the POP exposure profile of a representative Korean population based on KoNEHS Cycle 3 (2015–2017) data. Relatively high HCB serum levels were observed in the survey, which warrants further surveillance for the identification of risk groups and determinants of the sources of exposure. It should be noted that the POP concentrations available for some countries are based on the surveys conducted years before the present study, and may not allow direct comparison with those of the present study. In addition, since the present study was a cross-sectional study, it had limitations in terms of explaining the causal relationships between POPs in human serum and the factors influencing them. Therefore, additional analyses are required to determine the factors influencing the levels of exposure to POPs.

5. Conclusions

Korean adults are exposed to a wide range of POPs. Although most POPs were detected at levels similar to or lower than the levels reported in other national biomonitoring programs, HCB levels were several folds higher among Korean adults. Several demographic and behavioral factors were identified as influencing the serum POP levels among Korean adults. The present report will help facilitate the identification and prioritization of the chemicals of concern that warrant further environmental health management efforts. Major POPs will be added in the list of target chemicals in Cycle 5 (2021–2023) which will be measured among the general population. Such biomonitoring efforts would help identifying priority POPs among the general population of Korea and develop, exposure reduction policies.

Declaration

The results and conclusions in this report are those of the authors and do not necessarily represent the views of the Ministry of Environment and the National Institute of Environmental Research of Korea. The authors declare no competing financial interest.

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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.2021.113779.

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Healthcare provider satisfaction with environmental conditions in rural healthcare facilities of 14 low- and middle-income countries

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ABSTRACT

Healthcare provider (HCP) satisfaction is important for staff retention and effective health service delivery. Inadequate resources, understaffing, and ineffective organizational structure may reduce HCP satisfaction in low- and middle-income countries (LMICs). Some qualitative studies have described links between environmental conditions and job satisfaction in HCPs; however, few studies have explored this link using survey data. This study explores associations between HCP satisfaction and water, sanitation, and hygiene (WaSH) infrastructure, cleanliness, and infection prevention and control (IPC) practices in rural healthcare facilities (HCFs) in LMICs.

This study analyzes 2002 HCFs in rural areas of 14 LMICs. Generalized linear mixed-effects logistic regression models were used to analyze the association between HCP satisfaction, WaSH infrastructure, and cleanliness and IPC practices.

Most respondents reported that they were unsatisfied with water (65%), sanitation (68%), and hygiene infrastructure (54%) at their HCF. Insufficient supply and poor quality of WaSH resources were the most commonly reported reasons for provider dissatisfaction. Respondents were less likely to report dissatisfaction with cleanliness and IPC practices (36%). Dissatisfaction with cleanliness and IPC were most reported because patients and staff did not wash their hands at the correct times or with proper materials, or because the facility was not clean. Several characteristics of the WaSH environment were significantly associated with provider satisfaction at their HCFs, including acceptable water quality, readily available supply of water (on premises and improved), accessible supply of WaSH infrastructure to people with reduced mobility, accessible supplies of sanitation and hygiene materials, and sufficient training and budgeting for WaSH or IPC needs.

Our results suggest that the provision of on premises, improved water service accessible to people with reduced mobility, interventions that prioritize the acceptability of sanitation facilities within the local context, and the provision of hygienic materials are key interventions to improve HCP satisfaction. Dedicated funding and oversight should be established at the HCF level to ensure access to consumable hygiene and IPC products and maintenance of WASH infrastructure. Improvements to WaSH in HCF may improve HCP satisfaction and ultimately patient outcomes.

1. Introduction

Job satisfaction is "the attitude towards one's work and the related emotions, beliefs, and behavior," and "results from complex interactions between on-the-job experience, organizational environment, and motivation" (Peters et al., 2010). Healthcare providers (HCPs) who are more

Abbreviations: Healthcare provider, (HCP); healthcare facilities, (HCFs); water sanitation and hygiene, (WaSH); infection prevention and control, (IPC).

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satisfied with their job and work environment are more likely to demonstrate higher levels of effort towards quality improvement, provide lower-risk care for patients, and have less turnover (Alhassan et al., 2013; Mbaruku et al., 2014; Stewart et al., 2011).

The job of HCPs in rural areas of low- and middle-income countries (LMICs) can be especially demanding, and they often have little resources and institutional support (O'Neill and Sheffel, 2013). Increased pressure in the work environment affects job satisfaction, worker retention and health service delivery in rural settings (Mbaruku et al., 2014). Understaffing can lead to job dissatisfaction, in turn causing further staff shortages, labor unrest, and absenteeism (Gross et al., 2012; Mubyazi et al., 2012; Rowe et al., 2005). Dissatisfaction on the part of service providers can be passed on to patients in the form of impatient, distracted, or uncourteous behavior (Kruk et al., 2009). Improving the job satisfaction of HCPs in these settings is critical for improved patient and provider outcomes in healthcare facilities (HCFs).

The physical work environment – such as water, sanitation, and hygiene (WaSH) infrastructure, lighting, and infection control supplies – affects HCP job satisfaction. Peters et al. found that more than 90% of HCPs in India reported that "good physical conditions" were important in the "ideal job" and often ranked "good physical conditions" above "income" in importance (Peters et al., 2010). HCP dissatisfaction with staffing, poorer cleanliness and less orderliness of the workplace were associated with a greater risk for needlestick injury and pathogen exposure for HCPs (Lundstrom et al., 2002). Inadequate environmental conditions were linked to employee job frustration in HCFs in South Africa; rural HCPs had worse service delivery outcomes and expressed that transferring to another location with more resources would allow them to do their job more effectively (Tawana et al., 2019). In Ghana, HCPs in public and private facilities expressed that a major source of job dissatisfaction was due to the physical work environment of their clinics, and the low availability of resources and drugs (Alhassan et al., 2013). Insufficient resources were cited as a reason for job dissatisfaction for HCPs in government and public HCFs in Kenya, who were less likely to report adequate resources and safe water in the workplace (Ojakaa et al., 2014).

Understanding how the physical work environment impacts job satisfaction in HCPs can improve healthcare service delivery. Despite some qualitative evidence on job satisfaction in HCPs in rural, LMIC settings, few studies have explored this using survey data. We analyzed data from surveys conducted in 14 LMICs to explore the relationship between environmental conditions and HCP job satisfaction in HCFs, and to understand the factors influencing satisfaction with WaSH infrastructure and cleanliness and infection prevention and control (IPC) practices in rural HCFs in LMICs.

2. Materials and methods

Between July and December 2017, an evaluation of WaSH conditions in 14 low- and middle-income countries was conducted for the international non-governmental organization World Vision. As part of this evaluation, public clinics (such as health posts and health centers) in rural areas of Ethiopia, Ghana, Honduras, India, Kenya, Malawi, Mali, Mozambique, Niger, Rwanda, Tanzania, Uganda, Zambia, and Zimbabwe were assessed. Survey methods are described in detail by A. Z. Guo and Bartram (2019) but are summarized here briefly.

2.1. Study design and data collection

HCF sampling was nested within a population-based, cluster-randomized household survey design. Within the household survey sampling area in each country, 100 HCFs in areas where World Vision has programs and 100 HCFs in areas where World Vision does not have programs were randomly selected. Where fewer than 100 HCFs were identified in each of these categories, all HCFs in the sampling areas were surveyed.

Teams of trained enumerators travelled to each of the selected HCFs and attempted to interview a health provider at the facility – preferably the head nurse (42%), though a head doctor (13%) or a nurse who had worked at the facility for more than two years (23%) or other nurse (21%) could also respond if the head doctor was unavailable. Surveys were administered in the local language, and included both direct questions for respondents and enumerator observations of HCF characteristics, water service, sanitation and hygiene facilities, healthcare waste management and cleanliness practices. Definitions of all selected variables and a link to the full survey are available in Table 1. All responses were recorded in mWater (New York, NY, USA), a mobile survey tool which allowed for real-time quality checks by supervisors and researchers during the data collection period, including spot checking based on photos and checking for reasonable responses, duration of survey, and GPS location of surveys.

This study was approved by the UNC-Chapel Hill Institutional Review Board (IRB #17–0663) as well as agencies within each country (The Water Institute at UNC, 2019). Free and informed consent was obtained from all respondents in their own language before beginning the survey.

Full reports on data collection, survey methodologies and results, and surveys used are available at: http://waterinstitute.unc.edu/publication/world-vision-14-country-wash-evaluation/

2.2. Data processing and analysis

Data were cleaned and analyzed using R version 4.0.2 (R Core Team, 2020). Multivariable mixed-effects regression models were fitted using the lme4 package, version 1.1–23 (Bates, Mächler, Bolker and Walker, 2015). A general linear mixed model was used with a logit link function under a binomial family distribution for binary outcome variables. Satisfaction with water service, sanitation facilities, hygiene facilities, and cleanliness and IPC practices were coded as binary outcome variables (e.g., "satisfied with water" versus "not satisfied with water"). For each of these outcomes, approximately 20–40 binary, categorical, or ordered variables were selected *a priori* for testing based on plausible relationships with the outcome variables (Supplement 1). Random effects due to the country of survey were controlled for in each model and the variation in satisfaction attributable to country effects was assessed for each outcome according to the intraclass correlation coefficient (ICC). Responses of "do not know," "decline to state," and other non-responses were uncommon (<5% of responses) and imputed to the reference category.

Univariable logistic regressions were conducted to identify the variables with statistically significant relationships with each outcome variable ($p < 0.05$). For each outcome, all significantly associated variables were incorporated into a single corresponding multivariable mixed-effects logistic regression model as described above. Variables in these models were eliminated using a monitored stepwise procedure until all remaining variables had a corresponding p-value less than or equal to the significance-to-stay value (0.05) (Dietz et al., 2000; Suárez, Pérez, Rivera and Martínez, 2017). Pearson's correlation coefficient and the variance inflation factor were used to verify that none of the variables in the final models were highly correlated.

3. Results

Across the 14 studied countries, respondents at 2002 HCFs (over 98% of those contacted) consented to survey. The characteristics of facilities in the final sample are discussed in detail elsewhere (A. Z. Guo and Bartram, 2019). Respondents from India and Honduras reported consistently high satisfaction with environmental conditions compared to study countries in sub-Saharan Africa and in particular Malawi, Zambia, and Uganda, where respondents reported consistently low satisfaction with environmental conditions. A table displaying satisfaction with each category of environmental conditions (water, sanitation,

Table 1
Selected data on HCF characteristics, water service, sanitation and hygiene facilities, healthcare waste management and cleanliness practices, and administration and training associated with satisfaction with environmental conditions.

Grouping	Variable	Type	Definition
HCF characteristics	Electricity	Observed	Electricity available at the health facility on the day of visit
Water service	Source location	Reported	Location of the main water point (on premises, within 500 m, further than 500 m, other)
	Source availability	Reported	Water available from the main water point, on premises, at time of sampling
	Source type	Reported	Main water point is of an improved type according to JMP classifications at the time of analysis (WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation (JMP), 2019)
	Source condition	Reported	Main water point has not been out of service in the past two weeks
	Source continuity	Reported	Water is available from the main point 24 h per day
	Accessibility	Reported	Characteristics which would make water facilities difficult for people with limited mobility to use are present (e.g., path is difficult to navigate, steps at entrance, no handrails, etc.)
	Treatment	Reported	Health facility does "something" to make the water safer (e.g., boiling, chlorine, filtration, ceramic filter, other)
Sanitation and hygiene facilities	Facility number	Reported	Number of sanitation facilities
	Facility odor	Observed	Any sanitation facility has a "bad smell"
	Facility security	Observed	At least one sanitation facility has a door which can be locked from the inside
	Facility accessibility	Observed	Characteristics which would make sanitation facilities difficult for people with limited mobility to use are present (e.g., path is difficult to navigate, steps at entrance, no handrails, etc.)
	Open defecation	Reported	Open defecation occurs at the facility
	Hygiene near sanitation	Observed	Hand hygiene stations are available in or near (within 5 meters of) all sanitation facilities
	Soap and water	Observed	Soap and water available in all hygiene facilities
	Drying materials	Observed	Hygienic drying materials available in all hygiene facilities
	Staff hand rub	Reported	Medical staff carry alcohol-based hand rub or sanitizer while on duty
Healthcare waste management and cleanliness	Linen cleaning	Reported	Mattresses, pillows, and linens are cleaned between each patient with detergent
	Bins	Observed	Waste is segregated into at least three labeled bins at all points of care (sharps, infectious, and non-infectious general waste)
	Infectious waste	Reported	The mode of facility waste disposal is reported, then segregated into proper (e.g., "autoclaved") vs improper (e.g., "not treated and added to general waste") categories
	Surface cleaning	Reported	Floors, surfaces, and sanitation facilities are cleaned at least once a day with water and detergent or disinfectant
Administration and training	WaSH training	Reported	Water and sanitation training at the facility in the past 12 months
	WaSH budgeting	Reported	Annual budget for the facility which includes funding for WaSH and infection prevention/ control infrastructure, services, and personnel (and is sufficient to meet the needs of the facility)
	WaSH committee	Reported	Infection prevention and control, WaSH, or hygiene committee that employees belong to at the facility (and has met in the past 6 months)
	Oversight committee	Reported	Community-composed oversight committee at the facility (and has met in the past 6 months)
	IPC policy	Reported	Infection control policy, procedure, or document in place

and hygiene infrastructure and cleanliness and ICP practices) can be found in the supplementary materials (Supplement 2).

Most respondents reported that they were unsatisfied with WaSH infrastructure at their HCF. Overall, 68% of respondents were unsatisfied with the sanitation facilities, 65% were unsatisfied with the water service, and 54% were unsatisfied with the hygiene facilities. Survey respondent type was included in each model; head doctors were more likely to be dissatisfied with hygiene facilities (OR = 0.66, p = 0.028) than other respondents; nurses working more than 2 years at their respective facility were more likely to be satisfied with water services (OR = 1.40, p = 0.034) than other respondents; and head nurses as a survey respondent type were not significantly associated with any changes in satisfaction. Insufficient water quantity was the most frequently cited reason for dissatisfaction with WaSH infrastructure, followed by poor water quality (Fig. 1). Respondents were less likely to report that they were unsatisfied with cleanliness and IPC practices (36%). Dissatisfaction with cleanliness and IPC practices was most commonly reported because patients (40%) and other staff (23%) did not wash hands at the correct times, patients (33%) and other staff (28%) did not wash hands with proper materials, or because the facility was not cleaned well (34%). Reasons for HCP dissatisfaction with WaSH infrastructure and cleanliness and IPC practices are presented tabularly in the supplementary materials, alongside additional selected descriptive statistics from surveyed healthcare facilities (Supplement 3).

3.1. Satisfaction with water service

In a mixed-effects logistic regression model, satisfaction with water services at HCFs was significantly associated with continuous water service (OR = 2.81, p < 0.001); water available at the time of survey (OR = 2.21, p < 0.001); a water source on premises (OR = 2.01, p < 0.001); a water source that had not broken down in the last two weeks

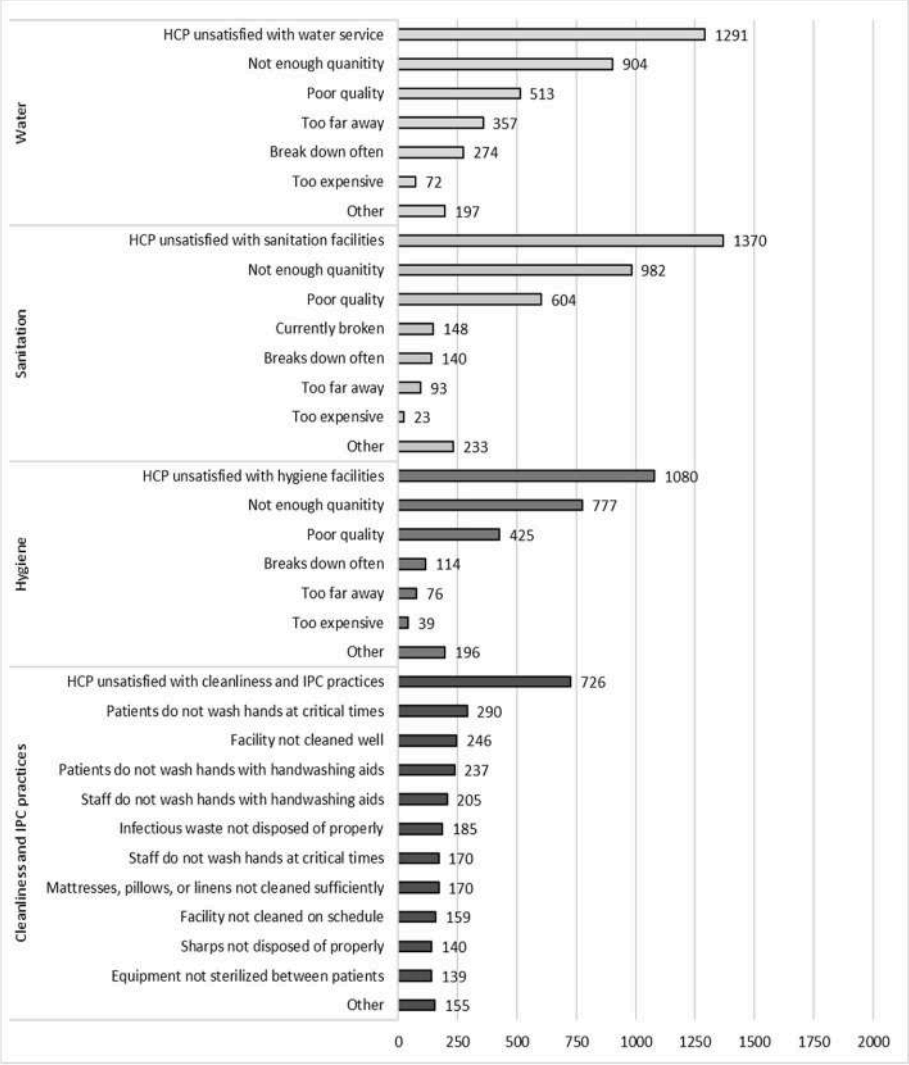

Fig. 1. Number of healthcare facilities dissatisfied with environmental conditions and the reported reasons for dissatisfaction (N = 2002).

(OR = 1.79, p < 0.001); and an improved main water source type (OR = 1.73, p = 0.007) (Table 2). Other than these direct water source characteristics, WaSH training for HCPs (OR = 1.41, p < 0.001) and working electricity (OR = 1.30, p = 0.030) were associated with increased satisfaction with water services. Satisfaction with water services was highest in Honduras (53%) and was lowest in Ethiopia (22%). Approximately 4% of variation in water satisfaction was attributable to differences by country (ICC = 0.039).

3.2. Satisfaction with sanitation facilities

Quantity, quality, and privacy of sanitation facilities were associated with satisfaction with sanitation services at HCFs (Table 3). Respondents were more likely to report that they were satisfied with sanitation facilities at HCFs where no open defecation was reported to occur (OR = 1.88, p < 0.001); at least one facility had a door that locked from the inside (OR = 1.62, p < 0.001); no sanitation facilities were reported to have a bad odor (OR = 1.42, p = 0.004); and had a greater number of hygiene facilities (OR = 1.08, p < 0.001). Respondents were more likely to be satisfied with sanitation where sanitation facilities (OR = 1.77, p < 0.001) and water points (OR = 1.40, p = 0.013) were accessible by people with reduced mobility. Other than direct characteristics of sanitation, hand hygiene stations with soap and water nearby (OR = 1.70, p < 0.001) and a sufficient budget for WaSH and IPC (OR = 1.75, p < 0.001) were associated with increased satisfaction with sanitation services. Satisfaction with sanitation facilities was highest in India (53%) and lowest in Zambia (15%). About 6% of variation in sanitation satisfaction was attributable to differences by country (ICC = 0.061).

3.3. Satisfaction with hygiene facilities

Satisfaction with hygiene facilities was associated with accessible water, the presence of hand hygiene stations and supplies, and institutional support (Table 4). An on premises water source (OR = 1.57, p = 0.003), a water source accessible for people with reduced mobility (OR = 1.57, p < 0.001) and a continuous water service (OR = 1.38, p = 0.012) were associated with increased hygiene satisfaction, as were alcohol-based hand rub carried by staff (OR = 1.66, p < 0.001); the presence of hygienic drying materials (OR = 1.63, p = 0.027) or hand hygiene stations with soap and water within 5 m of all sanitation facilities (OR = 1.39, p = 0.020); and hand hygiene stations with soap and water for at least one point of care (OR = 1.54, p < 0.001). A sufficient budget (OR = 1.73, p = 0.001) and an IPC or WaSH committee that had met in the past six months (OR = 1.33, p = 0.011) were associated with higher hygiene satisfaction. Satisfaction with hygiene facilities was highest in India (76%) and lowest in Ghana (29%). About 9% of variation in hygiene infrastructure satisfaction was attributable to differences by country (ICC = 0.088).

Table 2
Factors associated with statistically significant changes in reported satisfaction with water service within surveyed healthcare facilities (N = 2002).

Factor	Odds Ratio	95% CI	p-value
Survey respondent type			
Other nurse	Ref		
Head doctor	1.26	(0.87, 1.83)	0.219
Head nurse	1.27	(0.96, 1.67)	0.097
Nurse who worked at facility >2 years	1.40	(1.03, 1.90)	0.034
Location of main water source			
Further than 500 m	Ref		
Within 500 m	1.06	(0.70, 1.54)	0.779
On premises	2.01	(1.32, 2.79)	<0.001
Main water source is available during the survey			
No	ref		
Yes (reported, but not observed)	1.09	(0.65, 1.83)	0.741
Yes (reported and observed)	2.21	(1.48, 3.31)	<0.001
Electricity working on the day of the survey	1.30	(1.03, 1.64)	0.030
Facility provides WaSH training to healthcare providers	1.41	(1.14, 1.73)	<0.001
Main water source is an improved type	1.73	(1.16, 2.56)	0.007
Main water source has not broken down in the past 2 weeks	1.79	(1.30, 2.44)	<0.001
Main water source provides continuous (24-h) service	2.81	(2.03, 3.88)	<0.001

Table 3
Factors associated with statistically significant changes in reported satisfaction with sanitation facilities within surveyed healthcare facilities (N = 2002).

Factor	Odds Ratio	95% CI	p-value
Survey respondent type			
Other nurse	ref		
Head doctor	0.20	(0.47, 1.05)	0.085
Head nurse	0.71	(0.68, 1.22)	0.554
Nurse who worked at facility >2 years	1.05	(0.76, 1.45)	0.774
Facility has a budget that includes funding for WaSH/IPC needs			
No budget exists	ref		
Yes (insufficient)	0.87	(0.66, 1.15)	0.329
Yes (sufficient)	1.75	(1.26, 2.42)	<0.001
Number of sanitation facilities present	1.08	(1.04, 1.11)	<0.001
Water accessible for people with reduced mobility	1.40	(1.07, 1.83)	0.013
No sanitation facilities have a bad odor	1.42	(1.12, 1.80)	0.004
At least one sanitation facility has a door that locks from the inside	1.62	(1.26, 2.09)	<0.001
Hand hygiene stations with water and soap are available near all sanitation	1.70	(1.31, 2.20)	<0.001
At least one sanitation facility is accessible by people with reduced mobility	1.77	(1.30, 2.40)	<0.001
Open defecation does not occur at the facility	1.88	(1.34, 2.62)	<0.001

Table 4
Factors associated with statistically significant changes in reported satisfaction with hygiene facilities within surveyed healthcare facilities (N = 2002.

Factor	Odds Ratio	95% CI	p-value
Survey respondent type			
Other nurse	ref		
Head doctor	0.66	(0.46, 0.96)	0.028
Head nurse	0.90	(0.69, 1.18)	0.447
Nurse who worked at facility >2 years	0.95	(0.70, 1.27)	0.710
Location of main water source			
Further than 500 m	ref		
Within 500 m	1.09	(0.75, 2.43)	0.628
On premises	1.57	(1.17, 2.12)	0.003
None available	1.35	(0.77, 1.52)	0.322
Facility has a budget that includes funding for WaSH/IPC needs			
No budget exists	Ref		
Yes (insufficient)	0.98	(0.77, 1.26)	0.890
Yes (sufficient)	1.73	(1.24, 2.40)	0.001
Facility has an IPC/WaSH committee			
No	ref		
Yes, but they have not met in the past 6 months	1.00	(0.71, 1.41)	0.979
Yes, and they have met in the past 6 months	1.33	(1.07, 1.66)	0.011
Main water source provides continuous (24-h) service	1.38	(1.07, 1.79)	0.012
Hand hygiene stations with soap and water are available near all sanitation	1.39	(1.05, 1.83)	0.020
At least one point of care has a hand hygiene station with soap and water	1.54	(1.25, 1.91)	<0.001
Water accessible for people with reduced mobility	1.57	(1.22, 2.02)	<0.001
Hand hygiene stations with hygienic drying materials are available near all sanitation	1.63	(1.06, 2.51)	0.027
Medical staff carry alcohol-based hand rub while on duty	1.66	(1.33, 2.06)	<0.001

3.4. Satisfaction with cleanliness and IPC practices

Satisfaction with cleanliness and IPC practices in HCFs was associated with three determinant categories: availability of infrastructure and supplies, reported cleaning practices, and institutional support (Table 5). For infrastructure and supplies, presence of water accessible to people with reduced mobility (OR = 1.69, $p < 0.001$); a main water source within 500 m of the HCF (OR = 1.54, $p = 0.014$); water treatment (OR = 1.43, $p = 0.002$); working electricity (OR = 1.36, $p = 0.011$); and presence of bins for waste segregation at points of care (OR = 1.35, $p = 0.018$) were associated with increased satisfaction with cleanliness and IPC practices. Regarding reported cleaning practices, respondents were more likely to be satisfied with cleanliness and IPC practices if floors, surfaces, and sanitation facilities were cleaned with water, detergent, or disinfectant (OR = 1.54, $p = 0.002$); no open defecation was reported to occur at the facility (OR = 1.54, $p = 0.002$); mattresses, pillows, and linens were cleaned only intermittently (OR = 1.96, $p = 0.031$); and if infectious waste was disposed of using a safe method such as autoclaving or incineration (OR = 1.48, $p < 0.001$). Sufficient budgeting (OR = 2.14, $p < 0.001$); a community-composed oversight committee that had met in the past six months (OR = 1.54, $p < 0.001$); and an IPC policy, procedure, or document in place by the facility (OR = 1.62, $p < 0.001$) were all aspects of institutional support associated with increased satisfaction with cleanliness and IPC practices. Satisfaction with cleanliness and IPC practices was highest in India (87%) and lowest in Malawi (39%). Over

Table 5
Factors associated with statistically significant changes in reported satisfaction with cleanliness and infection prevention and control practices within surveyed healthcare facilities (N = 2002).

Factor	Odds Ratio	95% CI	p-value
Survey respondent type			
Other nurse	Ref		
Head doctor	0.81	(0.55, 1.18)	0.270
Head nurse	1.08	(0.81, 1.42)	0.610
Nurse who worked at facility >2 years	1.09	(0.80, 1.50)	0.572
Location of main water source			
Further than 500 m	ref		
Within 500 m, but not on premises	1.54	(1.09, 2.17)	0.014
On premises	1.23	(0.89, 1.68)	0.207
None available	1.18	(0.65, 2.17)	0.584
Facility does not clean mattresses, pillows, or linens with detergent			
No cleaning	Ref		
Linens, mattresses, or pillows are provided by the facility	1.25	(0.68, 2.36)	0.468
Cleaned, but not between every patient	1.96	(1.06, 3.70)	0.031
Cleaned, between every patient	1.03	(0.56, 1.89)	0.917
Facility has a budget that includes funding for WaSH/IPC needs			
No budget exists	Ref		
Yes (insufficient)	1.29	(0.99, 1.67)	0.060
Yes (sufficient)	2.14	(1.45, 3.15)	<0.001
Facility has a community-composed oversight committee			
No	ref		
Yes, but they have not met in the past 6 months	1.17	(0.82, 1.66)	0.379
Yes, and they have met in the past 6 months	1.54	(1.21, 1.95)	<0.001
Bins available for segregation of waste at all points of care	1.35	(1.05, 1.73)	0.018
Electricity working on the day of the survey	1.36	(1.07, 1.72)	0.011
Facility treats water to make it safer	1.43	(1.14, 1.82)	0.002
Infectious waste is disposed safely	1.48	(1.18, 1.84)	<0.001
Open defecation does not occur at the facility	1.54	(1.16, 2.04)	0.002
Facility has an IPC policy, procedure, or document	1.62	(1.28, 2.05)	<0.001
Water accessible for people with reduced mobility	1.69	(1.32, 2.17)	<0.001
Facility cleans floors, surfaces, and sanitation facilities with water, detergent, or disinfectant	1.85	(0.22, 2.78)	0.004

13% of variation in satisfaction with cleanliness and IPC was attributable to differences by country (ICC = 0.134).

4. Discussion

We assessed HCP satisfaction with WaSH infrastructure and IPC practices and cleanliness at 2002 rural HCFs across 14 LMICs. This is one of the first studies to quantitatively assess HCP satisfaction with water, sanitation and hygiene infrastructure and IPC practices in LMICs. HCP satisfaction directly affects patient outcomes (Alhassan et al., 2013; Mbaruku et al., 2014; Stewart et al., 2011) and may be especially important in rural and LMIC settings where a small staff with few financial resources determines the quality of healthcare available.

Our results demonstrate that most HCPs are unsatisfied with the water, sanitation, and hygiene infrastructure at their rural HCF, though they were somewhat less likely to report they were dissatisfied with cleanliness and IPC practices. Several indirect and direct characteristics of water, sanitation, and hygiene infrastructure were significantly associated with provider satisfaction at their HCFs, including high quality supply, readily available and accessible supply, and sufficient training and budgeting.

Most HCPs reported that they were unsatisfied with both the quantity and quality of water services at their HCFs. Previous assessments of water services in rural HCFs have shown that only 51% of HCFs in sub-Saharan Africa and 58% of those in Honduras have an improved water source type on premises (WHO/UNICEF, 2019) (data not available for India). Further, an assessment of HCFs in sub-Saharan Africa showed that 27% of rural HCFs lacked continuous water service, and that only 42% had access to a secondary source (A. Guo, Bowling, Bartram and Kayser, 2017).

Having a continuous (24-h) water service (OR = 2.81) was the variable most strongly associated with HCP satisfaction with water services. However, the location of the main water source was significantly associated with multiple aspects of HCP satisfaction, including: satisfaction with water service (on premises, OR = 2.01), hygiene infrastructure (on premises, OR = 1.57) and IPC practice and cleanliness (within 500 m but not on premises, OR = 1.54). The importance of water source location is supported by household water literature. Previous studies have shown that having water on premises increases quantity of household water use (Brown et al., 2013; Overbo et al., 2016), and improves water quality by reducing the risk of contamination during transport from the source and household storage (Overbo et al., 2016; Usman et al., 2018). Presence of a water source accessible for people with reduced mobility is also significantly associated with HCP satisfaction with sanitation facilities (OR = 1.4), hygiene facilities (OR = 1.57) and IPC practices and cleanliness (OR = 1.69).

As with water services, HCPs reported dissatisfaction with both the quality and quantity of sanitation facilities. Previous assessments of sanitation services in rural HCFs have shown that only 23% of HCFs in sub-Saharan Africa and 1% of those in Honduras have basic sanitation service, defined as an improved-type sanitation facility that is acceptable to users, accessible to people with limited mobility and sufficiently available (WHO/UNICEF, 2019) (data not available for India). Further, an assessment of rural HCFs in sub-Saharan Africa showed that 44% of sanitation facilities in HCFs faced issues with toilet privacy, cleanliness, or functionality (A. Guo et al., 2017).

Satisfaction with sanitation facilities was most strongly associated with whether open defecation occurs at the HCF (OR = 1.88). Open defecation was also significantly associated with satisfaction with IPC practices and cleanliness (OR = 1.54). People are motivated to practice open defecation by both active choice and compulsion (Bhatt et al., 2019), meaning that open defecation at HCFs could result from cultural norms (Thys et al., 2015), concerns about privacy (Bhatt et al., 2019), cleanliness, or other reasons (WHO/UNICEF, 2019). One previous study that found that 69% of people who used sanitation facilities at HCFs in Lesotho were dissatisfied with the toilet condition (WHO/UNICEF, 2019). One previous study found that 69% of people who used sanitation facilities at HCFs in Lesotho were dissatisfied with the toilet condition (WHO/UNICEF, 2019). Another study in sub-Saharan Africa found that, on average, 17% of toilets had privacy or cleanliness issues reported and that 20% of toilets were in need of repair (A. Guo et al., 2017). Any of these factors could affect decision-making about open defecation at the HCF for both HCPs and patients affecting both HCP satisfaction and related health outcomes. Accessibility of sanitation facilities for people with limited mobility was also strongly associated with HCP satisfaction with sanitation facilities (OR = 1.77).

HCP satisfaction with hygiene facilities was slightly better than satisfaction with water service or sanitation facilities. The factors most

strongly associated with HCP satisfaction with hygiene facilities were having a sufficient budget for WASH/IPC needs (OR = 1.73), medical staff that carry alcohol-based hand rub while on duty (OR = 1.66) and hand hygiene stations with hygienic drying materials available near all sanitation facilities (OR = 1.63). These results suggest that HCP satisfaction with hygiene is largely dependent on the availability of supplies, their amount/location, and a system which prioritizes funding for supplies, as opposed to the hygiene knowledge of HCPs. This finding is supported by previous literature which found associations between sufficient hygiene practices and hygiene materials such as soap, drying materials, and alcohol-based hand rub (Erasmus et al., 2010), but no association with theoretical knowledge, social influence, or moral obligation (De Wandel, Maes, Labeau, Vereecken and Blot, 2010; Naughton et al., 2015).

Respondents less often reported dissatisfaction with cleanliness and IPC practices than with WaSH infrastructure. However, dissatisfaction was still reported by 36% of respondents, and a number of factors – availability of infrastructure and supplies, reported cleaning practices, and institutional support – were associated with HCP satisfaction with cleanliness and IPC practices. Characteristics of water at the HCF, including proximal main water sources (OR = 1.54), water treatment (OR = 1.43), and accessibility of water to people with reduced mobility (OR = 1.69) were some of the most important infrastructure characteristics for satisfaction with cleanliness and ICP practices, similarly to HCP satisfaction with other environmental conditions studied here.

Cleaning practices, including the cleaning of floors, surfaces, and sanitation facilities with water, detergent, or disinfectant (OR = 1.54) and intermittent cleaning of mattresses, pillows, and linens (OR = 1.96) were also significantly associated with HCP job satisfaction. These specific factors do not yet have well-established links in the literature, however the importance of cleanliness to job satisfaction has been demonstrated (Lundstrom et al., 2002; Peters et al., 2010). Institutional support for cleanliness and IPC practices at the HCFs were closely associated with HCP satisfaction, including sufficient budgeting (OR = 2.14), a community-composed oversight committee that had met in the past six months (OR = 1.54), and an IPC procedure, or document in place by the HCF (OR = 1.62). This finding is supported by previous literature from Ghana indicating that oversight and organizational commitment are directly tied to job satisfaction in HCPs (Bonenberger et al., 2014), and analysis showing the importance of budgeting for cleanliness and IPC to maternity wards in HCFs of the same countries studied here (Cronk et al., 2021). This coincides with findings about oversight and dedicated budgets improving satisfaction with other environmental services like sanitation and hygiene infrastructure.

4.1. Limitations

This study was based on cross-sectional data, so our ability to infer causality is limited. Although sample size was comparatively large and variance assumptions were met, reducing survey information to binary or categorical variables reduced the amount of information available for models and thus the power of analyses. Spurious results due to logistic regression testing of large sets of variables was reduced by examining selected variables and results with subject-matter experts and alongside current literature. While surveys were conducted in private (away from patients, other staff, etc.) and respondents were informed that responses would be kept anonymous, respondents' answers may have been influenced by courtesy or social desirability bias due to face-to-face surveying. Only public clinics in rural areas of LMICs are studied here, so findings should not be interpreted for private, non-profit, or urban centers, or places with specialized care. We did not assess the impact of the presence of alcohol-based hand rub at the point of care (e.g., bedside) because this was not included in the survey, nor were other aspects of HCP satisfaction not available from the survey included in the analysis.

5. Conclusions

Our study suggests that governments and non-governmental organizations (NGOs) should facilitate substantial improvements to HCF WaSH infrastructure in order to improve both HCP satisfaction and patient outcomes in public, rural clinics of LMICs. With regards to water, our findings suggest that governments and NGOs working in this space should prioritize provision of on premises, improved-type water service accessible to people with reduced mobility at HCFs. This single intervention could improve the quantity of water available for all purposes, as well as increase HCP satisfaction with HCF water, hygiene, and cleanliness and IPC services. With regards to sanitation services, it is critical that future interventions prioritize the acceptability of sanitation facilities within the local context in order to prevent patients choosing to defecate in the open. Finally, our results suggest that the provision of hygienic materials may be more effective than the provision of hygiene education in improving handwashing compliance and HCP satisfaction, though provision of hygiene materials and education are often delivered together.

At the HCF level, we recommend that oversight committees include dedicated funding for WaSH and IPC needs. The sustainability of WASH infrastructure and services at HCFs depends on the ability of the service provider to ensure accessibility of the consumable hygiene and IPC products and the maintenance of WASH infrastructure. The inclusion of sufficient funding for WASH and IPC needs in the HCF budget affected HCP satisfaction with sanitation, hygiene and IPC practices and cleanliness.

Declaration of competing interest

Authors JBT and OO are both employed by World Vision, the sponsor for the original collection of data, but do not declare any influence from World Vision in their role in this manuscript. The other authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijheh.2021.113802.

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Maternal $PM_{2.5}$ exposure associated with stillbirth: A large birth cohort study in seven Chinese cities

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ABSTRACT

Background: Maternal exposure to fine particulate matter ($PM_{2.5}$) has been associated with a few adverse birth outcomes. However, its effect on stillbirth remains unknown in China, especially the susceptible windows and potential modifiers.

Objective: This study aimed to evaluate the associations between maternal $PM_{2.5}$ exposure and stillbirth in seven Chinese cities.

Methods: We used birth cohort data of 1,273,924 mother-and-birth pairs in seven cities in southern China between 2014 and 2017 to examine these associations. Pregnant women were recruited in the cohort at their first visit to a doctor for pregnancy, and stillbirths were recorded at the time of birth. Air pollution exposures were assessed through linking daily air pollutant concentrations from nearby monitoring stations to the mother's residential community. Cox regression models were applied to determine the associations between $PM_{2.5}$ and stillbirth for different gestational periods.

Results: Among the participants, 3150 (2.47‰) were identified as stillbirth cases. The hazard ratio (HR) of stillbirths was 1.52 (95% CI: 1.42, 1.62) for each 10 $\mu g/m^3$ increase in $PM_{2.5}$ during the entire pregnancy after controlling for some important covariates. Relatively stronger associations were observed during the second trimester [adjusted HR = 1.67 (95% CI: 1.57, 1.77)] than trimesters 1 [HR = 1.44 (95% CI: 1.37, 1.52)] and trimester 3 [HR = 1.23 (95% CI: 1.16, 1.30)]. Stratified analyses also showed a stronger association among pregnant women without previous pregnancy and previous delivery experiences.

Conclusion: The study indicates that maternal exposure to $PM_{2.5}$, especially during the midpoint period of pregnancy, might increase the risk of stillbirths. Maternal previous pregnancy and delivery may modify this association.

1. Introduction

Stillbirth is one of the important adverse birth outcomes (Blencowe et al., 2016; Yakoob et al., 2010). It is estimated that about 2.6 million stillbirths occurred internationally in 2015, with the majority occurring in developing countries (Lawn et al., 2016). A stillbirth can lead to tremendous societal and familial burden, such as the mental health problems and an increased risk of recurrent stillbirths (Heazell et al., 2016; Lamont et al., 2015).

Previous studies have identified a few important risk factors for stillbirth, such as genetics, childbirth or pregnancy complications, fetal growth restriction, and congenital abnormalities (Flenady et al., 2011; Jason et al., 2013). However, most of these factors are not easily preventable or modifiable, and thus are difficult for specific intervention

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measures (Mendola et al., 2017). Ambient air pollution exposure is another potential risk factor, which has been linked to a few adverse birth outcomes, such as preterm birth (Guo et al., 2018; Xiao et al., 2018) and low birth weight (Basu et al., 2014; Harris et al., 2014). However, only a few studies have assessed the association between stillbirths and $PM_{2.5}$ (Green et al., 2015; Siddika et al., 2016). One study conducted in California suggested that $PM_{2.5}$ exposure throughout pregnancy was associated with an increased risk of stillbirth (Green et al., 2015). Another study in Ohio showed $PM_{2.5}$ exposure during the third trimester of pregnancy was associated with stillbirth (DeFranco et al., 2015). At the same time, a few studies reported a non-significant association between $PM_{2.5}$ and stillbirths (Faiz et al., 2012, 2013). In China, only one study has found an increased risk of stillbirth associated with high $PM_{2.5}$ levels during pregnancy (Yang et al., 2018).

We investigated the associations between maternal $PM_{2.5}$ exposures and stillbirths in seven Chinese cities using a birth cohort study design. We also performed stratified analyses to explore potential modifiers, such as maternal age, previous pregnancy, and previous delivery condition.

2. Methods and materials

2.1. Study settings

This study was conducted in seven cities in the Pearl River Delta (PRD) region, Dongguan, Foshan, Huizhou, Guangzhou, Jiangmen, Zhaoqing, and Zhongshan, between January 1, 2014 and December 31, 2017 (Fig. 1). Participants were recruited in this cohort from their first hospital visit after becoming pregnant until delivery. Our birth cohort was linked to the birth registry datasets in order to track the occurrence of stillbirths. In this registry system, birth information is acquired from all midwifery clinics and hospitals in Guangdong province. In accordance with previous studies (Darrow et al., 2009; He et al., 2016), all singleton births that occurred at 20–42 weeks of gestation were included in the data. We also collected maternal and birth information, including date of birth, maternal age, gestational age at birth, the infant's sex, birth weight, birth outcome, mode of delivery, and parity. Gestational age was determined by ultrasound examinations during pregnancy (Fu and Yu, 2011). In the absence of ultrasound information, the gestational age was determined according to the last menstrual period (He et al., 2016).

Approval to perform the study was obtained from the institutional ethical committee of Guangdong Women and Children Hospital (Approval no 202101138). There was no identification information at an individual level in the dataset and all information was anonymous.

2.2. Stillbirths

Stillbirths were collected through the Guangdong Maternal and Child Health Management Information System. The definition of stillbirth used for this study is a fetus born between 20 weeks' and 42 weeks' gestation with no evidence of life such as breathing, heartbeat, or movement, as originally defined by the World Health Organization (WHO) (Yang et al., 2018).

2.3. Air pollution exposure assessment

Ambient air pollution data were collected at each city's air monitoring stations from February 1, 2013 to December 31, 2017. There are ten stations in Guangzhou, five in Dongguan, eight in Foshan, four in Zhongshan, five in Huizhou, three in Zhaoqing, and four in Jiangmen (Fig. 1).

All of the monitoring stations are located in areas with both commercial and residential activities. The air pollutant samples were collected from about 10 to 20 m above ground level. Daily 24 h mean concentrations of $PM_{2.5}$, nitrogen dioxide (NO_2), and sulfur dioxide (SO_2) as well as 8 h mean ozone (O_3) were obtained from each station. The quality control and quality assurance were processed according to

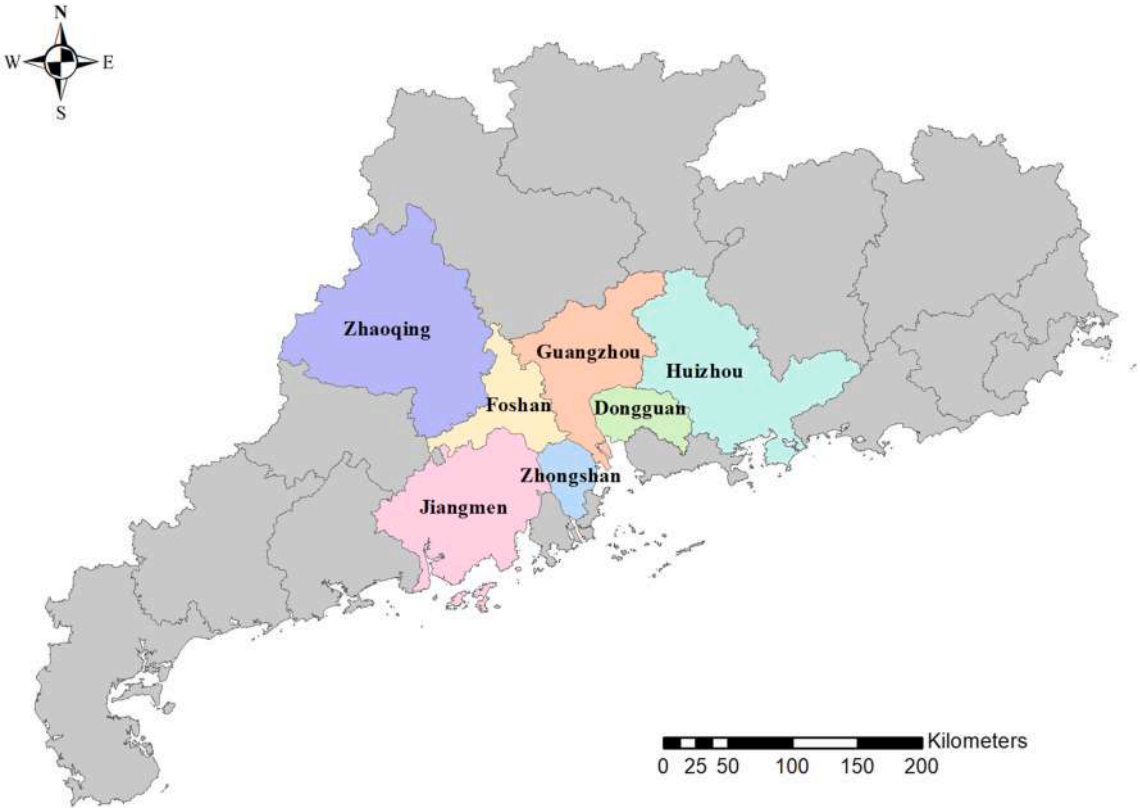

Fig. 1. The geographical distribution of the seven study cities in China.

the National Environmental Protection Administration of China (Lin et al., 2016). The missing data (fewer than 5%) were imputed based on the "na.approx" function in R (Vivian Chit et al., 2014). Air pollution exposure for each participant was estimated by matching the data from air monitoring stations to the mothers. Specifically, exposures were calculated based on the mother's residential district during pregnancy, and those districts without a monitoring station within a radius of about 5 km were excluded from the study, for a final total of 25 districts. To examine the effects at each of different trimesters of pregnancy (the first trimester from 0 to 12 weeks, the second trimester from 13 to 28 weeks, and the third trimester from after 28 weeks), the average concentrations during each trimester as well as over the entire gestation period were calculated (Chen et al., 2018). Participants who gave birth before or at 28 gestational weeks were excluded in the analyses for trimester 3.

We collected daily meteorological data from the National Weather Data Sharing System (http://cdc.cma.gov.cn/home.do). Daily mean temperature (°C) and relative humidity (%) were collected from one automatic weather observation station in each city. Meteorological data were matched to each participant through the same method used with air pollution and as described above.

Other covariates used in this study were continuous variables including gestational age (weeks), maternal age (years), and birth weight (grams) as well as categorical variables including baby gender, previous pregnancy and delivery condition.

3. Statistical analysis

A Cox proportional hazards model was applied to estimate the associations between $PM_{2.5}$ exposures during different trimesters and stillbirth, with gestational age as the time axis and stillbirth as the outcome (Wang et al., 2013). Before the formal analysis, we performed Schoenfeld residuals to assess the proportional hazards assumption and there were no violations.

To account for non-linear effects, both mean temperature and relative humidity were adjusted in model 1 using natural cubic splines with degrees of freedom of 6 and 3, respectively (Liang et al., 2019). Other covariates were also controlled in the model 2 analyses, including the maternal age, newborn gender, previous pregnancy, and season of conception (spring: March–May; summer: June–August; fall: September–November; winter: December–February) (Qian et al., 2016). We estimated the effects for different trimesters of pregnancy using an individual model of each trimester.

We examined these associations by both single-pollutant and two-pollutants in model 2. $PM_{2.5}$ was included alone in the single-pollutant model; while $PM_{2.5}$ and SO_2 (or NO_2, O_3) were included in the two-pollutant models (Lin et al., 2016). The associations were shown in hazard ratios (HRs) and 95% confidence intervals (95% CI) for each 10 $\mu g/m^3$ increase in $PM_{2.5}$ concentrations.

In our stratified analyses, the potential effect modifications were analyzed by variables such as baby's gender, maternal age group (<35 years or ≥35 years), previous pregnancy (first pregnancy or not), and previous delivery condition (first delivery or not). The statistical differences of associations between different subgroups were calculated by using the following strata:

$$(b_1 - b_2) \pm 1.96\sqrt{(se_1)^2 + (se_2)^2}$$

In this strata, b_1 and b_2 are presented for the effect estimates for each stratum, and the se_1 and se_2 represent their corresponding standard errors (Lin et al., 2016).

The robustness of these effects was examined by several sensitivity analyses. For the model 2, we conducted these analyses by changing the degrees of freedom for mean temperature (5–7 degrees of freedom) and relative humidity (2–4 degrees of freedom). We also performed one analysis by excluding the season of conception covariate in the model 2 to check the robustness of the findings. All data analyses were performed using R software (version 3.4.4). The "smoothHR" package was used to fix the Cox models.

4. Results

Our cohort included a total of 1,273,924 singleton live births at between gestational age of 20–42 weeks. Among those, 3150 (2.47‰) were stillbirths. Table 1 summarizes the characteristics. Among all the pregnant women, 54.29% had been previously pregnant and 43.68% had previously delivered a baby. A higher risk of stillbirth was found among women younger than 35 years and those with pregnancy experience. Mothers delivered male babies had higher risk of stillbirths.

Table 2 displays the meteorological factors and air pollution during pregnancy for all participants. The mean concentration of $PM_{2.5}$ during the entire gestation period was 36.78 $\mu g/m^3$, and the mean of daily average temperature and relative humidity during the entire pregnancy was 22.72 °C and 78.92% respectively. The results of the relation between air pollutants and meteorological data are showed in Supplementary Table S1. Both pollutants were positively correlated with $PM_{2.5}$ and SO_2 (Pearson's correlation coefficient: r ranged from 0.04 to 0.65), and a negative correlation between O_3 and NO_2 ($r = -0.16$). The daily average temperature and relative humidity were both negatively correlated with air pollutants except for O_3 and NO_2 with $r = 0.46$ and $r = 0.01$, respectively.

We observed associations between $PM_{2.5}$ exposure and stillbirths during all of the study periods in both single-pollutant (Table 3) and two-pollutant models (Table 4). For example, the associations between $PM_{2.5}$ and stillbirth in single-pollutant models were relatively higher in the second trimester for each 10 $\mu g/m^3$ increase in $PM_{2.5}$ concentration, with a HR of 1.61 (95% CI: 1.52, 1.71) in model 1 and an HR of 1.67 (95% CI: 1.57, 1.77) in model 2, while the HR was 1.44 (95% CI: 1.37, 1.52) for trimester 1, 1.23 (95% CI: 1.16, 1.30) for trimester 3, and 1.52 (95% CI: 1.42, 1.62) for the entire gestation period in model 2. Similar results were obtained in the two-pollutant models, with an HR of 1.59 (95% CI: 1.49, 1.70) after adjusting for O_3, an HR of 1.44 (95% CI: 1.33, 1.56) after adjusting for NO_2 and an HR of 1.89 (95% CI: 1.72, 2.08) after adjusting for SO_2 during the entire pregnancy.

Table 5 displays the effects of stratified analyses by sex, maternal age, previous pregnancy, and previous delivery condition. The associations varied by sex, maternal age, previous pregnancy and previous delivery experience, but only previous pregnancy and delivery were different at a statistically significant level during the entire pregnancy.

Table 1
Characteristics of all participants in seven cities of PRD region (2014–2017).

Variables	Live births (n = 1,270,774)	Stillbirths (n = 3,150)	Total births (n = 1,273,924)
Baby gender			
Male	681281 (53.61%)	1618 (51.37%)	682899 (53.61%)
Female	589377 (46.37%)	1498 (47.55%)	590875 (46.38%)
Uncertain	116 (0.01%)	34 (1.08%)	150 (0.01%)
Maternal age			
<35 years	1083764 (85.28%)	2593 (82.32%)	1086357 (85.28%)
≥35 years	186568 (14.68%)	551 (17.49%)	187119 (14.69%)
Missing	442 (0.03%)	6 (0.19%)	448 (0.03%)
Previous pregnancy			
Yes	689492 (54.26%)	2124 (67.43%)	691616 (54.29%)
No	333981 (26.28%)	1026 (32.57%)	335007 (26.30%)
Unrecorded	247301 (19.46%)	0	247301 (19.41%)
Previous delivery			
Yes	554838 (43.66%)	1567 (49.75%)	556405 (43.68%)
No	468741 (36.89%)	1583 (50.25%)	470324 (36.92%)
Unrecorded	247195 (19.45%)	0	247195 (19.40%)
Season of conception			
Spring	280823 (22.10%)	717 (22.76%)	281540 (22.10%)
Summer	285603 (22.47%)	708 (22.48%)	286311 (22.47%)
Fall	325760 (25.64%)	836 (26.54%)	326596 (25.64%)
Winter	378588 (29.79%)	889 (28.22%)	379477 (29.79%)

Table 2
Summary of air pollution and meteorological variables during pregnancy of all participants.

Variables	Mean	Min	Max	Percentiles		
				25th	50th	75th
$PM_{2.5}$ (μg/m^3)	36.78	19.17	73.46	32.73	36.24	40.99
O_3 (μg/m^3)	80.70	37.81	125.35	73.38	81.67	87.92
NO_2 (μg/m^3)	41.20	12.91	75.55	33.37	41.64	48.95
SO_2 (μg/m^3)	13.42	6.75	45.23	10.91	12.50	14.95
Temperature (°C)	22.72	14.17	28.36	21.20	22.54	24.35
Relative humidity (%)	78.92	64.09	88.32	76.79	79.03	81.13

Table 3
The HRs (95% CIs) for stillbirths with each 10 μg/m^3 increase in $PM_{2.5}$ concentration during study periods in single-pollutant models.

Trimesters	Model 1[a]	Model 2[b]
Trimester 1	1.45 (1.38, 1.53)	1.44 (1.37, 1.52)
Trimester 2	1.61 (1.52, 1.71)	1.67 (1.57, 1.77)
Trimester 3	1.19 (1.13, 1.26)	1.23 (1.16, 1.30)
Entire pregnancy	1.23 (1.16, 1.31)	1.52 (1.42, 1.62)

Note: HR, hazard ratio; CI, confidence interval.
[a] All models adjusted for mean ambient temperature and relative humidity with degrees of freedom of 6 and 3, respectively.
[b] All models adjusted for maternal age, newborn gender, season of conception, previous pregnancy and previous delivery condition, in addition to Model 1.

Table 4
The HRs (95% CIs) for stillbirths with each 10 μg/m^3 increase in $PM_{2.5}$ concentration during study periods in two-pollutant models.

Pollutants	Model 2[a]
Trimester 1	
$PM_{2.5}$	1.44 (1.37, 1.52)
$+O_3$	1.51 (1.43, 1.60)
$+NO_2$	1.47 (1.38, 1.56)
$+SO_2$	1.41 (1.32, 1.50)
Trimester 2	
$PM_{2.5}$	1.67 (1.57, 1.77)
$+O_3$	1.77 (1.66, 1.89)
$+NO_2$	1.63 (1.52, 1.75)
$+SO_2$	1.60 (1.48, 1.72)
Trimester 3	
$PM_{2.5}$	1.23 (1.16, 1.30)
$+O_3$	1.23 (1.17, 1.31)
$+NO_2$	1.09 (1.01, 1.16)
$+SO_2$	1.35 (1.26, 1.45)
Entire pregnancy	
$PM_{2.5}$	1.52 (1.42, 1.62)
$+O_3$	1.59 (1.49, 1.70)
$+NO_2$	1.44 (1.33, 1.56)
$+SO_2$	1.89 (1.72, 2.08)

Note: HR, hazard ratio; CI, confidence interval.
[a] All models adjusted for maternal age, newborn gender, season of conception, previous pregnancy and previous delivery condition, mean ambient temperature and relative humidity with degrees of freedom of 6 and 3.

Over an average during the entire pregnancy, the estimated effect of $PM_{2.5}$ was higher among pregnant women who had not been pregnant previously than among women who had been previously pregnant with an adjusted HR of 2.02 (95% CI: 1.78, 2.28) vs 1.33 (95% CI: 1.23, 1.45), and among pregnant women without previous delivery than those with previous delivery with an adjusted HR of 1.84 (95% CI: 1.66, 2.03) vs 1.28 (95% CI: 1.17, 1.41).

Altering the degrees of freedom for the adjustment of temperature and relative humidity did not substantially change effect estimates for the association of $PM_{2.5}$ to stillbirth. HR estimates for stillbirth of entire pregnancy ranged from 1.52 to 1.53 for the adjustment of temperature and from 1.52 to 1.56 for the adjustment of relative humidity (See Supplementary Table S2). Further excluding the season of conception covariate did not alter the effect estimates (Supplementary Table S3), for example, the strongest HR of including the season of conception was 1.67 (95% CI: 1.57, 1.77) for each 10 μg/m^3 increase in $PM_{2.5}$ at trimester 2, which similarly to the HR of excluding the season of conception covariate 1.63 (95% CI: 1.54, 1.73). The HRs (95% CIs) of other adjusting covariates for stillbirths during the entire pregnancy are showed in Supplementary Table S4.

5. Discussion

To our knowledge, this study is among the largest multi-city birth cohort study to investigate the association between $PM_{2.5}$ and stillbirths among a Chinese population. Based on our cohort of 1.27 million births, this study illustrated that gestational exposure to $PM_{2.5}$ was associated with increased risk of stillbirths. Our study also demonstrated that the second trimester might be more vulnerable exposure window than the first trimester and the third trimester.

The above findings are generally consistent with the results of previous literature which also showed the significant associations between $PM_{2.5}$ exposure and increased stillbirth (Arroyo et al., 2016; DeFranco et al., 2015; Green et al., 2015). For example, Arroyo's study in Spain and DeFranco's study in the United States both revealed that the risk of stillbirths increased with high concentrations of $PM_{2.5}$ exposure (Arroyo et al., 2016; DeFranco et al., 2015). Green's study also indicated an association between exposure to high level $PM_{2.5}$ and an increased stillbirth throughout pregnancy in California (Green et al., 2015). However, other two studies in USA and one study in China found no positive association between $PM_{2.5}$ and stillbirths (Faiz et al., 2012, 2013; Hou et al., 2014). Inconsistent findings across these studies might be due to the complexity of $PM_{2.5}$ composition, the difference of air pollutant concentrations, and the different susceptibilities and confounders of the underlying populations. Thus, large multicity birth cohort studies focused on identifying the association of different air pollutant and the risk of stillbirths are necessary to prevent the adverse effect of air pollutants and make effective measurements.

The present study suggested that $PM_{2.5}$ was a potential risk factor of stillbirths; however, the mechanism by which $PM_{2.5}$ causes stillbirths remains unsolved. One study found that exposure to $PM_{2.5}$ might cause hypercoagulability with vascular thrombosis and placental inflammation, which would affect placental development (Liu et al., 2016). Another study found that prenatal traffic exposure might increase the risk of preeclampsia and lead to placental abruption (Yorifuji et al., 2015). This placental abnormality is a main cause of stillbirths (Tikkanen, 2011). Experiments conducted on animals show that $PM_{2.5}$ exposure could exacerbate the deviation of Th1 (type 1 helper T cell) or Th2 (type 2 helper T cell) and affect the immune system (Hong et al., 2013). Immune or endocrine system dysfunction could affect fetal growth, leading to a stillbirth (Ebisu et al., 2018). Toxic pollutants may also affect blood flow and, thereby, lessen nutrition transfer from the mother to the fetus (Maisonet et al., 2004), which heightens the risk of stillbirths.

Trimester-specific susceptibility to air pollution exposure is another factor that remains unanswered. The sensitive window to air pollutant exposure is inconsistent across studies (Arroyo et al., 2016; DeFranco et al., 2015; Hwang et al., 2011). Yang's study in China reported that air pollutant exposure in the third trimester was significantly associated with stillbirth (Yang et al., 2018). In the present study, the second trimester was shown to be the sensitive window of stillbirth to $PM_{2.5}$ exposure. This variation in susceptibilities may be caused by different air pollutant exposures at different period of pregnancy, and associations may also fluctuate with seasonal changes. Different sample sizes, study design, air pollution levels, and methodologies might also contribute to

Table 5
Baby's sex, maternal age group, previous pregnancy, and previous delivery condition specific HRs (95%CIs) for stillbirths associated with each 10 μg/m^3 increase in $PM_{2.5}$ in the study population.

Variables	Model 1[a]				Model 2[b]			
	Trimester 1	Trimester 2	Trimester 3	Entire pregnancy	Trimester 1	Trimester 2	Trimester 3	Entire pregnancy
Baby gender								
Male	1.49 (1.38, 1.61)	**1.69 (1.55, 1.83)**	**1.26 (1.17, 1.36)**	1.26 (1.16, 1.37)	1.47 (1.37, 1.58)	**1.74 (1.60, 1.89)**	**1.32 (1.22, 1.43)**	1.55 (1.42, 1.71)
Female	1.36 (1.26, 1.47)	**1.48 (1.35, 1.61)**	**1.11 (1.03, 1.21)**	1.20 (1.09, 1.31)	1.36 (1.26, 1.47)	**1.53 (1.40, 1.67)**	**1.13 (1.04, 1.23)**	1.47 (1.33, 1.62)
Maternal age								
<35 years	1.48 (1.40, 1.57)	**1.66 (1.56, 1.78)**	**1.23(1.16, 1.30)**	1.25 (1.17, 1.34)	1.46 (1.38, 1.55)	**1.72 (1.61, 1.84)**	1.26 (1.19, 1.34)	1.53 (1.42, 1.65)
≥35 years	1.31 (1.15, 1.49)	**1.34 (1.16, 1.56)**	**1.06 (0.93, 1.20)**	1.17 (1.01, 1.36)	1.33 (1.17, 1.52)	**1.45 (1.24, 1.69)**	1.11 (0.97, 1.28)	1.50 (1.27, 1.76)
Previous pregnancy								
Yes	**1.30 (1.23 1.39)**	**1.46 (1.36, 1.57)**	1.19 (1.11, 1.26)	**1.25 (1.16, 1.35)**	**1.28 (1.20, 1.37)**	**1.47 (1.36, 1.58)**	1.18 (1.11, 1.27)	**1.33 (1.23, 1.45)**
No	**1.85 (1.69, 2.02)**	**2.15 (1.95, 2.38)**	1.31 (1.18, 1.44)	**1.75 (1.57, 1.96)**	**1.83 (1.67, 2.00)**	**2.24 (2.01, 2.49)**	1.32 (1.19, 1.46)	**2.02 (1.78, 2.28)**
Previous delivery								
Yes	**1.30 (1.20, 1.39)**	**1.46 (1.35, 1.58)**	1.17 (1.09, 1.26)	**1.22 (1.12, 1.33)**	**1.27 (1.18, 1.37)**	**1.47 (1.35, 1.60)**	1.18 (1.09, 1.27)	**1.28 (1.17, 1.41)**
No	**1.64 (1.52, 1.76)**	**1.88 (1.73, 2.04)**	1.30 (1.20, 1.41)	**1.60 (1.46, 1.76)**	**1.62 (1.51, 1.75)**	**1.92 (1.76, 2.09)**	1.30 (1.19, 1.41)	**1.84 (1.66, 2.03)**

Note: Bolded text indicates statistically significant values ($p < 0.05$), HR, hazard ratio; CI, confidence interval.
[a] All models adjusted for mean ambient temperature and relative humidity with degrees of freedom of 6 and 3, respectively.
[b] All models adjusted for maternal age, newborn gender, season of conception, previous pregnancy and previous delivery condition, in addition to Model 1.

differing results across ours and previous studies. For example, our study included a large sample size of participants who had been exposed to high $PM_{2.5}$ pollution levels in seven cities in the PRD region. Another example is that the mean concentrations of air pollutants at each trimester of pregnancy were considered in our analysis by using Cox proportional hazards models.

The observed associations between $PM_{2.5}$ and stillbirth seemed not to be confounded by other air pollutants, expect for SO_2. It might be due to the relatively high correlations between $PM_{2.5}$ and SO_2 ($r = 0.65$, shown in Table S1), which change the HR of $PM_{2.5}$ most after controlling for SO_2 in the model.

In this study, we examined potential effect modification and found higher estimated risk of $PM_{2.5}$ exposure on stillbirth among pregnant women without previous experiences of pregnancy and delivery. This finding had some important public health implications, particularly, special air pollution protection should be considered for these women. The underlying reasons for these findings remain unknown. It is possible that women who had not previously been pregnant and or delivered a child were less experienced on how to protect themselves and were exposed to more outdoor air pollution.

Our study was a population based birth cohort study which included 1,273,924 newborns across in seven cities in the PRD region. The air pollution levels in the studied region are relatively higher than that in the previously studied regions. A study in U.S. found the $PM_{2.5}$ concentration during the entire pregnancy ranged from 9.9 μg/m^3 in Florida to 18.7 μg/m^3 in California (Sun et al., 2016). In this study, the mean concentration of $PM_{2.5}$ during the entire pregnancy was 36.78 μg/m^3. Our study also added new evidence for the adverse effects of maternal $PM_{2.5}$ exposures on neonatal health. Pregnant women seemed to be more sensitive to the adverse effect of air pollutants, and thus effective air quality control policies and stricter enforcement are necessary to improve maternal and neonatal health. More studies focused on air pollutants and neonatal health are important for building a comprehensive understanding of their associations and interactions.

Our study was subject to a few limitations. First, exposure misclassification was possible, since the air pollutant data from nearby air monitoring stations was used, future studies are suggested to have a better exposure assessment at the individual level (Copeland et al., 1977). Secondly, we did not consider residence mobility of some participants during their pregnancy because of the unavailability of this information. We also lacked information on other important factors, such as genetics, childbirth or pregnancy complications, fetal growth restrictions, cogential abnormalties, maternal nutritional status and socio-economic status (Goldenberg et al., 2008). Furthermore, some previous studies on stillbirth treated exposure to indoor cigarette smoking and alcohol consumption as potential confounders, but this study did not have categorized parental occupations and behaviors available in our data (Hao et al., 2016; Woodruff et al., 2009).

6. Conclusions

Our study demonstrates that maternal $PM_{2.5}$ exposure might be an important risk factor for stillbirths, especially at the middle stage of pregnancy. Our analysis also suggests that whether the mother has been pregnant and/or delivered a child before might modify the associations between $PM_{2.5}$ and stillbirths.

Contributors

ZJ.L, HL. L and GC.L designed this study and wrote the manuscript. ZJ.L and J. Y collected the health data and analyzed the data. Y.Y and HL. L collected the air pollution and weather data. ZM. Q, ZL. Z, SE. M and E.L revised the manuscript and were involved in the discussion of the results.

Declaration of competing interest

The authors declare they have no actual or potential competing financial 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.2021.113795.

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Review

Substitutes mimic the exposure behaviour of REACH regulated phthalates – A review of the German HBM system on the example of plasticizers

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ABSTRACT

The population is constantly exposed to potentially harmful substances present in the environment, including inter alia food and drinking water, consumer products, and indoor air. Human biomonitoring (HBM) is a valuable tool to determine the integral, internal exposure of the general population, including vulnerable subgroups, to provide the basis for risk assessment and policy advice. The German HBM system comprises of five pillars: (1) the development of suitable analytical methods for new substances of concern, (2) cross-sectional population-representative German Environmental Surveys (GerES), (3) time trend analyses using archived samples from the Environmental Specimen Bank (ESB), (4) the derivation of health-based guidance values as a risk assessment tool, and (5) transfer of data into the European cooperation network HBM4EU. The goal of this paper is to present the complementary elements of the German HBM system and to show its strengths and limitations on the example of plasticizers. Plasticizers have been identified by EU services and HBM4EU partners as priority substances for chemical policy at EU level. Using the complementary elements of the German HBM system, the internal exposure to classical phthalates and novel alternative plasticizers can be reliably monitored. It is shown that market changes, due to regulation of certain phthalates and the rise of substitutes, are rapidly reflected in the internal exposure of the population. It was shown that exposure to DEHP, DiBP, DnBP, and BBzP decreased considerably, whereas exposure to the novel substitutes such as DPHP, DEHTP, and Hexamoll®DINCH has increased significantly. While health-based guidance values for several phthalates (esp. DnBP, DiBP, DEHP) were exceeded quite often at the turn of the millennium, exceedances today have become rarer. Still, also the latest GerES reveals the ubiquitous and concurrent exposures to many plasticizers. Of concern is that the youngest children showed the highest exposures to most of the investigated plasticizers and in some cases their levels of DiBP and DnBP still exceeded health-based guidance values. Over the last years, mixture exposures are increasingly recognized as relevant, especially if the toxicological modes of action are similar. This is supported by a cumulative risk assessment for four endocrine active phthalates which confirms the still concerning cumulative exposure in many young children. Given the adverse health effects of some phthalates and the limited toxicological knowledge of substitutes, exposure reduction and surveillance are needed on German and EU-level. Substitutes need to be monitored, to intervene if exposures are threatening to exceed acceptable levels, or if new toxicological data question their appropriateness. It is strongly recommended to reconsider the use of plastics and plasticizers.

1. Introduction

Although chemical safety has been continuously improved within the last century, public concern about the exposure to environmental chemicals, the number of insufficiently investigated substances as well as the aggregated exposure remains high. Challenging the task to inform the public and support a science-based chemical policy, the German Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) has established a comprehensive human biomonitoring (HBM) system at the German Environment Agency (UBA). The aims of the HBM system are the monitoring of internal and aggregated exposure to

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Abbreviations

BMU	German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety
BBzP	butylbenzyl phthalate
5cx-MEPP	mono(2-ethyl-5-carboxy-pentyl) phthalate
5cx-MEPTP	mono(2-ethyl-5-carboxyl-pentyl) benzene-1,4-dicarboxylate
cx-MiDP	mono(2,7-methyl-7-carboxy-heptyl) phthalate
cx-MINCH	cyclohexane-1,2-dicarboxylic mono carboxyisooctyl ester
DEP	diethyl phthalate
DEHA	di(2-ethylhexyl) adipate
DEHP	di(2-ethylhexyl) phthalate
DEHTP	diethylhexyl terephthalate
DI	daily intake
DiBP	di-iso-butyl phthalate
DiDP	di-iso-decyl phthalate
DINCH	di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate
DINA	diisononyl adipate
DiNP	di-iso-nonyl phthalate
DnBA	di-n-butyl adipate
DnBP	di-n-butyl phthalate
DMP	dimethyl phthalate
DPHP	di(2-propylheptyl) phthalate
ECHA	European Chemicals Agency
ESB	German Environmental Specimen Bank
GerES	German Environmental Survey
HBM	human biomonitoring
HBM4EU	European Human Biomonitoring Initiative
HBM-I value	human biomonitoring value I
HBM-II value	human biomonitoring value II
HBM-GV	human biomonitoring guidance value
HMW	high molecular weight
REACH	registration, evaluation, authorisation and restriction of chemicals
LMW	low molecular weight
MBzP	monobenzyl phthalate
MEP	monoethyl phthalate
MEHP	mono(2-ethylhexyl) phthalate
MiBP	mono-iso-butyl phthalate
MMP	monomethyl phthalate
MnBP	mono-n-butyl phthalate
5OH-MEHP	mono(2-ethyl-5-hydroxy-hexyl) phthalate
OH-MiDP	6-OH-mono-propyl-heptyl phthalate
OH-MiNP	7-OH-(mono-methyl-octyl) phthalate
OH-MINCH	cyclohexane-1,2-dicarboxylic mono hydroxyisononyl ester
OH-MPHP	mono(2-propyl-6-hydroxy-heptyl) phthalate
oxo-MPHP	mono(2-propyl-6-oxo-heptyl) phthalate
5oxo-MEHP	mono(2-ethyl-5-oxo-hexyl) phthalate
oxo-MiDP	6-oxo-mono-propyl-heptyl phthalate
oxo-MiNP	7-oxo-(mono-methyl-octyl) phthalate
PVC	polyvinyl chloride
TDI	tolerable daily intake
TOTM	tris(2-ethylhexyl) tri-mellitate
UBA	German Environment Agency
VCI	German Chemical Industry Association (Verband der Chemischen Industrie)

chemicals, the identification of potential health risks, and building of scientific bases for political actions.

The most prominent instrument of the German HBM system is the regular analysis of environmental chemicals and/or their metabolites in human matrices, mostly blood and urine. Thus, the internal exposure to certain chemicals and cumulative exposure to multiple chemicals simultaneously can be assessed accurately. The origin of the German HBM activities dates back to the early 1970s, where, after a massive lead intoxication of livestock in Nordenham (Germany), an HBM research project was initiated with the aim to assess the lead levels of the population (Kolossa-Gehring et al., 2012a). Successively, new instruments have been added to the German HBM toolbox and have continuously been developed further, resulting in a highly valuable instrument for exposure and risk assessment of the population.

Today, the German HBM system comprises five complementing components, providing strong scientific evidence for policy advice and information of the public (cf. Fig. 1): (1) the development of new analytical methods for the monitoring of substances of health relevance - to which the population might be exposed to a considerable extent and for which no sufficiently sensitive and specific HBM methods yet exist - in a co-operation between the BMU and the German Chemical Industry Association (VCI); (2) population-representative cross-sectional German Environmental Surveys (GerES) to assess the exposure of the general population, to identify exposure sources, exposure-relevant habits, higher exposed subgroups, and thus, to develop exposure reduction measures; (3) time-trend analyses using samples from the Environmental Specimen Bank (ESB) to identify trends in exposure and to monitor the effectiveness of exposure reduction measures and policy actions; (4) the toxicological risk assessment by independent experts from various disciplines in the German Human Biomonitoring Commission allowing for the evaluation and interpretation of HBM results in the context of health risks; (5) the sharing of experience, methodologies, and results for harmonisation at the EU level in the HBM network of the European Joint Programme HBM4EU.

This review aims at presenting the progress and achievements of the German HBM system, using the monitoring of exposure to phthalates and alternative plasticizers as an example. Plasticizers were identified by EU services and HBM4EU partners as priority substances for which open policy-relevant research questions still have to be answered to support chemical policy at the EU level (Ganzleben et al., 2017).

The strengths and limitations of the single components of the German HBM system are discussed in detail and conclusions for plasticizers risk assessment and exposure mitigation are drawn. The example is also used to show the progress of the German HBM system in the last decade, to emphasize the importance of its components, and to illustrate its contribution to European chemical policy. By using multiple tools complementing each other, conclusions can be drawn which allow for deriving recommendations for policy makers.

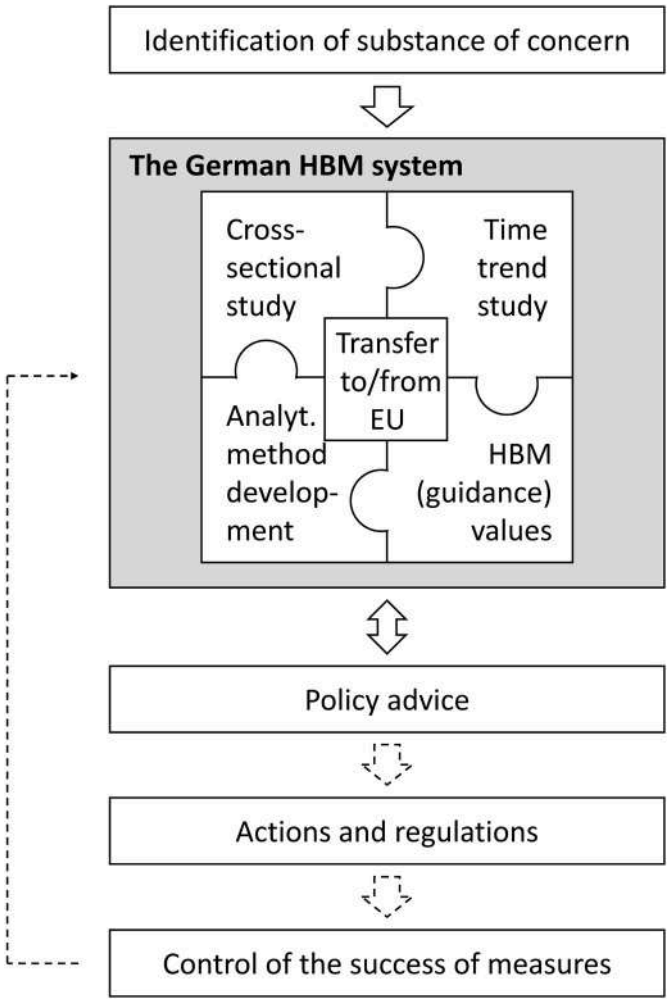

Fig. 1. The German HBM system and its embedding in the process from identification of substances of concern to policy advice and action.

2. The German HBM system

From the multitude of chemicals used in Europe only a selection can be monitored in HBM studies because of logistical and financial limitations. The criteria for the prioritisation of compounds for inclusion in HBM are their production volume, toxicological properties and potential impact on human health, accumulation potential and persistency, occurrence in the environment, and the availability of sensitive and specific biomarkers preferably in blood or urine. High tonnage compounds with critical toxicological characteristics such as carcinogenicity, mutagenicity, reproductive toxicity (CMR) or endocrine disrupting (ED) properties, as well as long term and organ specific effects are of high priority for investigation in HBM studies.

One major bottleneck of HBM is the availability of suitable analytical methods for emerging substances of concern and newly introduced substitutes for regulated chemicals like the reprotoxic phthalates. To close this gap, a cooperation between BMU and VCI with clearly separated responsibilities started in 2010 (Kolossa-Gehring et al., 2017; Leng and Gries, 2017). Within this BMU/VCI cooperation, up to 50 new HBM methods were to be developed for substances (1) for which substantial exposure of the general population is to be expected, (2) that might have health-relevant effects on the human organism, and (3) for which no specific and sensitive HBM methods exist. More than 20 HBM methods have already been developed and validated, inter alia for phthalates and alternative plasticizers (Gries et al., 2012a; Höllerer et al., 2018b; Kuhlmann et al., 2021; Leng and Gries, 2017; Lessmann et al., 2016a; Schütze et al., 2012). After analytical method validation and peer-reviewed publication, the respective methods are applied to samples of the two German HBM studies at federal level: the German Environmental Survey (GerES) and the German Environmental Specimen Bank (ESB).

GerES is a population-representative cross-sectional study investigating the exposure of the population in Germany to chemicals. It has repeatedly been carried out since 1985 (Kolossa-Gehring et al., 2012b; Schulz et al., 2007). GerES has always been conducted in close cooperation with the German health interview and examination surveys of the Robert Koch Institute (RKI) (Kamtsiuris et al., 2007; Kurth et al., 2008; Mauz et al., 2017). The latest cycle, GerES V (2014–2017), examined children and adolescents aged 3–17 years (Schulz et al., 2017). Some of the former GerES cycles focussed on other age groups. The HBM of GerES is complemented, inter alia, by the monitoring of indoor air, house dust, and drinking water. It is also supplemented by questionnaire data on dietary behaviour, lifestyle, and living environment and can be supplemented by the data derived by the health surveys of RKI. Thus, allowing for the identification of potential exposure pathways.

The ESB is an archive for human samples collected annually (Kolossa-Gehring et al., 2012a; Wiesmüller et al., 2007) and specimen of various environmental compartments (ESB, 2021a). Collection of human samples started in 1981. While a base set of substances (mainly heavy metals) is routinely analysed in all samples, cryo-archived samples are used for retrospective analyses of relevant substances if there is a need and an analytical method exists, becomes newly available or is sufficiently refined to detect exposure levels originating from environmental exposure. Thus, time trends of pollutant concentrations, e.g. in human blood, plasma, or urine can be analysed and the effects of the marketing of chemicals and their potential regulation on human internal exposure can be investigated. The ESB investigates a non-representative group of 20–29-year-old students at four sampling locations. The strength of the ESB is to supply valuable information on the development of exposure over time in continuously collected, comparable samples investigated by standardized procedures and methods (ESB, 2021a, b) – while ESB samples (not population-representative, from not specifically exposed volunteers, limited to 20–29 year old adults) are not suited to draw conclusions for other subgroups and living conditions of the whole population in Germany (as does GerES). A unique selling point of the ESB is the option for thorough time trend analyses dating back even to times before a substance was of concern. The time trends can help to interpret GerES results when exposure levels measured in GerES at different time points shall be compared. ESB and GerES together allow a complementary cross-sectional and time trend assessment of the chemical body burden of the population in Germany.

The results of the HBM studies are evaluated by using the health-based HBM values derived by the German HBM Commission (German HBM Commission, 1996) whenever available. The Commission derives so-called HBM-I and HBM-II values. The HBM-I value is defined as the concentration of a substance or its biomarker(s) at or below which there is no risk for human health, according to current knowledge. The HBM-II value represents the concentration of a substance or its biomarker(s) in human biological material at or above which there is an increased risk for adverse health effects, according to current knowledge (Apel et al., 2017). If the exposure levels are at or below HBM-I value, there is no need for action. If there is an exceedance of the HBM-II value, a health risk is anticipated. Thus, the HBM-I value can be regarded as a control value and the HBM-II value as an intervention value (Angerer et al., 2011; Apel et al., 2017).

In 2017, the European Human Biomonitoring Initiative (HBM4EU) has been set up. HBM4EU is a joint effort of 30 countries and the European Environment Agency, co-funded under the European Commission's Horizon 2020 program. HBM4EU, coordinated by UBA, creates a European network that improves the knowledge and factual basis for the European Union's environmental and chemical policy by harmonizing the planning and implementation of HBM studies, sample analysis and

data analysis across national borders (Ganzleben et al., 2017). One key aim is the knowledge transfer from countries with a long tradition in HBM to countries without own national programs to advance and implement HBM on a European scale and to provide scientific evidence to improve chemical policy making. Another goal of the project is to bring together already existing data, including the results of the GerES and ESB studies, and evaluate them according to uniform criteria. Moreover, new joint studies are conducted. For this purpose, questions, procedures, analytical methods, and evaluations are standardized and quality assured. HBM4EU also aims at harmonizing health risk assessments by deriving human biomonitoring guidance values (HBM-GVs) in a coordinated manner (Apel et al., 2020b). HBM-GVs derived for the general population correspond to the HBM-I values of the German HBM Commission and provide a valuable addition and partial update of already existing HBM-I values.

Plasticizers were chosen as an example to show the complementary elements of the German HBM system. The full scope of activities on plasticizers within the German HBM system is given in Table 1.

3. Human biomonitoring of phthalates and substitute plasticizers

Phthalates are esters of phthalic acid (benzene-1,2-dicarboxylic acid) and are widely used in industrial applications, e.g. as solvents, formulating agents, and plasticizers for soft polyvinyl chloride (PVC) (Koch et al., 2017). The first phthalates were introduced into the market in the 1920s and production skyrocketed after di-(2-ethylhexyl) phthalate (DEHP) was synthesized in 1933 and the PVC industry started growing (Graham, 1973). As of 2011, 8 mio tons of phthalates per year were consumed worldwide and about 840 ktons in Europe (ECHA, 2013), decreasing to 810 ktons in 2017 (European Plasticisers initiative CEFIC sector group, 2018).

High molecular weight (HMW) phthalates such as DEHP, di-iso-nonyl phthalate (DiNP), di-iso-decyl phthalate (DiDP) and di-(2-propylheptyl) phthalate (DPHP) were mostly used as plasticizers for the production of PVC (Fréry et al., 2020). Low molecular weight (LMW) phthalates like di-*n*-butyl phthalate (DnBP), di-*iso*-butyl phthalate (DiBP), butylbenzyl phthalate (BBzP), diethyl phthalate (DEP) and dimethyl phthalate (DMP) used to be contained in consumer products like textiles, pesticides, paints, adhesives and cosmetics (Fréry et al., 2020).

Until 2000, DEHP was the most commonly used phthalate in Europe with over 400 ktons of annual consumption (Bizzari et al., 2013). However, from the late 1990s, the use of DiNP, DiDP and DPHP started increasing and partially replaced DEHP on the plasticizer market (ECHA, 2013).

Health impacts of phthalates raised increasing concern after their reprotoxic properties were identified. Even more so as ubiquitous exposure of humans was observed due to the high production volumes and the use in products in close contact to consumers (Koch et al., 2017; Wittassek et al., 2011). Especially phthalates with a backbone of three to six carbon atoms such as DiBP, DnBP, and BBzP, and the HMW phthalate DEHP show endocrine disrupting properties related to the so-called phthalate syndrome (Koch et al., 2017). The phthalate syndrome includes developmental effects and structural as well as functional damage of the male reproductive system when the foetus is exposed during a critical window of sexual development (Apel et al., 2020a). Among others, the observed effects include malformations of the testes, epididymis and gubernaculum testis, cryptorchidism, hypospadias, reduced anogenital distance and reduced semen count (summarised in e.g. NRC, 2008; US CPSC, 2014). As phthalates are not chemically bound to the carrier material, they are easily released into the environment,

Table 1
Overview of German HBM activities on phthalates and substitute plasticizers – monitoring, assessment values, method development.

	Population-representative monitoring		Time trends	HBM (guidance) values	Analytical methods
	GerES IV	GerES V	ESB		BMU/VCI cooperation
Phthalates					
DMP, DEP, DiDP, DnOP, DCHP, DnPeP		Schwedler et al. (2020c)	2007–2015: Koch et al. (2017)		
BBzP, DnBP, DiBP	Becker et al. (2009)	Schwedler et al. (2020c)	1988–2015: Apel et al. (2020a); Göen et al. (2011); Koch et al. (2017); Wittassek et al. (2007b)	HBM-GV: Lange et al. (2021)	
DEHP	Becker et al. (2009)	Schwedler et al. (2020c)	1988–2015: Apel et al. (2020a); Göen et al. (2011); Koch et al. (2017); Wittassek et al. (2007b)	HBM-I: Apel et al. (2017), HBM-GV: Apel and Ougier (2017)	
DiNP	Becker et al. (2009)	Schwedler et al. (2020c)	1988–2015: Apel et al. (2020a); Göen et al. (2011); Koch et al. (2017); Wittassek et al. (2007b)		
DPHP		Schwedler et al. (2020a)	1999–2017: Schmidtkunz et al. (2019); Schütze et al. (2015)	HBM-I: Apel et al. (2017), HBM-GV: Lange et al. (2021)	Gries et al. (2012b); Leng and Gries (2017)
Substitute plasticizers					
DEHTP		Schwedler et al. (2020b)	1999–2017: Lessmann et al. (2019)	HBM-I: Apel et al. (2017)	Lessmann et al. (2016a)
DINCH		Schwedler et al. (2020a)	1999–2017: Kasper-Sonnenberg et al. (2019); Schütze et al. (2014)	HBM-I: Apel et al. (2017), HBM-GV: Apel and Ougier (2017)	Schütze et al. (2012)
TOTM		Murawski et al. (2021)			Höllerer et al. (2018b); Kuhlmann et al. (2021)
DEHA					Nehring et al. (2019)
DnBA					Ringbeck et al. (2020)
DINA					Gotthardt et al. (2021)

GerES IV: 2003–06, children (3–14 years), N = 599; GerES V: 2015–17, children and adolescents (3–17 years), N = 439–2256; ESB: young adults (20–29 years), N = 60/year.

DMP: Dimethyl phthalate, DEP: Diethyl phthalate, DiDP: Di-iso-decyl phthalate, DnOP: Di-n-octyl phthalate, DCHP: Dicyclohexyl phthalate, DnPeP: Di-n-pentyl phthalate, BBzP: Butylbenzyl phthalate, DnBP: Di-n-butyl phthalate, DiBP: Di-iso-butyl phthalate, DEHP: Di(2-ethylhexyl) phthalate, DiNP: Di-iso-nonyl phthalate, DPHP: Di(2-propylheptyl) phthalate, DEHTP: Diethylhexyl terephthalate, DINCH: Di-iso-nonyl-cyclohexane-1,2-dicarboxylat, TOTM: Tri-(2-ethylhexyl) trimellitate, DEHA: Di(2-ethylhexyl) adipate, DnBA: Di-n-butyl adipate, DINA: Diisononyl adipate.

resulting in ubiquitous exposure of the population, e.g. by uptake via food, indoor air, and dust (Bui et al., 2016; Fréry et al., 2020; Schwedler et al., 2020c). After implementation of different steps of regulation, HBM studies were conducted to evaluate the success of the reduction measures. First mitigation measures were already enacted in 1999 (ECHA, 2013). The classification of DnBP, DEHP, BBzP and later DiBP as reproductive toxicants (Regulation (EC) No 1272/2008, EU, 2008) was followed by further restrictions and bans, e.g. use in toys and childcare articles (Directive, 2009/48/EC, EU, 2009a), cosmetics (Directive, 2009/48/EC, EU, 2009b) and food contact materials (Regulation (EC) 10/2011, EU, 2011). These four phthalates are regulated under REACH (registration, evaluation, authorisation and restriction of chemicals, Regulation (EC) No 1907/2006, EU, 2006) after classification as substances of very high concern (SVHC) (Schwedler et al., 2020c) and since 2015 authorisation is required for DiBP, DnBP, BBzP, and DEHP (REACH Annex XIV) (ECHA, 2013; Koch et al., 2017; Schwedler et al., 2020c). The latest amendment of REACH Annex XVII took effect in July 2020, restricting the general use of DiBP, DnBP, BBzP, and DEHP in consumer articles (ECHA, 2020).

One effect of the increasing regulation of certain phthalates is the decrease in use of the regulated phthalates and a shift to the use of non-regulated phthalates or substitute substances (Apel et al., 2020a; Frederiksen et al., 2020; Koch et al., 2017). DEHP is the most prominent regulated phthalate. The regulation resulted in a shift to the less regulated phthalates DiNP and DiDP, which in turn were substituted by DPHP, diethylhexyl terephthalate (DEHTP), di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate (Hexamoll®DINCH, abbr. DINCH), tris (2-ethylhexyl) tri-mellitate (TOTM) and di (2-ethylhexyl) adipate (DEHA). This effect is apparent in the increasing consumption of DINCH and DEHTP in Europe, rocketing from 9 to 2 ktons in 2002 to 55 and 100 ktons in 2014, respectively (Kasper-Sonnenberg et al., 2019; Lessmann et al., 2019). In contrast to the substituted phthalates, the substitutes thus far are not classified as toxic to reproduction or as endocrine disruptors. DINCH, introduced into the marked in 2002, did not show reproductive toxicity or developmental toxicity in animal studies (German HBM Commission, 2014). Effects on the kidneys were considered most relevant (EFSA, 2006). For DPHP, EFSA and also the German HBM Commission based their guidance values on the combined chronic and carcinogenicity dietary study in rats by Deyo (2008), in which effects on the retina were considered critical (Apel et al., 2017; Deyo, 2008; EFSA, 2008). Adverse systemic effects for TOTM following short-term and sub-chronic exposure refer to haematology, clinical chemistry, liver and spleen. Developmental toxicity considered to be biologically significant or substance-induced was not observed. However, toxicity to reproduction of TOTM remains to be elucidated as studies show contradictory results (CPSC, 2018).

DINCH and DEHTP are not only used as substitute plasticizers for reprotoxic HMW phthalates like DEHP, but also for DnBP, DiBP, BBzP, especially in regulated products and products resulting in potentially high exposure, e.g. toys, food contact materials, and medical applications (Kasper-Sonnenberg et al., 2019; Lessmann et al., 2019). DINCH and DEHTP, like other plasticizers, are not bound to the polymer and can easily migrate. A considerable exposure of the population is the result (Lessmann et al., 2016a; Schütze et al., 2012). The shift in the market and its consequences for human exposure and health require the inclusion of substitute compounds into the monitoring of human internal exposure.

4. Analytical methods for phthalate determination

Early methods for phthalate determination in urine could not distinguish between different specific phthalates and were replaced by liquid chromatography coupled to mass spectrometry (Blount et al., 2000; Kato et al., 2005; Koch et al., 2003b), which also allowed the simultaneous determination of various specific phthalate metabolites (Barr et al., 2003). Additionally, this method prevented interference with contamination by the ubiquitous parent compounds. Multi-methods enabling the quantification of many different phthalates are frequently expanded to include an even higher number of urinary metabolites. Nowadays, routine measurements of more than 10 different phthalates are available (Koch et al., 2017). Due to the short half-lives, phthalates and their substitutes, DINCH, DEHTP, DEHA, di-n-butyl adipate (DnBA) and diisononyl adipate (DINA), are rapidly eliminated from the body (Anderson et al., 2001a, 2011; Gotthardt et al., 2021; Koch et al., 2012, 2013; Lessmann et al., 2016b; Nehring et al., 2019; Ringbeck et al., 2020), whereas TOTM has a longer elimination half-life due to the molecular structure (most of the 2-ethylhexanol group is eliminated within 6 days; trimellitic acid core is excreted slowly) (CPSC, 2018). Thus, HBM measurements for the phthalates and their substitutes reflect only very recent exposure, except for TOTM which has a slower urinary excretion rate than e.g. DEHP with metabolites still detectable after 48 h and 72 h post-exposure (Höllerer et al., 2018a).

To keep up with the market development and the introduction of non-regulated substitute plasticizers, new analytical methods for the biomonitoring of these emerging substances were needed. Within the German BMU/VCI cooperation, new methods for the determination of DEHTP (Lessmann et al., 2016a), DINCH (Schütze et al., 2012), TOTM (Höllerer et al., 2018b; Kuhlmann et al., 2021), DEHA (Nehring et al., 2019), DnBA (Ringbeck et al., 2020) and DINA (Gotthardt et al., 2021) have been developed, thereby greatly expanding the toolbox of plasticizers' and phthalate substitutes' monitoring in the general population. Moreover, existing methods were improved to allow for the distinction of the structurally similar phthalates DiDP and DPHP (Gries et al., 2012b; Leng and Gries, 2017).

5. Internal exposure levels reflect regulation of phthalates and the emergence of substitute plasticizers

ESB and GerES provide complementary information on the population's exposure to pollutants: GerES supplies information on the exposure distribution in sub-groups and the whole society, on potential risk groups, risk factors, and on sources of exposure, while the ESB answers the question if either a new regulation has to be considered because of increasing exposure time trends or if implemented measures where sufficiently successful. Additionally, this archive allows for the retrospective analyses of newly emerging substances.

HBM data on 15 phthalates and plasticizer substitutes are to date available from ESB and GerES. An overview is given in Table 1. Daily intakes (DI) derived from ESB and GerES data are presented in Fig. 2. DI from 24-h-urine samples of the ESB were derived using equation (1) as also described by Apel et al. (2020a). For the first-morning void urine samples of GerES, a creatinine excretion-based approach described by Wittassek et al. (2007a) was applied as shown in equation (2).

$$\mathrm{DI}(\mu g / kg_{bw} / day) = \frac{UE_{vol,sum} \cdot uv}{F_{UE} \cdot bw} \cdot MW \quad (1)$$

$$\mathrm{DI}(\mu g / kg_{bw} / day) = \frac{UE_{crea,sum} \cdot CE}{F_{UE} \cdot bw} \cdot MW \quad (2)$$

$UE_{vol,sum}$ - molar urinary excretion sums of specific metabolites in µmol/L; $UE_{crea,sum}$ – molar urinary excretion sums of specific metabolites in µmol/g_{crea}; uv – 24-h urine volume; CE – sex- and body height-specific daily creatinine excretion given by Remer et al. (2002); F_{UE} – summarised urinary excretion fraction for all considered metabolites given in Table 2; bw – bodyweight; MW – molecular weight of respective substance.

Exposure to BBzP, DnBP, DEHP and, much later, DiBP decreased after regulatory measures were taken (Koch et al., 2017), reflecting the decrease of consumption (German HBM Commission, 2011). A very strong correlation between production and daily intake of DEHP was also already revealed by Helm (2007), indicating that human internal

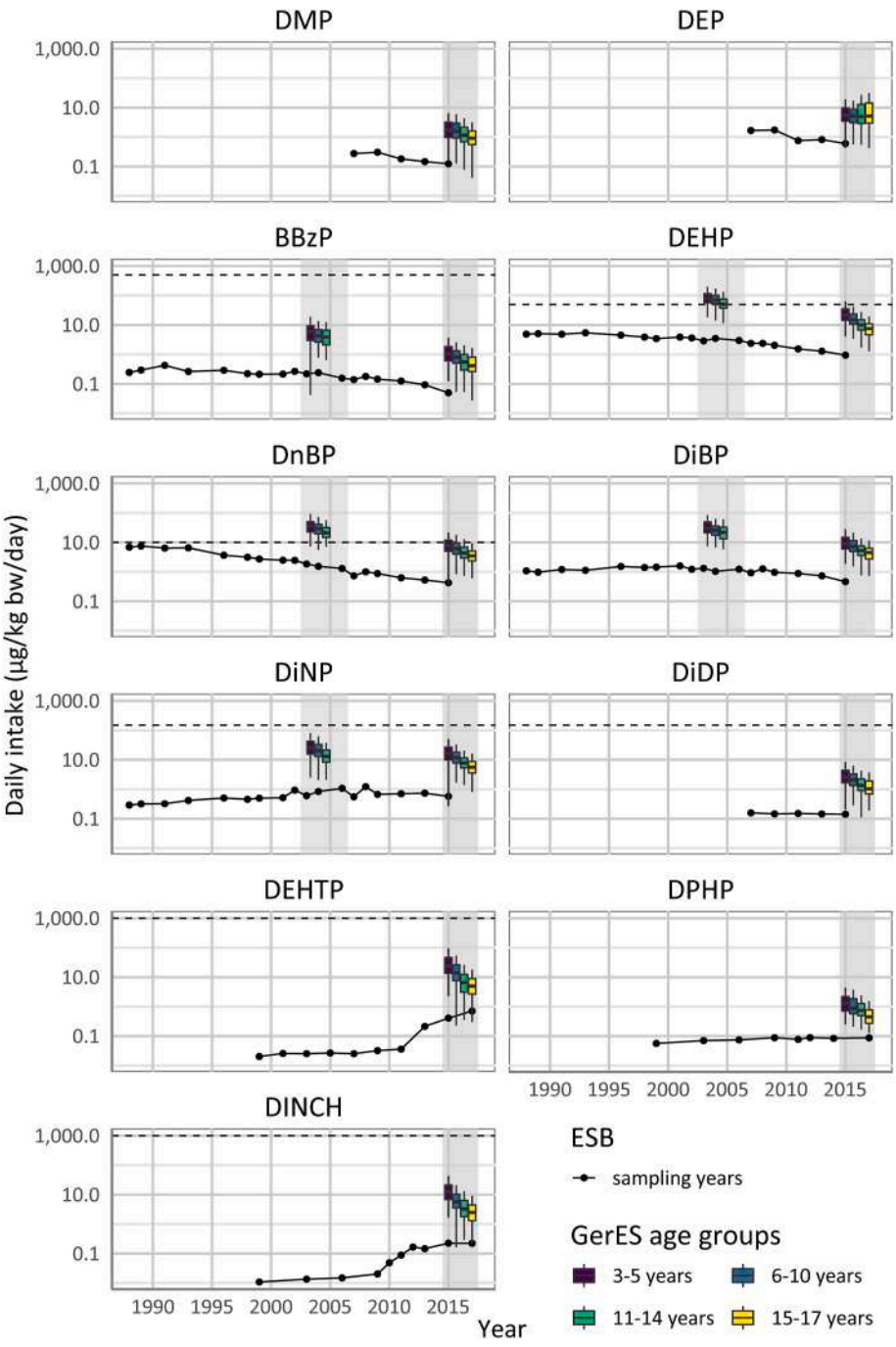

Fig. 2. Daily intake (µg/kg_{bw}/day) of plasticizers measured in samples of the ESB, GerES IV, and GerES V. Annual median values for ESB are presented by line-connected dots. GerES data are presented by colour-coded boxplots for each age group (whiskers spanning the 1.5-fold interquartile range at max, outliers are not shown). Grey shaded areas denote the sampling period of the respective GerES cycle (GerES IV – 2003–2006, GerES V – 2015–2017). The position of boxplots within the grey shaded area is for presentation purposes only; each boxplot represents the full sampling period of the respective GerES. Horizontal dashed lines denote the TDI of the substance (not available for DMP, DEP, DiBP, and DPHP). DCHP, DnPeP, DnOP, and TOTM are not shown as too few samples contained quantifiable amounts for statistical summaries. Note the logarithmic scale!

exposure follows production of plasticizers closely and immediately. Simultaneously with the decrease of regulated plasticizers, new phthalates, and substitute plasticizers such as DPHP, DEHTP, DINCH, DEHA and TOTM emerged, leading to an increasing internal human exposure to these new substances. The time trends provided by the ESB reveal strong increases in exposure to the substitute plasticizers DINCH (Schütze et al., 2014) and DETHP (Lessmann et al., 2019) since their market entry, resulting in a nowadays ubiquitous exposure of the population. These time trends are qualitatively mirrored by the comparison of data from two GerES cycles, GerES IV and GerES V. This is also apparent in Table 3, which shows the fraction of ESB and GerES participants with urinary plasticizer concentrations above the respective quantification limits. Since the market entry in 2002, exposure to DINCH steadily increased along with increasing production, resulting in quantifiable internal exposure levels in all GerES V participants. The same holds true for DEHTP. Rapidly increasing production volumes are reflected in increasing internal exposure levels, leading to quantifiable urinary DEHTP levels in each GerES V sample. While TOTM is not yet used widely and is consequently found in only few individuals (Murawski et al., 2021), the annual consumption of DPHP is clearly related to the detection rates of its main metabolite oxo-MPHP in human urine (Schmidtkunz et al., 2019).

6. Identification of subgroups of high exposure – GerES

A decrease of BBzP, DiBP, DnBP, and DEHP levels in ESB samples – and corresponding daily intake – after regulation (Apel et al., 2020a; Koch et al., 2017), as shown in Fig. 2, was similarly observed by

Table 2
Urinary excretion fractions (F_{UE}) and respective metabolites used for daily intake calculation of plasticizers.

Plasticizer	F_{UE}	Metabolites considered	Source
DMP	0.69	MMP	US Consumer Product Safety Commission (2014)
DEP	0.69	MEP	Koch et al. (2003a)
BBzP	0.73	MBzP	Anderson et al. (2001b)
DiBP	0.707	MiBP	Koch et al. (2012)
DnBP	0.691	MnBP	Anderson et al. (2001b)
DEHP	0.471	MEHP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP	Anderson et al. (2011), summarised by Apel et al. (2020a)
DiNP	0.189	OH-MiNP, oxo-MiNP	Anderson et al. (2011), summarised by Apel et al. (2020a)
DiDP	0.34	OH-MiDP, oxo-MiDP, cx-MiDP	summarised in Wittassek et al. (2011)
DEHTP	0.13	cx-MEPTP	summarised in Lessmann et al. (2019)
DPHP	0.1392	OH-MPHP, oxo-MPHP	average of Leng et al. (2014) and Klein et al. (2018), as also used by Lange et al. (2021)
DINCH	0.1276	OH-MINCH, cx-MINCH	Koch et al. (2013)

comparison of GerES IV and GerES V data (Schwedler et al., 2020c). Fig. 2 presents the GerES data for different age groups, showing a significant age gradient for most plasticizers with the highest exposure of young children (3–5 years) compared to older children and adolescents (15–17 years).

The daily intakes of children and adolescents were an order of magnitude higher than those of young adults. Apart from age-dependent metabolic differences and a relatively higher intake of food, drinks, and inhaled air per kg body weight by younger children compared to adults, strong associations between plasticizer concentrations in urine and house dust samples were observed in GerES V (Schwedler et al., 2020a, 2020b, 2020c). As young children tend to ingest dust due to hand-to-mouth contacts (Salthammer et al., 2018), exposure via house dust might additionally contribute to the observed age gradient. Furthermore, as many toys made of plastic contain plasticizers, and especially small children tend to put them in their mouth, further uptake of plasticizers by small children is likely.

For the interpretation of the results from ESB and GerES and a comparison of daily intakes methodological differences and some limitations have to be considered. The general sampling design differs, as described above (ESB: convenience sample of young adults, GerES: two-stage population-representative sample of children and adolescents in Germany), as well as the sampling method of the analysed urine samples (ESB: 24-h-urine, GerES: first-morning void urine). While the total

Table 3
Percentage of ESB and GerES participants with measurements above the limit of quantification (% > LOQ). Percentages for GerES refer to the full survey period. For ESB, percentages were derived for each individual sample year and displayed ranges refer to the range of annual values in the displayed time period.

Parent compound	BBzP	DEHP					DnBP		DiBP		DiNP		
Metabolite(s)	MBzP	MEHP	5OH-MEHP	5cx-MEPP	5oxo-MEHP	2cx-MMHP	MnBP	OH–MnBP	MiBP	OH-MiBP	7OH-MiNP	7oxo-MiNP	7cx-MiNP
LOQ (µg/L) [a]	0.5/0.2	0.5	0.5/0.2	0.5/0.2	0.5/0.2	0.5/–	2.0/1.0	–/0.25	2.0/1.0	–/0.25	0.5/0.2	0.5/0.2	0.5/0.2
GerES IV (2003–2006)	100	100	100	100	100	100	100	–	100	–	100	98	100
GerES V (2015–2017)	99	86	100	100	100	–	100	99	100	100	100	99	100
ESB (2003–2006)[b]	100	97–98	100	100	100	100	100	–	100	–	97–98	91–93	92–93
ESB (2015–2017)[b]	98	90	100	100	100	–	100	92	100	100	100	100	100
ESB before 2002	98–100	93–100	100	100	100	100	100	–	100	–	90–100	58–86	–
ESB from 2002	96–100	90–100	100	100	100	100	100	92–100	100	100	96–100	91–100	92–100

Parent compound	**DMP**	**DEP**	**DiDP**			**DCHP**	**DnPeP**	**DnOP**
Metabolite(s)	MMP	MEP	OH-MiDP	oxo-MiDP	cx-MiDP	MCHP	MnPeP	MnOP
LOQ (µg/L)	1.0	0.5	0.2	0.2	0.2	0.2	0.2	0.2
GerES V (2015–2017)	97	100	98	88	97	6	6	0
ESB (2015–2017)[b]	95	100	97	57	82	5	2	0
ESB from 2007	88–98	100	90–97	53–67	77–100	0–5	0–5	0–3

Parent compound	**DINCH**				**DPHP**			**DEHTP**				**TOTM**[c]
Metabolite(s)	MINCH	OH-MINCH	oxo-MINCH	cx-MINCH	OH-MPHP	oxo-MPHP	cx-MPHP	5OH-MEHTP	5oxo-MEHTP	5cx-MEPTP	2cx-MMHTP	
LOQ (µg/L)	0.1	0.05	0.05	0.05	0.3	0.25	0.15	0.3	0.2	0.2	0.4	0.09–0.26
GerES V (2015–2017)	–	100	97	99	50	62	1	67	79	100	20	0–3
ESB (2003–2006)[b]	0	0–7	0–5	0–3	0	0	0	0	0	0–3	0	–
ESB (2015–2017)[b]	–	100	95–97	80–87	2	18	0	22–47	18–40	98–100	2–3	–
ESB before 2002	0	0	0	0	0	0	0	0	0–2	3–10	0	–
ESB from 2002	0–5	0–100	0–100	0–88	0–3	0–23	0	0–47	0–40	0–100	0–3	–

[a] First values refer to ESB sampling years until 2006, 2008, and GerES IV; second values refer to ESB sampling years 2007, 2009–2015, and GerES V.

[b] Not every year in the given period is covered, for sample years of ESB see Fig. 2 or refer to the original publications.

[c] Six metabolites, for more information see Murawski et al. (2021).

Table 4
Percentage of ESB and GerES participants exceeding HBM values of the German HBM commission (HBM-I; Apel et al., 2017) or HBM guidance values derived within the framework of HBM4EU (HBM-GV; Lange et al., 2021). Percentages for GerES refer to the full survey period. For ESB, percentages were derived for each individual sample year and displayed ranges refer to the range of annual values in the displayed time period.

	HBM-I				HBM-GV						
Parent compound	DEHP	DEHTP	DINCH	DPHP	BBzP	DEHP	DEHP	DnBP	DiBP	DINCH	DPHP
Metabolite(s)	5oxo-MEHP + 5OH-MEHP	5cx-MEPTP	OH-MINCH + cx-MINCH	oxo-MPHP + OH-MPHP	MBzP	5cx-MEPP + 5OH-MEHP	5oxo-MEHP + 5OH-MEHP	MnBP	MiBP	OH-MINCH + cx-MINCH	oxo-MPHP + OH-MPHP
HBM value (mg/L)											
- children (<14 years)	0.5	1.8	3.0	1.0	2.0	0.38	0.34	0.12	0.16	3.0	0.33
- women/men	0.3/0.75	2.8	4.5	1.5	3.0	0.57	0.5	0.19	0.23	4.5	0.5
GerES IV (2003–2006)	1.55%	–	–	–	0%	4.23%	3.41%	34.10%	19.37%	–	–
GerES V (2015–2017)	0%	0%	0.04%	0%	0%	0.05%	0%	1.18%	1.76%	0.04%	0%
ESB (2003–2006)[a]	0%	0%	0%	0%	0%	0%	0%	1.72%–5.00%	0%–3.45%	0%	0%
ESB (2015–2017)[a]	0%	0%	0%	0%	0%	0%	0%	0%	1.67%	0%	0%
ESB before 2002[b]	0%–1.67%	0%	0%	0%	0%	0%–1.67%	0%–1.67%	10.00%–48.33%	0%–6.67%	0%	0%
ESB from 2002[c]	0%–1.67%	0%	0%	0%	0%	0%	0%	0%–5.00%	0%–3.45%	0%	0%

[a] Not every year in the given period is covered, for sample years of ESB see Fig. 2 or refer to the original publications.

[b] Earliest samples measured are from 1999 (DEHTP, DINCH, DPHP), and 1988 (BBzP, DEHP, DnBP, DiBP).

[c] Latest samples measured are from 2017 (DEHTP, DINCH, DPHP), and 2015 (BBzP, DEHP, DnBP, DiBP), respectively.

amount of daily metabolite excretion is known in ESB's 24-h-urine samples, daily intake calculation from GerES data requires various assumptions. Based on the first-morning void creatinine content, the excretion was extrapolated using generalised daily creatinine excretion totals given by Remer et al. (2002). Thus, only qualitative comparisons can be made between ESB and GerES results, while the comparability between the two cycles of GerES is mostly warranted.

The recent population representative study FLEHS IV from Belgium investigated the internal exposure of 14–15-year-olds to phthalates and compared geometric means (GM) of urinary concentrations of different studies (Bastiaensen et al., 2021). The internal exposure to various phthalate metabolites was in the same range as observed in GerES V and this was also true for studies from USA and Canada, which investigated 12–19-year-olds. These studies, investigating adolescents, had somewhat lower GM than observed for the GerES V population, which presumably is due to the difference in age. Studies from Sweden, Poland and Portugal which examined the internal exposure of younger children found considerably higher concentrations for some phthalates (summarised in Bastiaensen et al., 2021).

Given the reprotoxic properties of some phthalates and the higher exposure of young children to these substances, special attention is needed. Since highest exposure for all investigated phthalates and substitutes appears in small children, a cause for highest concern is given and indicates the necessity for further action.

7. Health-based risk assessment

HBM values derived by the German HBM Commission for DEHP, DEHTP, DINCH, and DPHP (Apel et al., 2017), as well as the HBM guidance values for DEHP, DINCH, DiBP, DnBP, BBzP, and DPHP derived within the framework of HBM4EU (Apel and Ougier, 2017; Lange et al., 2021) are well suited to evaluate possible health risks of the exposure. A limitation of this assessment lies in the approach to assess the risk only for a single substance and not for the real simultaneous exposure to a mixture. Table 4 summarises the percentage of participants exceeding the respective HBM-I value or HBM-GV. Urinary concentrations of BBzP, DEHTP, and DPHP were below the HBM-I values and HBM-GVs in ESB samples and samples of GerES IV and GerES V, which is in line with daily intakes being below the respective tolerable daily intakes (TDIs) (cf. Fig. 2). With a view on decreasing exposure levels to BBzP observed in the ESB data, the exposure to this phthalate can, according to today's knowledge, be classified as not of current concern, but is still found in nearly all samples. The current DEHTP and DINCH levels reach, up to now, only in exceptional cases the HBM-I value and can, therefore, be counted as below the level of health concern. However, the fact that their metabolites were found ubiquitously in the most recent samples of GerES V and increasing concentration trends are observed in ESB data require continuous attention and a further development of a mixture risk assessment, especially as the maximum exposure to DINCH has reached the range of mg/L in urine.

The HBM-I value for DEHP was exceeded by 1.55% of GerES IV and up to 1.67% of ESB participants, but not by GerES V participants. Up to 1.67% of ESB participants in sampling years before 2002 exceeded the HBM-GVs (adults: 500 μg/L for 5oxo-MEHP + 5OH-MEHP and 570 μg/L for 5cx-MEPP + 5OH-MEHP; Apel and Ougier, 2017) but none exceeded the HBM-GVs after 2002. The new HBM-GVs for DEHP for children, considering two different metabolite compositions, are lower (children: 380 μg/L for 5cx-MEPP + 5OH-MEHP and 340 μg/L for 5oxo-MEHP + 5OH-MEHP; Apel and Ougier, 2017) than the HBM-I values derived in 2007 (children: 500 μg/L for 5OH-MEHP + 5oxo-MEHP; Apel et al., 2017). The HBM-GVs were exceeded by 4.23% and 3.41% of GerES IV participants, but only by 0.05% and 0% of the GerES V population, respectively. In the GerES V sample, which was collected after several steps of regulation, still up to 0.05% exceeded the HBM-GV, which represents about 5690 children and adolescents in Germany. However, the decrease in exceedances also reflects the success of the increasingly

strict regulation. Yet, even more than a decade after the first regulatory steps all samples contained quantifiable amounts of DEHP and some individuals still had concerningly high exposure levels.

The HBM-GVs for DiBP and DnBP were exceeded by substantial fractions of the GerES IV population (DiBP: 19.37%, DnBP: 34.10%), but only by 1.76% (DiBP) and 1.18% (DnBP) of the participants of GerES V. This shows the considerable progress in reducing exposure to these reprotoxic and endocrine disrupting phthalates, but they are still present in all samples analysed. Again, the higher exposure of small children is still of concern as they represent a vulnerable group. ESB samples collected in the years of GerES IV sampling (2003–2006) exceeded the HBM-GVs in at most 3.45% and 5.00% of the participants. For comparison, in 1988–2001 DnBP concentrations exceeded the HBM-GV in 10.00%–48.33% and from 2002 in, at maximum, 5.00%. This again reflects a successful reduction of exposure, but also emphasises that young adults reached lower exposure levels much earlier than children.

Considering the differences between the physiology of children and adults, it is recommendable to apply the HBM-I values and HBM guidance values to children under 6 years of age only if the metabolite ratio in young children is the same as in adults. Therefore, the comparison with exposure data used here can only be used as an approximation for rough guidance (German HBM Commission, 2007).

Health based guidance values (HBM-I and HBM-GV) allow for assessing the exposure risk of single substances only, but many phthalates share similar effects and modes of action (Apel et al., 2020a). Therefore, mixture effects must be considered to account for the impact of the real-life exposure to multiple phthalates simultaneously, which for phthalates are mainly the antiandrogenic effects. Based on reproductive and liver effects, EFSA derived a group-TDI of 50 µg/kg_{bw}/day for DEHP, DnBP, BBzP, and DiNP expressed as DEHP equivalents (EFSA, 2019). While median exposure of ESB participants was below the group-TDI already from 1988 on (Fig. 3), virtually the whole GerES IV population of children was above the group-TDI with median values of individual age groups ranging between 150 and 300 µg/kg_{bw}/day. Despite the observed decrease of exposure, the youngest age groups (3-5- and 6-10-year-olds) of GerES V (2014–2017) were on average still exceeding the group-TDI of 50 µg/kg_{bw}/day. Within the framework of HBM4EU, a mixture risk assessment approach using HBM guidance values is currently developed, evaluating the exposure based on internal concentrations. Thus, avoiding the conversion of HBM exposure levels into daily intake estimates to compare to external health-based guidance values.

In summary, a remarkable, successful reduction is seen in the exposure to critical, regulated phthalates. However, the overall exposure to plasticizers (phthalates and their substitutes) remains high. As long as regulated substances are replaced by new ones with presumably better toxicological properties derived from animal studies, but no long-time knowledge in humans (epidemiological studies), and human exposure only shifts from high exposure levels of a few substances to medium exposure levels of a wider mixture of substances, the desired success in exposure reduction may not be gained. For some of the substitutes investigated (e.g. DEHTP), exposure amplitudes might even surpass those of their predecessors very soon, with no net effect on exposure reduction.

Such developments might not fulfil the demands of a precautionary use of chemicals as well as the zero pollution ambition of the EU (European Commission, 2019), considering that the plasticizers discussed in this paper are all anthropogenic, synthetic chemicals. Regular monitoring of exposure levels, their time trends and subsequent cumulative risk assessment are therefore the key instruments to achieve a further improvement of chemical policy, including improvement of use, safe substitution and potentially reduction measures for plasticizers if needed.

8. Conclusions

Germany has established a unique HBM system at federal level allowing for the multidimensional assessment of chemical exposure. Due to the complementary application of cross-sectional and time trend studies, combined health and HBM surveillance, and additionally environmental monitoring, evidence-based conclusions on the chemical burden of the population, sources of exposure, vulnerable groups, and the efficacy of regulations can be drawn. The bidirectional interaction between science and policy strengthens the appropriate regulatory initiatives on a national and European level. The development of new HBM methods, especially for substitute plasticizers, enabled a more complete assessment of the exposure to plasticizers after the market shift to non-phthalate alternatives. The achievements in reducing the exposure to single reprotoxic phthalates were put into perspective when considering the increasing exposure to substitute substances. EU-wide regulated phthalates are still detectable in every sample analysed. The overall exposure to plasticizers remains at a very high level and regulated compounds have only been substituted by new chemicals with net exposures not changing much, but exposure profiles becoming more complex regarding mixtures. The German HBM studies show that decreasing numbers of participants exceed HBM assessment values for single plasticizers. However, the cumulative risk assessment revealed toxicologically relevant mixture exposures, which are particularly concerning for small children.

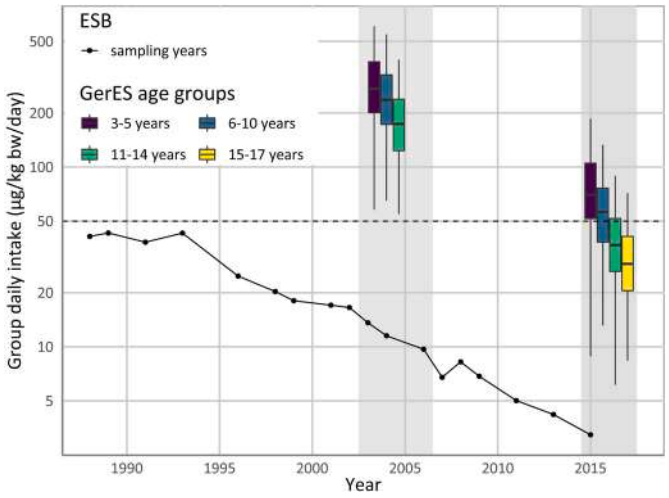

Fig. 3. Group-TDI for BBzP, DnBP, DEHP, and DiNP for ESB, GerES IV, and GerES V. Annual median values for ESB are presented by line-connected dots. GerES data are presented by colour-coded boxplots for each age group (whiskers spanning the 1.5-fold interquartile range at max, outliers are not shown). Grey shaded areas denote the sampling period of the respective GerES cycle (GerES IV – 2003–2006, GerES V – 2015–2017). The position of boxplots within the grey shaded area is for presentation purposes only; each boxplot represents the full sampling period of the respective GerES. Horizontal dashed line denotes the group-TDI of 50 µg/kg_{bw}/day. Note the logarithmic scale!

Based on current knowledge, the substitutes clearly have a preferable toxicological profile over the regulated phthalates which shows in lower risk estimates of their exposure levels. However, regarding exposure levels, overall plasticizer exposures seem stable, but the composition has changed and the complexity of mixtures has increased.

Therefore, in the future, investigations of the whole group of plasticizers are needed, not only single substance exposure and risk assessments. To sustainably reduce exposure to plasticizers, chemicals regulation yet in place might not be sufficient. The reduction of plasticizer use in consumer products, especially marketed for children, must be encouraged and policy makers, industry, and consumers have to reconsider their use of plastics and plasticizers.

HBM studies remain a key instrument in monitoring of the chemical market and controlling preventive and risk reduction measures within health and environmental policy programmes. However, the time between the detection of potentially critical levels of a substance in

humans and the respective regulatory actions is still too long. Regulation often starts much too late when harmful substances are already ubiquitously present in the population. To protect the population from harmful chemicals, a chemical policy in line with the precautionary principle and the zero pollution ambition (European Commission, 2019) is needed. Up to now, HBM has increasingly been established as an instrument to inform policy makers and the public about the exposure to environmental chemicals. Several European countries but also countries worldwide have set up national HBM programs (see e.g. WHO, 2015). A comprehensive HBM on a broader scale can only be achieved by burden sharing and channeling efforts into transnational projects. Therefore, HBM4EU, as a network of 30 countries, has been set up to accomplish this goal on a European level. The advances in HBM gained from the German HBM tools are shared within HBM4EU, which in return provides the broader expertise for harmonised risk assessment, hereby implementing a scientific base for chemical policy making in the EU.

Declaration of competing interest

The authors declare no conflict of interest related to this work.

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Corrigendum to "Substitutes mimic the exposure behaviour of REACH regulated phthalates – A review of the German HBM system on the example of plasticizers" [Int. J. Hyg. Environ. Health 236 (2021) 113780]

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The authors regret that some statements in chapter 3 "Human biomonitoring of phthalates and substitute plasticizers" are wrong due to missing text in the previously published version. Below is the corrected statement with changes in bold font.

For DPHP, **effects on the thyroid gland and pituitary gland, both observed in a subchronic toxicity study in rats, are considered critical (BfR, 2011; Bhat et al., 2014; German HBM Commission, 2015). DPHP is currently being under assessment for endocrine disruption (ECHA, 2021b). DEHTP is not considered toxic for reproduction and no warning for potential endocrine disrupting properties were identified under the regulatory management option analysis (ECHA, 2021a).** EFSA and also the German HBM Commission based their guidance values on the combined chronic and carcinogenicity dietary study in rats by Deyo (2008), in which effects on the retina were considered critical (Apel et al., 2017; Deyo, 2008; EFSA, 2008).

The authors would like to apologise for any inconvenience caused.

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Successes, challenges, and support for men versus women implementers in water, sanitation, and hygiene programs: A qualitative study in rural Nepal

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ABSTRACT

Introduction: Women's active participation is important for inclusive water, sanitation, and hygiene (WaSH) programs, yet gender roles that limit women's access to formal education and employment may reduce their skills, experience, and capacity for implementation. This paper explores differences between men and women implementers of rural WaSH programs in implementation approaches, challenges, and sources of support for implementation, and success in achieving program quality outcomes.

Methods: We interviewed 18 men and 13 women in community-based implementation roles in four districts of Nepal. We identified challenges and sources of support for implementation in four domains—informational, tangible, emotional, or companionship—following social support theory. We assessed successes at achieving intermediate implementation outcomes (e.g., adoption, appropriateness, sustainability) and long-term intervention outcomes (e.g., community cleanliness, health improvements).

Results: Women used relational approaches and leveraged social ties to encourage behavior change, while men used formative research to identify behavior drivers and sanctions to drive behavior change. Women experienced stigma for working outside the home, which was perceived as a traditionally male role. Companionship and emotional support from other women and male community leaders helped mitigate stigma and lack of informational support. Women were also more likely to receive no or low financial compensation for work and had fewer opportunities for feedback and training compared to men. Despite lack of support, women were motivated to work by a desire to build their social status, gain new knowledge, and break conventional gender roles.

Conclusions: Both men and women perceived that women were more effective than men at mobilizing widespread, sustained WaSH improvements, which was attributed to their successes using relational approaches and leveraging social ties to deliver acceptable and appropriate messages. Their skills for motivating collective action indicate that they can be highly effective WaSH implementers despite lack of technical experience and training, and that women's active participation is important for achieving transformative community change.

1. Introduction

Women's active participation is important for safe, effective, and inclusive water, sanitation, and hygiene (WaSH) programs. Women and girls are disproportionately affected by cultural norms for modesty and privacy during defecation and hygiene (Caruso et al., 2015). They are typically the duty-bearers for WaSH-related tasks within the household, such as water collection (Graham et al., 2016) and child feces management (Majorin et al., 2019). These duties expose women to additional health risks, such as exposure to pathogens or injury from carrying heavy water containers (Sevilimedu et al., 2017; Sorenson et al., 2011). The Sustainable Development Goals (SDGs) recognize the importance of women's participation in WaSH through Goal 5, which sets targets for increasing women's participation in leadership roles and decision making, and ending discrimination against women and girls, and Goal 6, which calls for universal access to WaSH "for all" and

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emphasizes "paying special attention to the needs of women and girls" (United Nations, 2015).

WaSH programs often rely on women for implementation at the local level. Community-based sanitation programs in low- and middle-income countries often emphasize recruitment of natural leaders from within the community and inclusion of women (WHO, 2018), and many countries have incorporated WaSH activities into the duties of female community health volunteers (female CHVs or FCHVs) (Perry et al., 2016). FCHV programs have extended basic health services to poor, rural areas that otherwise have little or no access (Bhutta et al., 2010; Perry et al., 2014). FCHVs are well suited to deliver health messaging to other women on sensitive topics such as menstruation that would be taboo for male healthcare providers (El Arifeen et al., 2013; Mumtaz et al., 2013; Panday et al., 2017). Mobilizing women's groups for participatory learning and action has been shown to reduce gender inequalities that impede access to proper care and improve maternal and neonatal health outcomes (Perry et al., 2014; Prost et al., 2013).

Women's active participation in WaSH and other natural resource management committees has been found to improve community trust in fee collection and financial management (Kelly et al., 2017), sustainable service delivery (Hoque et al., 1996; Westermann et al., 2005), collaboration and conflict management (Westermann et al., 2005), and recognition and resolution of problems in the community (Kelly et al., 2017). Women are often selected as implementers for community-based programming because they are familiar with the local context, well integrated into community social networks, and able to speak with other women on sensitive health topics (WHO, 2018).

However, recruiting women implementers from local communities has challenges. Communities targeted for health and development programs often have lower levels of formal education and other socioeconomic indicators (Kane et al., 2016). Gender norms that limit women's participation in formal education and employment can reduce their experience, skills, and capacity to effectively deliver health programming (Panday et al., 2017; Sarin and Lunsford, 2017). Furthermore, women may lack agency to participate in WaSH implementation (Wali et al., 2020) or be nominally included without valuing their contributions and concerns (Yerian et al., 2014). Although documentation is limited, some evidence suggests that meaningful inclusion of women in community-based implementation can improve the quality of programs, provided they receive appropriate support (Bhutta et al., 2010).

The purpose of this study was to explore how the gender of community implementers influences implementation at the local level for rural WaSH programs in Nepal. Specific objectives were to describe differences between men and women implementers in implementation approaches, challenges, and sources of support for implementation, and success in achieving program quality outcomes at the local level. We discuss the implications of these differences for improving program implementation, including opportunities to strengthen training and feedback systems, leverage women's social ties within communities, and reconsider compensation of unpaid workers.

2. Methods

2.1. Study design

We collected data through qualitative interviews with WaSH implementers in four districts in Nepal (Siraha, Mahottari, Surkhet, and Salyan) from June to August 2019. Interviews were conducted as part of a larger study on quality improvement and innovation in rural WaSH, which included implementers from the regional to community levels. Here, we analyzed a subset of interviews from men and women in community-based implementation roles to understand how implementers perceive approaches, challenges, support, and successes at the local level.

2.2. Conceptual frameworks

Implementation approaches are the activities and strategies used for program delivery. In implementing these approaches, men and women experience various challenges that hinder successful implementation and sources of support to mitigate those challenges. When challenges can be mitigated and adequate support is received, implementation approaches are expected to yield program success in terms of intermediate implementation outcomes (e.g., adoption and sustained practice of WaSH behaviors) and long-term impacts on target intervention outcomes (i.e., improvements in health and wellbeing). Fig. 1 depicts the conceptual model that we developed for this study.

We hypothesized that gender would influence the implementation approaches used by men versus women and the challenges and support systems experienced when implementing these approaches. In turn, we hypothesized that these approaches, challenges, and support systems would influence success at achieving implementation and intervention outcomes. We assessed implementation approaches, support and challenges, and implementation and program quality outcomes for all participants following the frameworks described below, then compared differences between men and women.

2.2.1. Implementation approaches

Activities conducted by village-level implementers primarily comprised behavior change messaging delivered in household and community settings. These activities in Nepal draw heavily from a community-led total sanitation (CLTS) approach (Department of Water Supply and Sewerage, 2011). CLTS uses participatory activities designed to elicit negative emotions such as shame and disgust at open defecation to "trigger" behavior change and collective action to make the community open defecation free (ODF) (Kar and Chambers, 2008). Similar approaches for behavior change triggering are also used to promote hygiene and other WaSH behaviors (e.g., food hygiene) post-ODF. Following triggering, local-level implementers conduct household and community-level follow up activities to give behavior change messages and create demand for WaSH.

For this study, we assessed which CLTS and related behavior change and demand creation activities were conducted by implementers, plus any alternative or additional activities delivered to households or communities. We did not assess implementation approaches related to capacity building or coordination of stakeholders, which were occurring at the local level but were rarely conducted by participants in our sample.

2.2.2. Challenges and support systems

We applied social support theory to categorize challenges and sources of support for implementation as either informational, tangible, emotional, or companionship. Informational support provides knowledge or guidance, typically to assist with problem solving. Tangible support provides physical goods, services, or money as a form of direct assistance. Emotional support provides encouragement through making an individual feel valued or self-confident, such as through expressions of empathy, concern, or caring. Companionship support provides a feeling of social belonging or presence of companions to engage in shared activities (Langford et al., 1997).

Social support theory has been used to explore determinants of successful implementation of health behaviors by lay individuals, households, and communities (Heaney and Israel, 2008; Kelly et al., 1991), similar to lay persons who serve as natural leaders under CLTS-style programs. Social support is particularly relevant for community-based WaSH implementers, who live in the community in which they work and have strong social ties to program recipients.

2.2.3. Program successes

We used outcomes defined by Proctor et al. (2011) as measures of program success: acceptability, adoption, appropriateness, cost,

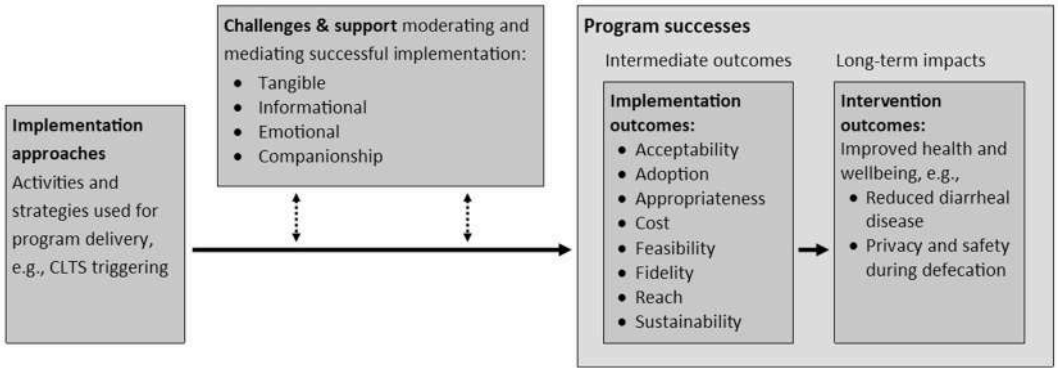

Fig. 1. Conceptual model of relationships between implementation approaches, challenges and sources of support for implementation, and program successes. Implementation approaches are expected to achieve program success measures where they are evidence-based, contextually appropriate, and receive appropriate support to overcome challenges. The model combines elements of social support theory (Langford et al., 1997) to describe challenges and support, and implementation outcomes and long-term impacts (Proctor et al., 2011) to describe successes.

feasibility, fidelity, penetration, and sustainability. Table 1 provides definitions. Proctor's framework proposes that implementation outcomes are precursors to achieving intervention outcomes, and that intervention outcomes may only be achieved if a program is implemented well. In the case of WaSH programs, relevant intervention outcomes include reductions in diarrheal disease or child mortality.

2.3. Study setting

WaSH implementers in Nepal include a diverse range of government, international and local non-governmental organizations (NGOs), multilateral organizations, private sector, and civil society organizations, such as women's, mothers', and journalist groups. We consider WaSH implementers to be anyone who participates in the delivery of program activities directly to beneficiary households (e.g., behavior change messaging) or the supervision, regulation, or technical support of these activities.

Sanitation and hygiene delivery in Nepal are governed at the federal level by the National Sanitation and Hygiene Master Plan. The Master Plan outlines responsibilities for different levels of government and defines "guiding principles"—such as representation of women on steering committees at a "minimum of 33% of the members, as appropriate." The details of specific activities or program plans are determined at the subnational level (Department of Water Supply and Sewerage, 2011).

District committees called "WaSH coordination committees" are responsible for supervising and harmonizing activities of implementers.

Table 1
Implementation outcomes as measures of program quality. Adapted from Proctor et al., (2011).

Outcome	Definition
Acceptability	Perception that a given WaSH program or its component parts is agreeable and satisfactory
Adoption	Intention, decision, or action to uptake a WaSH behavior or technology
Appropriateness	Perceived fit, relevance, or compatibility of the WaSH program with context, or perceived fit of the program to address a specific issue or problem
Cost	Cost of implementation efforts
Feasibility	The extent to which the WaSH program can be successfully delivered or used within a given context
Fidelity	The extent to which the WaSH program is delivered as originally developed and specified in program plans and protocols
Penetration	Coverage area and intensity of exposure to the WaSH program among the target population
Sustainability	The extent to which WaSH behaviors and technologies are maintained and institutionalized within the target population

Municipal committees plan and coordinate implementation activities in partnership with NGOs. Coordination committees are chaired by elected government officials, such as mayors, vice-mayors, and ward presidents. Elected government officials participate in the supervision and policy regulation but rarely deliver activities directly to beneficiary households. The majority of day-to-day behavior change programming at the local level is delivered to households by paid staff from local NGOs and unpaid community volunteers, including FCHVs and women's and mothers' groups. Technical support is provided by government employees, who are non-elected, hired specifically for their technical expertise. Multilateral agencies and international NGOs also provide technical support, including the World Health Organization (WHO), United Nations Children's Fund, United Nations Human Resettlement Program, and the SNV Netherlands Development Organization (Adhikari, 2012).

Men outnumber women in WaSH implementation particularly for roles in district, regional, and national supervision; private sector jobs for construction and small business owners; and engineering or technical jobs (Wali et al., 2020). Women more often fill unpaid roles at the local level as FCHVs and women's and mothers' group members. FCHVs, women's, and mothers' group members may receive reimbursement or a small stipend to cover travel and food allowances but otherwise do not receive formal wages (Khatri et al., 2017).

2.4. Study population and recruitment

Within each district, a program coordinator who had lived and worked in the district for at least the past five years assisted with recruitment. Program coordinators were employees of local or international NGOs. Their duties included supervising teams of NGO staff and unpaid volunteers (e.g., FCHVs, women's groups) across multiple villages and liaising with government and other partners.

Program coordinators were briefed on the research purpose and asked to identify a list of individuals who were highly involved in WaSH delivery at the district, municipal, and sub-municipal levels. From this list, we purposively sampled participants to represent a range of perspectives from government, multilateral organizations and NGOs (hereafter "development organizations"), and civil society organizations. When both men and women were available for interview for a particular stakeholder role, we preferentially recruited women to maintain a more equal gender balance.

For this study, we defined local-level as implementers at the sub-district level. We excluded regional- and national-level implementers, as their challenges, support, and successes differed substantially from those experienced at the local level. We also excluded participants from roles filled exclusively by men in the study sample—specifically WaSH

technicians and engineers, masons, journalists, and small business owners.

2.5. Data collection

A research team comprised of one interviewer and one note taker conducted interviews in private offices or meeting rooms at participants' workplace, or at the home or a nearby community center for participants without a formal office. During interviews, we asked participants to describe a novel WaSH solution or approach they used to improve program quality. We identified successes through questions that asked participants to describe whether they perceived their solution to be successful and how they defined and measured success, as well as questions that asked participants to describe successes and challenges for implementation and program improvement overall. We identified challenges and sources of support through questions that asked participants to describe barriers or facilitators to implementing and sustaining their solutions. Where participants described difficulties in implementing or sustaining solutions, we probed to explore sources of support for overcoming those difficulties.

Interview guides were developed in English, translated into Nepali, and pre-tested in a district bordering Siraha. Based on pre-testing, we reworded and reordered questions to improve the guide's ability to elicit information on key concepts and question flow. We also iteratively revised the interview guide throughout data collection to explore emergent themes. Interviews lasted approximately 1 h. We conducted interviews in English, Nepali, Hindi, or Maithili, following the preference of the participant. We audio recorded interviews where participants gave permission (n = 27, 87%) and transcribed recordings directly into English for analysis.

We also asked participants to complete a brief demographic questionnaire to identify their job title, years of work experience, and gender.

2.6. Analysis

We conducted template analysis using NVivo qualitative data analysis software (QSR International, Melbourne, Australia) for coding. We coded for *a priori* themes for implementation approaches as CLTS, behavior change, and demand creation activities. We coded challenges and sources of support in four domains (informational, tangible, emotional, and companionship) following social support theory (Langford et al., 1997). Finally, we coded successes at achieving the eight implementation outcomes described in Table 1 and a single code for intervention outcomes encompassing health improvements in WaSH-related diseases.

We developed an initial template for analysis using a subset of six transcripts, revising codes for *a priori* themes, and developing inductive codes for emergent themes, as necessary. We then applied the template to an additional six transcripts, iterating the reflection and revision process, before proceeding to coding the full dataset. After coding all interviews, we sorted codes by participants' self-identified gender to examine similarities and differences across men and women.

2.7. Ethics

This study was ruled as non-human subjects research by the Institutional Review Board of the University of North Carolina-Chapel Hill (IRB # 19–0945). Local approval for study activities was obtained from the Nepali Ministry of Water Supply. Participants were informed of the study purpose and provided written consent before enrollment.

3. Results

3.1. Sample characteristics

Our sample comprised 18 men and 13 women (Table 2). Participants were primarily elected officials (n = 6 men, 3 women) and development organization employees (n = 8 men, 3 women). Men ranged in age from 30 to 62 years (median 40.5) and had served an average of 3.0 years in their current implementation role. Women ranged in age from 23 to 46 years (median 38) and has served an average of 4.6 years in their current implementation role. Women more commonly held unpaid non-elected roles (n = 6, 46%) compared to men (n = 2, 11%).

Table 2
Participant demographics.

	Number of participants	
	Men (n = 18)	Women (n = 13)
Age (years)		
20-29	1	2
30-39	8	5
40-49	4	6
50+	5	–
Employment position		
Elected officials[a]	6	3
Government employees	1	–
NGO or multilateral employees	8	3
Teacher or school official	1	1
Social activist[a]	1	–
Disabled persons' organization representative[a]	1	–
Female community health volunteers[a]	–	3
Women's or mothers' group members[a]	–	3
Years in current position		
0-2	8	7
3-5	9	3
>5	1	3
Location		
Mahottari	5	3
Salyan	2	4
Siraha	8	1
Surkhet	3	5

[a] Denotes participants in unpaid roles.

We use the term "unpaid women" to describe women in non-elected, unpaid roles as FCHVs and women's and mothers' group members. We use the term "government implementers" to describe the combined efforts of government elected officials and employees, or differentiate between elected officials versus government employees as applicable.

3.2. Implementation approaches

Activities and messages used by community-based implementers were typically designed by development organizations. Content was similar to triggering techniques used under CLTS, designed to raise negative feelings about open defecation and awareness of its dangers. In some cases, content had been adapted to other settings, such as school-led total sanitation, where children were given similar messages and asked to pressure their parents to build toilets.

When WaSH activities were first implemented, implementers perceived strong community norms for open defecation and reported that households were often unwilling to construct toilets without a subsidy. Both men and women implementers perceived that messaging strategies were often initially unsuccessful at motivating behavior change and toilet construction, and WaSH activities did not meet participants' expectations for achieving rapid, community-wide behavior change. Responses to poor perceived success varied across government versus development organizations and men versus women.

Men from government in our sample commonly reported using "pressure" approaches. They insisted that individuals and households must comply with WaSH policies to meet government regulations. When these messages did not meet expectations for achieving rapid toilet construction, sanctions were the used, such as threatening to restrict or restricting government services (e.g., work permits and citizenship papers) for non-toilet owners or arresting and fining open defecators. Men

perceived that these approaches created tension with the community:

> "… Women and people used to curse me and abuse me. We have also abused them and dishonored them. We also seek the help of police to control them." -man, local government official

Technical advisors from development organization did not endorse sanctions approaches. Men in development organizations instead reported adjusting the content of messaging to more effectively motivate behavior change. For example, one man used radio jingles with voices of local people and two men developed new slogans to address locally relevant behavioral motivators.

In contrast to "pressure" approaches, women in all implementation roles used "convincing" approaches when they perceived households to be resistant to behavior change. Women did not demand that households must change behavior, but rather emphasized the benefits of WaSH for individuals, other members of the household, and community at-large. Women also recruited others in the community to show their support and deliver messages in groups:

> "When we say to the women, 'Sister, this toilet is for you,' they agreed and made the toilet. If the women do not understand, then we convince the mother in law by saying, 'Aunt, please make the toilet. If you made the toilet, it will be good for your daughter in law.' We convince the daughter in law by saying, 'It will be good for your son and daughters. Please look after the child and their health.'" -woman, development organization local implementer

3.3. Challenges and sources of support

Challenges for implementers arose due to differences in implementation approaches taken by men versus women, community and peer responses to these approaches, and the variance in support received to cope with challenges. The following sections categorize and describe these differences following the four domains of social support theory: informational, tangible, emotional, and companionship.

3.3.1. Informational support

3.3.1.1. Participation in program planning and training. Participants in all districts recognized women's participation in coordination committees as important. However, large committees, particularly at the district level, were perceived as unwieldy and inefficient, so smaller subcommittees were often formed for developing work plans. These committees included primarily government implementers and development organization technical advisors—roles typically filled by men. Our sample included three women elected government officials at the sub-municipal level, but they rarely were invited to participate in these subcommittee meetings at the municipal or district level.

No women reported participating in subcommittees where high-level planning occurred. Instead, they participated in meetings to receive training on how to deliver and monitor program activities that had already been designed. For women in unpaid roles, trainings typically lasted one day, with some lasting up to three days. One woman conducting training for local mothers' groups described how indicators for WaSH behaviors such as toilet use and handwashing were complex and difficult to understand with little training:

> "I took the training from the district and then give training to the mother groups. I have a banner of Total Sanitation [post-ODF WaSH promotion activities], and I used it to educate them. In the banner there are many pictures …. They could not understand all the indicators at once." -woman, elected official and former development organization implementer

Government implementers often overestimated FCHVs' knowledge and training. Government implementers relied on FCHVs to support developing program plans. However, FCHVs were not confident in their ability to design, deliver, and improve messages independently. In one area where development organization-supported programs had ended and no technical advisors were available, FCHVs and other community-based workers with little formal WaSH training were the primary source of technical expertise:

> "We have not yet given the technical knowledge to the *tole* [small municipal sub-unit] … [FCHVs and] health workers have the knowledge of sanitation and hygiene." -woman, local government representative

3.3.1.2. Systems for feedback and troubleshooting. Men and women employed by development organizations described the most robust communication and information sharing for addressing implementation challenges. They shared information between workers across geographic areas through phone and social media groups and met locally to discuss progress and challenges towards achieving program targets and to share good practices.

Outside of development organizations, men typically had more robust systems for feedback and troubleshooting. Men in government described participating in committees with representatives from other municipalities, where they would share progress and troubleshoot problems. Technical advisers and program supervisors from development organizations, who were predominately men, also participated in these committees.

Women in unpaid roles were less integrated into systems for feedback and troubleshooting. In some areas, women in unpaid roles reported challenges to municipal WaSH committees but were rarely engaged in developing solutions. In some cases, they received ongoing support for improving the content of program activities from a development organization or government adviser. However, this support was less stable and strongly linked to presence of funded programs, as technical advisors often left program areas or were reassigned to other projects when funding ended. One FCHV described how she was unsure of the quality of her work, because she had never received feedback:

> "I cannot say that all the works I have done are good because I have not seen some work output. I do my best, but there may be a chance that I am doing wrong. For example, if someone tells me that I have done wrong, then I can improve. Until now, no one has said that I have done anything wrong." -FCHV

3.3.1.3. Exposure to external learning and experience. Men had more prior employment experience prior to holding their current position and had worked more years in with their institution compared to women (6.6 versus 5.0 years). They described experiences working on previous programs or in other areas, which they applied for improving program quality in their current positions:

> "Our community was very backward. We used to go to Kathmandu, India, and other places. We have observed about the drainage system and other many things. And by seeing all these we have put the plan to the rural municipality." -man, social activist

In contrast, most women had little to no prior work experience before taking their current position. One woman, who was president of her local mothers' group, described how mobilizing women for WaSH campaigns was difficult because women had received little formal education, and some were "very young because of the early child marriage." Another woman described how opportunities to learn were limited because no other development organizations were working locally:

> "If they [other development organizations] came, we would get a chance to learn about new approaches. What is needed for the

community, only we cannot decide. If other organizations come, their recommendation and feedback will help." -woman, development organization local implementer

3.3.2. Tangible support

Insufficient funding and resources to deliver program activities was a challenge consistently reported across men and women. However, women were more likely to work as unpaid volunteers (46% of women versus 11% of men for non-elected positions in our sample). Programs relied heavily on FCHVs, women's and mothers' groups to conduct house-to-house behavior change activities and monitoring but did not offer formal wages to compensate their work. Unpaid women sometimes received allowances for snacks or travel, but two FCHVs indicated that these allowances were insufficient to cover the costs of their duties.

Four women in unpaid positions described challenges with balancing other responsibilities, such as managing domestic responsibilities in the home or field, or other income-generating activities. One FCHV indicated that with her time commitments to deliver non-WaSH programming under the FCHV curricula (e.g., contraceptive education) and hold paid employment, WaSH messages were only added to her work agenda seasonally, or as needed during water-borne disease outbreaks. One unpaid woman indicated that if she were paid, she could dedicate more time to WaSH work.

Unpaid women reported feeling less responsibility to improve program activities, because this was the responsibility of development organization workers who paid employees with more expertise. While unpaid women indicated that they would be willing to dedicate more time and effort to WaSH activities if they were paid, benefits such as increased opportunities for training and building social networks and status outside the home were still sufficient incentives to engage in WaSH work part-time.

3.3.3. Emotional support

Women implementers in all roles faced stigma for taking formal employment outside the home, which was perceived to violate traditional gender roles of women working only domestically. Stigma was particularly strong when programs were first implemented and decreased over time as communities perceived improvements to health and environmental cleanliness. This stigma was similar across women in both formal paid implementation roles and unpaid volunteers. Five women described receiving "abusive" words or confrontational reactions from men when delivering program activities. Particularly when programs were first implemented, these women reported criticism for attempting to educate and change behavior of men, which was perceived to overstep women's authority. One woman was told that her work disrespected her husband and family:

"Some say face-to-face that, 'You are rubbish, you only have the job to roam here and there. Your husband cooks the food by himself. You only roam here and there, and you do not respect your husband.' It was most challenging for me during the working period. All people laugh on me. Some in front, some in back. Many abuse me" -woman, development organization local implementer

In response to this criticism, women received support from family, development organizations, and progressive community leaders who publicly endorsed their work. One woman development organization implementer reported that her husband called a meeting of other men in the village to ask that they "at least hear her" before criticizing, which caused a positive response once men recognized the benefits of the program. For other women, development organizations spoke with community men to mitigate stigma:

"[Development organization workers] have said, 'You have done good work. We will look after those people who have abused you,' and after they have said something to them, and now there is no such issue." -FCHV

Women perceived that criticism decreased as communities observed improvements in community health and cleanliness, at which point women reported that they were recognized and praised for their work by community members. In lieu of compensation, unpaid women often received formal recognition for their work from local government, in the form of appreciation letters, ceremonies, and small gifts, such as shawls, which motivated them to continue working.

Themes related to emotional challenges and support were less commonly raised among men in our study sample. No man reported facing emotional challenges specifically related to their gender. Emotional challenges faced by men were typically experienced in relation to specific activities that they perceived to have low community acceptance, often sanctions-based approaches.

3.3.4. Companionship support

3.3.4.1. Companionship support through group messaging strategies. Companionship support was an important coping mechanism for women in response to many types of challenges. When faced with households resistant to behavior change messaging, the most common response from women was to recruit others to revisit households and deliver the same messages in a group. Groups were perceived to have more persuasive power than individuals. Group messaging strategies were particularly important for women in unpaid roles with less access to informational support. For these women, group messaging was the primary strategy for improving implementation, rather than refining the content of messages. Groups were commonly formed of women's peers from the community, particularly women's and mothers' group members:

"When the challenges came, they [women] do not move back. Their meeting was continuous, and unity was their strength. When a woman has problems, all the women move to that house and sort out that problem by explaining to them. They go in the groups and convince other people, that it is the benefit for the community, it is the benefit for us, it is for family. And this how people have gradually changed." -man, development organization local implementer

In some instances, women would also recruit support from men from development organizations, elected leaders, or informal opinion leaders, who were perceived to hold more power to enforce messages:

"We approach the community with the elected members, and the people follow them. If a ward-WaSH coordination committee makes any decision, the ward members have to follow …" -woman, development organization local implementer

Women proud of their achievements, and wanted to support other women to demonstrate that they could be skilled WaSH implementers. One woman who chaired a local all-women WaSH committee described the committee's accomplishments despite a man who questioned their ability to succeed:

"He does not want to support the women, and then we have decided that we will do anything to show this man that we are not so much weak. And we can run the committee without the men and make changes in the community. This is how we have done. And we are successful. You can ask the people that our drinking water committee is one of the best drinking water committees in this area. Now the women are supporting me very much." -woman, local elected official

Companionship support from peers was less important to men. None described using group messaging approaches as a strategy to cope with challenges.

3.3.4.2. Political support and rivalries. Men reported companionship challenges from political rivals who were unwilling to support or would actively undermine WaSH efforts. One development organization implementer reported that the locally elected leader refused to conduct activities with him and "create a pressure on [his] people" out of fear it would affect his re-election chances. Another man elected offical attributed WaSH budget shortfalls to political rivalries. In some cases, political affiliation aided implementation, where members of the same political group motivated each other:

> "I am supporter of the winning politician. He comes to monitor and says everything is good in my school. It motivates me to do the work. But to a non-supporter he always tries to find mistakes in his work. This can develop a negative attitude." -man, school headmaster, Siraha

Women rarely mentioned politics as a challenge or source of support. No women in non-government roles mentioned politics as a challenge. Our sample included only three women in elected government positions women in politics, and only one elected woman described political challenges when interacting with men. However, the same woman reported that politics did not influence her interactions with women:

> "The women are continuous supporting me. They do not have any concern with the political party. The men of opposite party are somehow angry with me. When I ask anything of them, like 'Brother, please help me in this,' they say 'You do in your time. We will do in our time when we win.'" -woman, elected official

3.4. Successes in achieving implementation and intervention outcomes

The following section describes men's and women's perceived successes at achieving implementation outcomes (i.e., acceptability, appropriateness, penetration, adoption, and sustainability) and intervention outcomes, as defined by Proctor et al. and outlined in Table 1. Implementation outcomes for feasibility, fidelity, and cost were not described by participants during interviews. We also describe how these perceived successes were driven by the implementation approaches men and women used, challenges encountered using those approaches, and the support received to mitigate those challenges, as shown in Fig. 1.

3.4.1. Acceptability

Implementers perceived that relational approaches more commonly used by women faced less resistance from the community. Women reported that their "convincing" style of messaging was more likely to be given with respect, asking that households please change rather than demanding change:

> "Women speak very humbly. They do not use any harsh word or speak tightly … If we have pressurized the people saying, 'Uncle!! You have to do it.' Then maybe they have not built the toilet. But when we respect with respective words, then they agree. This respect develops a good relation with us, and people get convinced easily." -woman, development organization implementer

In contrast, men using pressure approaches reported being "cursed" or "abused" by some members of the community. One woman attributed the difference in communication styles between men and women to social norms dictating that women give respect to men and household elders:

> "Women have habit of giving respect. After marriage they have to respect the mother- and father-in-law. Women have the habit of speaking calmly, humbly. If they speak harshly, it will be hard to change people. But from birth women and girls are reared in that manner that they have to speak humbly and respect the older ones." -woman, development organization implementer

3.4.2. Appropriateness

FCHVs and women's and mother's groups were recruited from local communities and were familiar with local WaSH norms and conditions. While not required to be hired from local communities, all the women working for development organization that we interviewed lived locally in the communities in which they worked. Because of their local ties, women reported that they well positioned to identify community needs and develop appropriate solutions. Women believed that they were more honest in identifying and addressing community needs with good intentions of improving program quality and community health, whereas men, particularly government implementers, were often perceived as acting for political gain:

> "In my view, the women understand [community needs] better. Because if men go, they behave like the [political] party workers. Some behave like officers, but the women think that because we are living here, our place should be clean. Many women understand the needs of the community better. Some people's works are biased with their political party. What the party says or where the voters are high, they work there. But the women live here. They have to live all day and night here. They know better what is needed. Women are honest for themselves, their husband and children." -woman, development organization local implementer

Men working for development organizations commonly worked in larger geographic areas or in communities where they did not live. Formative research and engagement with women's, mothers' and other local groups were some of the strategies used by men to overcome lack of local knowledge and to improve the appropriateness of their implementation approaches.

3.4.3. Penetration

Women's and mothers' groups included large proportions of the local population. Women reported using these groups to efficiently spread messages through the community, while men reported challenges with reaching all the households in their working area. Men and women participants also reported that women were the best population to spread WaSH messages, as WaSH duties within the home are traditionally assigned to women, and women were often home during the day to receive messages:

> "The message spreads fast through the women. In the home, the women are sanitation workers. Men do not do, how much you convince or fight with men, they do not do the sanitation work … That's why I thought how I clean my household, I will mobilize the women for the cleaning the village." -woman, local government representative

3.4.4. Adoption

Approaches that asked households to build a toilet simply to meet government targets were widely perceived by all study participants to be unsuccessful at prompting adoption of sanitation improvements. Poor success of this messaging led participants to try new approaches, including both pressure and convincing approaches. Of the approaches tried in response, both men and women perceived that their efforts led to improvement in adoption of toilets and other WaSH behaviors among the community.

Both men and women also perceived buy-in from community women as important for widespread adoption of WaSH, and that adoption of WaSH practices was faster and more widespread when women actively participated in implementation. One woman attributed this to ease of working with other women and women's shared understanding and experience of WaSH needs. She perceived that this shared experience and understanding promoted cooperation and collective action by women:

> "It is very easy to work through women, because all the work [related to WaSH] is done by the women. That's why if we can make the women understand about the sanitation, change comes very fast. If the women wills, then the program is completed fast … In my ward, I have achieved success in less time working with the women of the ward. What I have experienced in life, almost all women are also affected by those problems." – woman, elected official and former development organization implementer

3.4.5. Sustainability

Women-led activities were perceived by both men and women to be more sustainable, and four participants identified women's leadership specifically as a solution to improve sustainability:

> "In the women-led areas, the activities are still continued. When we have gone for follow up, we observed that they are doing follow up of their activities. If you go and monitor, you will find that they are continuing. And the area where there was pressure, or where we had to push for work …. In some places we have seen that people are going back." -man, development organization implementer

Participants proposed several reasons for the sustainability of women-led programs. Women's and mothers' groups were not formal organizations within government or development organizations, so their activities had been sustained through government restructuring and program closeouts. Continual presence of women allowed for sustained delivery of program activities which reinforced habits in the community. Two women reported raising funds in the community to support continuing activities after formal program funding ended.

Implementers perceived that convincing approaches used by women to genuinely change social norms and attitudes regarding WaSH practices. Pressure approaches used by men were perceived as less sustainable: households would construct toilets to avoid sanctions, but individuals had not meaningfully changed perceptions, norms, and behaviors surrounding open defecation. One development organization implementer described how households had built toilets only to receive a "sanitation card" that entitled them to government services but continued openly defecating.

3.4.6. Intervention outcomes

We found no meaningful differences between men and women for intervention outcomes in terms of perceived impact on health or community cleanliness. Both men and women perceived that their efforts and those of others resulted in cleaner communities (e.g., less visible feces, solid waste) and improved health, specifically reducing diarrheal disease and child deaths. Most participants attributed changes to the program overall, noting the importance of a collective movement towards sanitation rather than specific activities or actions by individuals.

3.4.7. Other outcomes—women's empowerment

Traditional gender norms in Nepal dictate that women should not participate in formal employment outside the home. However, women consistently reported that participating in WaSH programs gave them access to non-domestic employment opportunities, education and training, larger social networks, and power and status in society. Opportunities to access training, build skills, and gain status outside the home were significant motivators to work in WaSH programs, despite not being paid:

> "Why I involved with FCHV was because the women are generally limited in the home, and this job have took me out from the house. That's why I always chose FCHV in first priority. It was my first opportunity." -FCHV

WaSH work built women's confidence to voice their opinions in the community and work in other programs and sectors. They reported happiness and pride that their work improved the community and that they had been recognized and praised locally and, in some cases, nationally and internationally, for their work. One woman described her experience being featured in a video campaign:

> "I have been recognized in many places, sir … many VDCs [village development committees] of the west, because in that area the program was implemented. The sisters and friends from that area have recognized me easily. When they have recognized, it developed a type of excitement in me, I can also do something. I was afraid of the video earlier. I was not able to speak publicly. After the video, I thought that I can speak more confidently in future." -FCHV

Participation in WaSH programs encouraged women to participate in social development and employment in non-WaSH areas. After their experience working on WaSH programs, two women in our sample ran for local office, and one woman started a bio-sand filter business and planned to offer training to other women on how to construct the filters. Two program coordinators reported increases in women's participation in other social programs, such as anti-child marriage, following their engagement in WaSH campaigns. One woman described how participation in the local WaSH program led to changes in how women expressed their views more openly:

> "Due to the participation, women started going to the meetings and came outside. The women's participation was increased in every program. If any NGO comes, like for the women's violence, the women now go and take the participation. Previously, women did not want to participate. … Now women support in such programs. If any NGO comes or any other person comes, women give time and put their views openly." -woman, development organization implementer

4. Discussion

This study explored differences in implementation approaches, challenges and sources of support for implementation, and successes in achieving program quality outcomes among men versus women in community-based WaSH implementation roles in Nepal. We found that men and women used different approaches for implementation, and each approach leveraged different sources of support within the community and faced unique challenges. In turn, these approaches, challenges, and sources of support influenced perceived program quality, as assessed using self-reported implementation and intervention outcomes (Proctor et al., 2011).

Men who were government implementers applied sanctions-based approaches in response to low adoption, while men in development organizations typically refined activity content to target behavioral determinants. Implementation approaches varied less among women, with women in all roles applying relational approaches to convince households to adopt WaSH practices for community wellbeing rather than aggressively demanding change. Women implementers also mobilized other women in the community to deliver messages in groups for more persuasive power.

Women's approaches to implementation were influenced by power dynamics within households and communities and patriarchal norms in which men are the primary decision makers. Women reported that they were raised to give respect to others, and these respectful messages had higher acceptance within the community than more confrontational approaches used by some men. Power dynamics within Nepali households where women have reduced autonomy are well documented (Wali et al., 2020). Women's success with respectful messaging is consistent with literature indicating that confrontational messaging is less effective at changing behaviors than other approaches (Hornik, 2002), and that respect and familiarity with social norms are important for successful program delivery by FCHVs (Mohajer and Singh, 2018). We also found that both men and women perceived WaSH as women's work and

women as more appropriate messengers to discuss domestic WaSH-related information. This suggests that women may have greater influence for decision making in WaSH compared to other areas, though further research to is needed to clarify how gender roles impact WaSH related decision making within the home.

We applied social support theory to examine challenges and sources of support for implementation as informational, tangible, emotional, and companionship. Many frameworks that examine the factors supporting effective implementation have been developed in a healthcare context to examine implementation by medical and allied health professionals (Aarons et al., 2011; Damschroder et al., 2009). They consider the role of the community only in terms of patient needs and do not account for how relationships between implementers and beneficiaries may influence implementation. However, this research suggests that social relationships between implementers and beneficiaries are an important determinant of implementation success, which are lacking from many implementation science frameworks. Future studies may incorporate constructs from social support theory or other frameworks for lay implementation to more holistically evaluate determinants of implementation success.

Relationships between women and the community were an important source of support and a driver of their success in delivering acceptable and appropriate messages. Women typically lived in the communities where they worked and leveraged their social ties to mobilize other women to join WaSH campaigns. In contrast, men worked in more widespread geographic areas, which may reduce their ability to build social ties and mobilize companionship support. The importance of social support and social capital for women to practice WaSH behaviors within the home is well documented (Friedrich et al., 2018; House et al., 2013; Malolo et al., 2021; Sahoo et al., 2015). Women are more likely to be engaged in community social networks, and to both provide and seek social support from others (Taylor, 2011; Taylor et al., 2000). Our study indicates that these trends extend to the professional setting and approaches to seeking and providing social support between WaSH implementers.

Some differences in challenges and sources of support between men and women may be more proximally attributable to the implementation roles they hold. This is likely the case for information and tangible support, where differences occur as much within genders as across genders. For example, women in development organizations reported being well integrated into training and feedback systems, while unpaid women did not. Poor integration into systems for feedback and learning are likely experienced by unpaid women not because of their gender directly, but because unpaid roles are less integrated into organizational structures that support program implementation. In contrast, emotional challenges and coping strategies of companionship support were consistently experienced and used across all women, suggesting that implementation role was not an important moderator.

While some differences in informational and tangible challenges and support may be proximally driven by the implementation roles, gender bias remains an important distal cause of women's underrepresentation in paid and senior positions and decision-making processes. Disproportionate numbers of women in unpaid WaSH implementation roles aligns with evidence of gender bias in hiring, promotion, and compensation of women in Nepal (Adhikary, 2016; Wali et al., 2020) and worldwide (Stamarski and Son Hing, 2015; Weichselbaumer, 2004; Weichselbaumer and Winter-Ebmer, 2005). Women in this study reported that they were expected to be the primary duty-bearer for cooking, cleaning, childcare, and other domestic chores even while holding employment outside the home. Other studies have similarly found that domestic responsibilities impede women's participation in WaSH work, and that support and willingness to share domestic chores from husbands and in-laws is necessary to give women the time and permission needed to engage in WaSH work outside the home (Leder et al., 2017; Rautanen and Baaniya, 2008).

Some have questioned the sustainability, appropriateness, and ethics of relying heavily on unpaid labor from CHVs for program implementation (Kasteng et al., 2015; WHO et al., 2007) and argued that dependency on unpaid female labor reinforces gender inequality (Panday et al., 2017). The WHO recommends that all trained workers, including CHVs, receive "adequate wages and/or other appropriate and commensurate incentives" to ensure sustainability (WHO et al., 2007). Reliance by development organizations and government on unpaid women not only fosters economic inequality, but also reinforces programmatic structures that treat women differently from their paid counterparts and reduce their opportunities for participation in decision making, training and promotions, and other sources of informational support.

Evidence suggests that while altruism is one motivator for engaging in unpaid community health work, benefits such as opportunities for training and building reputation are also important (Government of Nepal, 2014; Kasteng et al., 2015). In this study, women expected to gain knowledge, skills, and social status through WaSH work. However, as gender equality increases in Nepal, these women may be less willing to engage in unpaid work, as the perceived value of opportunities for skill building and status may decrease.

Consensus on how to design appropriate compensation for CHVs is lacking. Some studies suggest that offering formal wages to CHVs may undermine their credibility if the community views them as working only for personal profit rather than altruistic motives (Vareilles et al., 2017). Some Nepali policy makers perceive that formal wages would decrease FCHVs' motivations to work and decrease community respect, suggesting alternatives to formal wages such as free healthcare and education (Glenton et al., 2010). However, the assertion that pay will decrease community respect is predicated on the perception that woman holding formal employment is improper, and there is little evidence to suggest that payment would reduce motivation to work. These assumptions reinforce regressive gender norms and may no longer hold as gender equality has increased over the past ten years and continues to do so. FCHV participants in this study indicated that wages would increase their motivation to work, a finding which has been supported by other studies in South Asia (Alam et al., 2012). Ultimately, the design of appropriate compensation packages for FCHVs and other unpaid WaSH implementers is likely context specific and will require further research to understand and balance perceptions of policy makers, community members, and implementers themselves regarding what is appropriate, desirable, and best achieves programmatic goals.

Overall, women had less formal education and experience and fewer opportunities for training and feedback, yet women-led programs were perceived as having higher adoption and sustainability. Women achieved these successes regardless of their level of training or access to supervision and feedback. Sustainability of women-led activities suggests that relational group messaging techniques used by women were more effective at changing underlying behavioral drivers of sanitation behavior. This suggests that technical knowledge and experience may be less important than social skills to persuade peers and mobilize social groups, familiarity with local communities, and ability to navigate and communicate appropriately within social dynamics and structures.

Community-level action and collaboration between women is particularly important in light of evidence that household-level WaSH interventions are insufficient to realize health benefits (Pickering et al., 2019a), and calls for "transformative WaSH" as a more holistic effort to eliminate fecal contamination in the household and broader community environment (Pickering et al., 2019b). Collective efficacy (i.e., a group's belief in their ability to work together to achieve common goals) is important for community change necessary for transformative WaSH (De Shay et al., 2020; Dickin et al., 2017; Salinger et al., 2020). Group messaging strategies and willingness to support each other during implementation demonstrate collective efficacy among women to improve the health and wellbeing of their communities. This translated into implementation successes such as rapid and far-reaching dissemination of messages among women's and mothers' groups and sustained

commitment of these groups to deliver WaSH activities even where formally funded programs had ended. In contrast, political rivalries among men demonstrated how a lack of collective efficacy can negatively impact program acceptability and adoption. This study suggests women's ability to mobilize companionship support among peers may make them more effective at widespread community transformation than men.

4.1. Limitations

We assessed successes and challenges from the perspective of local-level implementers only. Participants' perceptions of successes and challenges related to community attitudes and norms may not fully reflect the range of perspectives within the community. For example, in working to reach ODF, implementers will spend a disproportionate amount of time interacting with households who are resistant to constructing toilets. This may lead to a skewed perception of acceptance and appropriateness of WaSH activities among the community.

In selecting the study sample, we prioritized recruiting a diverse range of perspectives across government, NGO, and multilateral and civil society organizations. This allowed for identifying differences within genders, as well as between genders. However, as a result, the sample size of sub-groups within genders (e.g., paid versus unpaid women) is small. Where gender is strongly associated with the type of implementation role participants fill, disentangling the influence of gender and other contextual factors is challenging. For example, we found that men were more likely to use sanctions, but men are also more likely to hold political office with the power to enforce sanctions (Thapa, 2019). Our sample did not contain enough women in government to conclude whether sanctions approaches would be used by women if they had political power to enact them.

Further research with more senior program officials at the regional and national levels may help to determine the influence of broader contextual factors versus gender. Additionally, recruiting participants from a narrow range of implementation roles could better identify the range of experiences of men and women within that role and disentangle the influences of gender versus other factors associated with implementation role. Similarly, research recruiting participants of only a single gender could achieve a richer representation from different implementation roles to understand how factors such as level of training, political affiliation, or financial compensation affect performance within genders.

4.2. Programmatic implications & recommendations

This study illustrates several opportunities to address gender-related challenges and to better support and leverage the abilities of men and women as rural WaSH implementers. At the household and community level, activities to normalize and build acceptance among family and community members for women in non-domestic employment may reduce emotional challenges experienced by women. Frameworks for gender mainstreaming highlight the need to include men as role models and champions for gender equality (Morgan et al., 2016; Tolhurst et al., 2012). Studies have shown that women are more likely to participate in WaSH leadership positions when they have husbands and parents in-law who encourage their work and share childcare and other domestic duties (Leder et al., 2017; Rautanen and Baaniya, 2008). Various frameworks and guidelines exist that can support programs in identifying opportunities and integrating programming to promote gender equality in WaSH (Cortobius and Kjellen, 2014; Gosling, 2012; Halcrow et al., 2010; Panda, 2007).

At the program management level, deemphasizing technical knowledge and prior WaSH experience as key criteria for hiring and promotion may help improve women's representation in paid roles without sacrificing the quality of activities. We found that women implementers achieved high adoption and sustainability even where they had received little formal training, and ability to mobilize peer support, not technical skills, was critical to that success. Yet, perceived lack of technical proficiency and skills to perform maintenance and repair tasks are barriers to hiring women in WaSH in Nepal (Bhandari et al., 2005). Systematic reviews have identified a variety of interventions to mitigate gender bias in hiring (Isaac et al., 2009), and further research would help identify which are most appropriate for rural WaSH programs.

This study suggests opportunities to strengthen training and feedback systems for non-development organization implementers, particularly for unpaid women who experience challenges related lack of training and poor integration into feedback systems. Training and feedback systems have been found to increase productivity, motivation, satisfaction among CHVs (Scott et al., 2018; Whidden et al., 2018), which is consistent with women in this study reporting training opportunities as a motivator for work. Women expressed a desire for additional technical training (e.g., making reusable menstrual pads), as well as training to develop leadership skills, overcome stigma, and empower themselves and others in their community to challenge traditional gender roles. This study also suggests opportunities to build men's communication and social mobilization skills. Training to build facilitation skills among both men and women community-based CLTS implementers has been shown to improve sanitation adoption (Crocker et al., 2016).

In the long-term, programs may ultimately need to plan for the possibility that women's willingness to work in unpaid roles may decrease as gender equality increases, and to reconsider the ethics, appropriateness, and sustainability of relying on unpaid labor. Unpaid women in this study reported that their motivation to work would increase with a formal salary, and studies of CHVs in other contexts have found that satisfaction with both monetary and non-monetary incentives is important for motivation and retention (Kasteng et al., 2015; Kok et al., 2015). Payment of all trained CHVs aligns with WHO recommendations to ensure sustainability of health programs (WHO et al., 2007) and addresses structural inequalities associated with reliance on women's unpaid labor (Panday et al., 2017). However, coordination will be needed between government and external funders to ensure that payment systems linked to specific WaSH activities are not disruptive of long-term CHV programs.

5. Conclusions

Women were more likely to be omitted from high-level planning, face stigma for engaging in work outside the home, and hold unpaid roles that are not well integrated into feedback and troubleshooting systems. Women also had less formal experience, education, and training. Social ties between women were robust sources of companionship and emotional support to mitigate challenges, and opportunities to build job skills, social status, and overcome traditional gender norms were important motivators for women to engage in WaSH work.

Both men and women perceived that women were more effective than men at mobilizing widespread, sustained WaSH improvements, which was attributed to their success using relational approaches and leveraging social ties to deliver acceptable and appropriate messages. Women's skills for motivating collective action indicate that they can be highly effective WaSH implementers despite lack of technical experience and training, and that women's active participation is important for achieving transformative community change.

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Declaration of competing interest

None.

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Systematic review of biomonitoring data on occupational exposure to hexavalent chromium

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ABSTRACT

Occupational exposure to hexavalent chromium (Cr(VI)) can cause serious adverse health effects such as lung cancer and irritation of the skin and airways. Although assessment of chromium (Cr) in urine is not specific for Cr (VI) exposure, the total amount of Cr in urine is the most used marker of exposure for biomonitoring of Cr(VI). The purpose of this systematic review was fourfold: (1) to assess current and recent biomonitoring levels in subjects occupationally exposed to Cr(VI), with a focus on urinary Cr levels at the end of a working week, (2) to identify variables influencing these biomonitoring levels, (3) to identify how urinary Cr levels correlate with other Cr(VI) exposure markers and (4) to identify gaps in the current research. To address these purposes, unpublished and published biomonitoring data were consulted: (i) unpublished biomonitoring data comprised urinary Cr levels (n = 3799) of workers from different industries in Belgium collected during 1998–2018, in combination with expert scores indicating jobs with Cr exposure and (ii) published biomonitoring data was extracted by conducting a systematic literature review. A linear mixed effect model was applied on the unpublished biomonitoring data, showing a decreasing time trend of 30% in urinary Cr levels. Considering the observed decreasing time trend, only articles published between January 1, 2010 and September 30, 2020 were included in the systematic literature search to assess current and recent biomonitoring levels. Twenty-five studies focusing on human biomonitoring of exposure to Cr(VI) in occupational settings were included. Overall, the results showed a decreasing time trend in urinary Cr levels and the need for more specific Cr(VI) biomarkers. Furthermore, this review indicated the importance of improved working conditions, efficient use of personal protective equipment, better exposure control and increased risk awareness to reduce Cr levels in biological matrices. Further investigation of the contribution of the different exposure routes is needed, so that better guidance on the use of control measures can be provided. In addition, this review support the call for more harmonization of human biomonitoring.

1. Introduction

Chromium (Cr) is a transition element that exists in oxidation states ranging from −2 to +6. The common stable ones in the environment are trivalent (Cr(III)) and hexavalent (Cr(VI)) chromium (Lunk, 2015). Cr (III) mostly occurs in nature, whereas Cr(VI) is mostly released from industrial processes. The main properties of Cr(VI) compounds are corrosion-resistance, durability and hardness. Exposure to Cr(VI) may occur when Cr(VI) compounds are manufactured as end-product (e.g. chromate production), when Cr(VI) compounds are used as start-product (e.g. electroplating) or when Cr(VI) compounds are formed as by-product (e.g. welding) (NIOSH, 2013). Cr(III) has limited toxicological properties (Anderson, 1997), whereas Cr(VI) is more toxic due to its oxidizing ability and high solubility leading to increased membrane permeability (Saha et al., 2011). Occupational exposure to Cr (VI) can cause serious adverse health effects such as cancer and irritation of the eyes, skin and airways (OSHA, 2006).

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Abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists
BAR	Biologischen Arbeitsstoff-Referenzwerte
BEI	Biological Exposure Indices
BLUPs	Best Linear Unbiased Prediction
BLV	Biological Limit Value
BMGV	Biological Monitoring Guidance Value
BRV	Biological Reference Value
Ca	Circa
Cr	Chromium
Cr(0)	Elemental Chromium
Cr(III)	Trivalent Chromium
Cr(VI)	Hexavalent Chromium
Cr-Air	Chromium level in Air
Cr–B	Chromium level in whole Blood
Cr-EBC	Chromium level in Exhaled Breath Condensate
Cr(III)-EBC	trivalent Chromium levels in Exhaled Breath Condensate
Cr(VI)-EBC	Hexavalent Chromium levels in Exhaled Breath Condensate
Cr-Fingernails	Chromium level in Fingernails
Cr-Hair	Chromium level in Hair
Cr-Hand	Chromium level on Hands
Cr–P	Chromium level in Plasma
Cr-RBC	Chromium level in Red Blood Cells
Cr–S	Chromium level in Serum
Cr-Surface	Chromium level on Surface
Cr–U	Chromium level in Urine
Creat	Creatinine
EBC	Exhaled Breath Condensate
EC	European Commission
EQA	External Quality Assurance
FCAW	Flux-Cored Arc Welding
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
GM	Geometric Mean
GMAW	Gas Metal Arc Welding
HBM	Human BioMonitoring
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ILC	InterLaboratory Comparison
ILO	International Standard Classification of Occupations
IQR	InterQuartile Range
JEM	Job-Exposure Matrix
LEV	Local Exhaust Ventilation
LOD	Limit Of Detection
LOQ	Limit Of Quantification
N°	Number of
NACE	Nomenclature statistique des Activités économiques dans la Communauté Européenne
OEL	Occupational Exposure Limit
OSH	Occupational Safety and Health
P_0	0^{th} percentile
P_{25}	25th percentile
P_{75}	75th percentile
P_{90}	90th percentile
P_{100}	100th percentile
PPE	Personal Protective Equipment
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RBC	Red Blood Cells
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
REML	REstricted Maximum Likelihood
RMMs	Risk Management Measures
RPE	Respiratory Protection Equipment
SD	Standard Deviation
SMAW	Shielded Metal Arc Welding
TIG	Tungsten Inert Gas
URL	Upper Reference Limit

Human biomonitoring (HBM) can be used for assessment of occupational exposure and involves measurement of the chemical or metabolites of this chemical in a biological medium such as urine, blood, breath, nails or hair (Scheepers et al., 2014). The measurement of Cr in urine is often used in the workplace to monitor Cr(VI) exposure and gives an indication of total Cr exposure as Cr(VI) is reduced inside the body to Cr(III) (Ray, 2016). Depending on the route of exposure, reported elimination half-lives differ. After exposure by inhalation, the excretion of Cr in urine is thought to follow a two- or tri-phasic process with elimination half-lives of 7 h, 15–30 days and 3–5 years (ATSDR, 2012; Hoet, 2005). In order to better interpret the biomonitoring results, several guidance values have been set up by authorities or advisory organisations for occupationally exposed population and general population with no known occupational exposure to Cr. Examples of these guidance values are shown in Table 1. Guidance values for occupationally exposed population are either based on a relationship between concentrations in biological exposure markers and health effects, between concentrations in biological exposure markers and occupational exposure limits (OELs) or on data collected from a representative set of workplaces with good control of exposure. (Worksafe, 2020). At EU level, no biological guidance value have been set up for Cr(VI) compounds (Hartwig et al., 2017). In the USA, the American Conference of Governmental Industrial Hygienists (ACGIH) has established two health-based biological exposure indices (BEI): one for the total Cr concentration increase in urine during the shift and one for the total Cr concentration at the end of the shift at the end of the working week. The values are respectively 10 μg/l and 25 μg/l (ACGIH, 2020). France has established the most stringent health-based biological limit value (BLV) for European workers, this being a BLV of 2.5 μg/l from its occupational exposure limit (OEL) of 1 μg/m^3 for Cr(VI) (ANSES, 2017). The same BLV is applied in the Netherlands (SZW, 2016). Finland has derived a health-based BLV of 10 μg/l corresponding to its OEL of 5 μg/m^3 for Cr (VI) (MSAH, 2012). In the UK, an occupational hygiene-based biological monitoring guidance value (BMGV) of 10 μmol/mol creatinine (approximately 6.3 μg/l) in post-shift urine has been established (HSE, 2020). Besides these occupational limit values, reference values exist for the general population with no known occupational exposure to Cr. These values represents the upper reference concentration of a biomarker in the general adult population without occupational exposure to the agent. These reference values are influenced by environment, lifestyle and medical factors. Therefore, these values for the general population may differ between regions (Hoet et al., 2013). Examples of such reference values are the German Biological reference values for chemical compounds in the work area (Biologischen Arbeitsstoff-Referenzwerte (BAR)), the Biological Reference Value (BRV) in France and the Upper Reference Limit (URL) in Belgium.

Due to required authorization of Cr(VI) compounds under Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) and the proposal of the European Commission (EC) to set a BLV for Cr (VI) (EC, 2004, 2017), we aimed to gather existing and recent occupational biomonitoring data concerning Cr(VI) and to identify gaps in the current research. Therefore, the goal of this review article was fourfold: (1) to identify the current and recent biomonitoring levels in subjects occupationally exposed to Cr(VI), with a focus on urinary Cr levels at the

Table 1
Biological monitoring guidance values for total Cr concentration in urine.

Type of guidance value	Definition	Sampling time	Guidance value	Authorities or advisory organisations
Occupationally exposed population				
Biological Guidance Value (BGV)	Genotoxic mechanism of action is likely or cannot be excluded		None	SCOEL - European Commission (Hartwig et al., 2017)
Biological Limit Value (BLV)	Health-based guidance value Derived corresponding to French OEL for Cr(VI) of 1 µg/m^3	End of shift at end of working week	2.5 µg/l [1.8 µg/g creatinine]	ANSES - France (ANSES, 2017)
Biological Limit Value (BLV)	Health-based guidance value. Derived corresponding to Finish OEL for Cr(VI) of 5 µg/m^3.	End of shift at end of working week	10 µg/l	Finland - MSAH (STM, 2018)
Biological Monitoring Guidance Value (BMGV)	Occupational hygiene-based guidance value. 90th percentile of data from workplaces with good control of exposure.	End of shift	10 µmol/mol creatinine [4.6 µg/g creatinine] [ca. 6.3 µg/l]	HSE - UK (HSE, 2020)
Biological Exposure Index (BEI)	On the basis of six studies.	Increase during shift	10 µg/l	ACGIH - USA (ACGIH, 2020)
	Health-based guidance value Derived corresponding to TLV-TWA of 0.05 mg/m^3 for airborne soluble Cr(VI) of µg/m^3.	End of shift at end of working week	25 µg/l	
General population with no known occupational exposure to Cr				
Biological reference values for chemical compounds in the work area ("Biologischen Arbeitsstoff-Referenzwerte", BARs)	95th percentile in German general population		0.6 µg/l	DFG - Germany (DFG, 2018)
Biological Reference Value (BRV)	95th percentile in French general population		0.65 µg/l (0.54 µg/g creatinine)	ANSES - France (ANSES, 2017)
Upper Reference Limit (URL)	upper limit of the 90% confidence interval of 97.5th percentile in Belgian general population		0.55 µg/l	UCLouvain - Belgium (Hoet et al., 2013)

Abbreviations: HBM4EU, Human Biomonitoring for Europe; MSAH: Ministry of Social Affairs and Health; OEL, Occupational Exposure Limit; TLV-TWA, Threshold Limit Value - Time-Weighted Average.

end of a working week, (2) to identify variables influencing these biomonitoring levels, (3) to identify how urinary Cr levels correlate with other Cr(VI) exposure markers and (4) to identify gaps in the current research. To achieve these objectives, we firstly analysed an unpublished dataset comprising urinary Cr measurements of workers from different industries in Belgium, in combination with another dataset comprising expert scores indicating jobs with Cr exposure based on risk assessments and exposure data. By combining these two datasets, we aimed to reveal recent exposure levels and time trends in exposure levels. In addition, published biomonitoring data was extracted by conducting a systematic literature review to evaluate the extent of current and recent exposure levels reported in literature and to identify possible correlations between different Cr(VI) exposure markers. The time period of the search strategy used for the literature review was based on the time trend of exposure levels reported in the unpublished dataset.

2. Methodology

2.1. Unpublished biomonitoring data

Two types of datasets regarding occupational exposure to Cr were obtained through a Belgian external occupational safety and health (OSH) service.

- The first dataset comprised of measurements on urinary Cr levels of workers from different industries across Belgium collected during 1998–2018. Workers under risk of exposure to hazardous chemicals had been examined periodically by the external OSH service. Post-shift urine samples were taken in the context of periodical examinations at the end of a working week. The data of these examinations were stored pseudonymized on a computerized database. Jobs were coded into ISCO-08, the International Standard Classification of Occupations. ISCO-08 is a system used to classify jobs into a set of groups according to the tasks and duties related to a job and the relevant necessary skills (ILO, 2016). Jobs are classified into 436 unit groups. These unit groups are aggregated into 130 minor groups, 43 sub-major groups and 10 major groups. This aggregation or classification is done based on their similarity. Each group is clearly defined and is designated by a code number. Major groups are indicated by a one-digit code, sub-major groups by a two-digit code, minor groups by a three-digit code and unit groups by a four-digit code. Therefore, ISCO-08 has a hierarchical structure and is composed of four levels, going from general to specific. This hierarchical use of the ISCO codes allows the generation of summary data for 10 occupational groups, indicated by major-groups, at the highest level of aggregation as well as relatively detailed data at the lowest level of aggregation, indicated by unit-groups. Industries were coded into NACE (Nomenclature statistique des Activités économiques dans la Communauté Européenne), the European industry standard classification system (Eurostat, 2008). NACE also uses four hierarchical levels. Industries are classified into 21 sections, 88 divisions, 272 groups and 629 classes. This aggregation is done based on similarity in business activities. Sections are indicated by an alphabetical letter (A to U), divisions by a two-digit code (01–99), minor groups by a three-digit code (01.1–99.0) and unit groups by a four-digit code (01.11–99.00). Data on date of birth, gender, ISCO-08 code, NACE code, sampling date and unit of measurement were extracted for the time period 1998–2018.
- The second dataset comprised of expert scores. Experts, namely occupational physicians of the external OSH service, rated (0 = unexposed and 1 = exposed) jobs based on data obtained through risk

assessments and exposure data obtained through periodical examinations. This dataset covered a wide range of exposure profiles or health outcomes linked to ISCO-08 codes. Data extraction that solely focused on Cr resulted in the identification of 47 jobs with Cr exposure. This data was used as the expert-based qualitative assessment on Cr exposure and incorporated in the statistical model *a priori* to combine urine measurements with expert knowledge (Peters et al., 2012; Vested et al., 2019). This approach of developing a job-exposure matrix (JEM) by combining measurement data and expert knowledge is especially helpful in situations where a limited number of measurements with high variability are available for a job. Otherwise, the resulting estimate from the limited number of measurements is characterized by a high uncertainty (Vested et al., 2019). Furthermore, JEM allows to estimate exposure levels when no measurements are available for a job.

2.1.1. Descriptive analysis of unpublished biomonitoring data

First, descriptive statistics were applied to biomonitoring data in order to find the number and the proportion of workers exceeding the BEIs set up by the ACGIH (ACGIH, 2020) and URL described previously for healthy Belgian adults (Hoet et al., 2013).

2.1.2. Temporal analysis of unpublished biomonitoring data

Subsequently, an empirical linear mixed effect model that combines urinary Cr measurements and expert decisions was applied in order to predict job and industry specific exposure levels. Year of measurement and expert score (0 = unexposed, 1 = exposed) were included as the fixed effects while job, industry and worker were included as random effect terms in the model. Random effects were incorporated in order to evaluate the possible effects of the variances between jobs, between workers and between industries on exposure levels. "Variance components" is selected as the covariance structure. The variance between workers and between jobs were assumed to be equal across all fixed determinants. Restricted maximum likelihood (REML) was used to estimate variance components and fixed effects. Data analysis was done using IBM Statistical Package for the Social Sciences (SPSS) Version 25.

The empirical model used for the estimation of exposure based on biomonitoring results is shown in equation (1) (adapted from Vested et al., 2019):

$$Ln(Y) = \beta 0 + \beta eE_{0\text{-}1} + \beta tT + bj_{1-56}J + bi_{1-101}I + bw_{1-606}W + \varepsilon \quad (1)$$

Where:

Ln(Y): Natural log-transformed urine chromium level
β0: Model intercept
βeE$_{0\text{-}1}$: Fixed effect term for expert score (0–1)
βtT: Fixed effect term for year of measurement (continuous)
bj$_{1-56}$J: Random effect term for job (1–56)
bi$_{1-101}$I: Random effect term for industry (1–101)
bw$_{1-606}$W: Random effect term for workers (1–606)
ε: Residual error

The model used an algorithm to predict estimates of covariance parameters for the random terms and mean levels for the fixed effect terms. A job with a limited number of measurements or high variable exposure data will result in an estimate of covariance parameter that is equal to 0, whereas a job with a larger number of exposure data or less variable exposure data will lead to an estimate for the exposure level that differentiate from the intercept (Vested et al., 2019). Modeled predicted geometric means were used to identify the jobs with highest exposure levels.

2.2. Published biomonitoring data: systematic literature review

2.2.1. Study identification

The databases PubMed, Scopus and Web of Science were systematically searched according to PRISMA guidelines (Moher et al., 2009) for articles published between January 1, 2010 and September 30, 2020 focusing on HBM of Cr(VI) in occupational settings. Search terms were developed using the keywords "workplace", "worker" or "occupation" in combination with "hexavalent chromium". The full search string used in each database is provided in the Supplementary Material S2. Additional articles were found by scanning the list of references of original publications and review articles.

2.2.2. Study selection

Studies meeting the following criteria were included in the review: 1) reported in a peer-reviewed journal, 2) in English, 3) full text article, 4) publication date between 01/01/2010 to 30/09/2020, 5) occupational exposure, 6) age of study characteristics between 18 and 65 years, 7) exposure to Cr(VI), 8) HBM and 9) at least two markers of exposure are reported.

2.2.3. Data extraction

For each study, information on the following variables was extracted in an MS Excel template: 1) Industry, 2) Study population characteristics, 3) Sample size, 4) Biomonitoring data, 5) Correlations between exposure markers, 6) Sampling time, 7) Sampling strategy (e.g. spot urine sample) and matrix adjustment (e.g. creatinine-adjusted or/and non-adjusted urinary Cr concentrations), 8) Technique (e.g. Inductively coupled plasma mass spectrometry (ICP-MS), Graphite furnace atomic absorption spectroscopy (GFAAS)), 9) Method characteristics (limit of detection (LOD)/limit of quantification (LOQ), stability, contamination (-free) and quality assurance), 10) Tasks with higher exposure/variables influencing exposure, 11) Risk management measures (RMMs) in place/to be applied, 12) Environmental monitoring (e.g. air, surfaces), 13) Health surveillance, 14) Biomarkers of effects, 15) Co-exposure characteristics and 16) Exposure characteristics.

2.2.4. Quality scoring

The quality of each study was then scored according to the LaKind scoring criteria (LaKind et al., 2014), adapted for Cr(VI) exposure (Supplementary Material S1). The LaKind criteria are designed to assess quality issues of exposure assessment by biomonitoring or environmental epidemiology for short-lived and non-persistent chemicals. In this review article, the LaKind criteria have been refined specifically for biomonitoring of Cr(VI), with these adaptations also taking into consideration a more recent scientific report related to HBM data collection from occupational exposure to pesticides (RPA HSL IEH, 2017). This report highlighted factors for standardisation in HBM studies. The following two assessment components were added to the existing LaKind criteria: study records and background levels. Both criteria are helpful for the interpretation of biomonitoring data. Study records provide contextual information. Background levels provide knowledge about exposure levels arising from non-occupational exposure. So in this review, the studies are assessed based on their study design (population size and selection of study participants), biomarker selection and measurement (exposure biomarker specificity, analytical technique, method sensitivity, biomarker stability, sample contamination, quality assurance and matrix adjustment), contextual information (study records) and interpretation (background levels). The study classification for each assessment component has to be accompanied by a justification for each decision to increase transparency. More information about the refined LaKind criteria is provided in Supplementary Material S1. For each study included in this review, eleven study assessment components are assessed by giving a score from 1 (Tier 1 = highest quality) to 3 (Tier 3 = lowest quality). Therefore, the possible total scores range from 11 (highest quality) to 33 (lowest quality).

Industry	Study Population country N° workers N° controls N° companies	Biomonitoring data - directly exposed workers Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	Key findings exposure assessment	LaKind scoring strengths and/or weaknesses	Reference
Welding	Germany 50 workers 0 controls 14 companies	● Cr–U: *0.*90 μg/g creat. (P_{25}: 0.56, P_{75}: 1.55) *0.*96 μg/l (P_{25}: 0.60, P_{75}: 1.60)	Significant positive correlations between: • Pre- and post-shift Cr–U ($r_S = 0.78$) • Creatinine-adjusted pre- and post-shift Cr–U ($r_S = 0.83$) • Respirable Cr(VI)-Air and post-shift Cr–U ($r_S = 0.25$, $P = 0.0008$) • Respirable Cr-Air and post-shift Cr–U ($r_S = 0.44$, $P \leq 0.0001$)	17 EQA	Pesch et al. (2018)
	Poland 67 workers 52 controls 5 companies	● Cr–U: *3.*81 μg/g creat. (P_{25}: 2.22, P_{75}: 6.70) ca. *5.*18 μg/l ca. (P_{25}: 3.02, P_{75}: 9.11) ● Cr–S: 1.25 μg/l (P_{25}: 0.12, P_{75}: 2.68) ● Cr-RBC: 0.09 μg/g Hb (P_{25}: 0.05, P_{75}: 0.15)	Significant positive correlations between: • Cr–U and inhalable Cr-Air ($r_S = 0.59$, $p < 0.0001$) • Cr–U and inhalable Cr(VI)-Air ($r_S = 0.58$, $p < 0.0001$) • Cr–U and inhalable Cr(III)-Air ($r_S = 0.64$, $p < 0.0001$) • Cr–S and inhalable Cr-Air ($r_S = 0.68$, $p < 0.0001$) • Cr–S and inhalable Cr(VI)-Air ($r_S = 0.67$, $p < 0.0001$) • Cr–S and inhalable Cr(III)-Air ($r_S = 0.67$, $p < 0.0001$)	17 EQA	Stanislawska et al. (2020)

3. Results

3.1. Unpublished biomonitoring data

3799 measurements on Cr collected during 1998–2018 were available among 1824 workers representing 56 minor groups of jobs. Jobs were coded into ISCO-08 which is characterized by a hierarchical structure (1–4 digits) as mentioned before. Measurements were distributed over unique ISCO-08 codes as follows (the maximum amount of unique codes in the ISCO-08 system is given between brackets): 9 (out of 10) unique one-digit codes, 26 (out of 43) unique two-digit codes, 56 (out of 130) unique three-digit codes and 83 (out of 436) unique four-digit codes. 101 (out of 629) unique NACE codes were represented by the measurement data. The urinary Cr levels ranged from 0.1 to 70 μg/l with a geometric mean of 0.88 μg/l 54.9% of the measurements exceeded the URL (0.55 μg/l) for Belgian adults and 0.8% of the measurements exceeded the occupational limit (25 μg/l) recommended by ACGIH. An overview on the characteristics of the data is presented in Table 2.

The linear mixed effect model applied at sub-major ISCO levels (three-digit) showed that about 15% of the total variance in Cr data was explained by the variance between jobs and industries.

There was a decreasing time trend of 30% in urinary Cr levels. The parameters of the linear mixed effect model are available in Table 3.

According to observed and model predicted urine levels, “sheet and structural metal workers, moulders and welders, and related workers”, “electronics and telecommunications installers and repairers”, “blacksmiths, toolmakers and related trades workers”, “metal processing and finishing plant operators” were found to have the highest Cr exposure (Table 4).

Table 2
Characteristics of data on urinary Cr levels.

Exposure matrix	Urine
Unit	μg/l
Number of measurements	3799
Total number of workers	1824
Median age (min.-max.)	40 (13–73)
Sex (F/M)	104/3701
Unique ISCO codes	
ISCO One-digit	9
ISCO Two-digit	26
ISCO Three-digit	56
ISCO Four-digit	83
Unique NACE codes	101
Years covered	1998–2018
Geometric mean (μg/l)	0.88
Geometric standard deviation (μg/l)	3.02
Min.-max. levels (μg/l)	0.1–70
Percentiles (μg/l)	$P_5 = 0.3$, $P_{10} = 0.4$, $P_{25} = 0.4$, $P_{50} = 0.6$, $P_{75} = 1.5$, $P_{90} = 4.6$, $P_{95} = 9.01$
Measurements under LOD (n, %)	684 (18%)
Measurements exceeding reference limits for Belgian adults[a] (n, %)	2089 (54.9%)
Measurements exceeding occupational limit[b] (n, %)	33 (0.8%)

[a] Upper Reference Limit (Hoet et al., 2013): 0.55 μg/l.
[b] Biological Exposure Index (ACGIH, 2020): 25 μg/l.

Table 3
Mixed effect model parameters applied to unpublished biomonitoring dataset

Estimates of fixed effects				
	β	SE	P	95 % CI
Intercept	7.63	0.52	**< 0.001**	6.61-8.66
Year	**-0.30**	0.02	**< 0.001**	-0.35-0.25
Riskpro				
Unexposed	-0.78	0.89	0.93	-1.84-1.68
Exposed (ref.)	—	—	—	—
Estimates of covariance parameters*				
	Estimate*	**SE**	**P**	**95 % CI**
ISCO (3 digit)	0.43	0.29	0.13	0.11-1.60
NACE	**1.99**	0.70	**0.005**	0.99-3.99
Workers	**4.79**	0.69	**< 0.001**	3.60-6.37
Residual	7.80	0.54	< 0.001	6.80-8.96

Abbreviations: Rikspro, Risk profile; ISCO, International Standard Classification of Occupations; NACE, Nomenclature statistique des Activités économiques dans la Communauté Européenne; β, fixed effects terms; SE, Standard error; p, p-value or probability value; CI, confidence interval.
*Job (ISCO), sector (NACE) and individual workers are parametrized as categorical variable and added as random effects in the model.

Table 4
Jobs with the highest chromium exposure, according to observed and model predicted geometric mean (GM) levels.

ISCO-08	Observed GM (μg/l)	Range of observed values [P_0 – P_{100}]	Model predicted GM (μg/l)	95% CI	Measurements (N)
721 Sheet and structural metal workers, moulders and welders, and related workers	3.75	0.60–23.80	3.70	2.32–5.08	658
742 Electronics and telecommunications installers and repairers	2.54	0.70–13.60	2.79	1.13–4.45	17
722 Blacksmiths, toolmakers and related trades workers	2.55	0.70–12.80	2.18	0.54–3.82	50
812 Metal processing and finishing plant operators	2.30	0.60–23.80	2.16	0.67–3.65	101

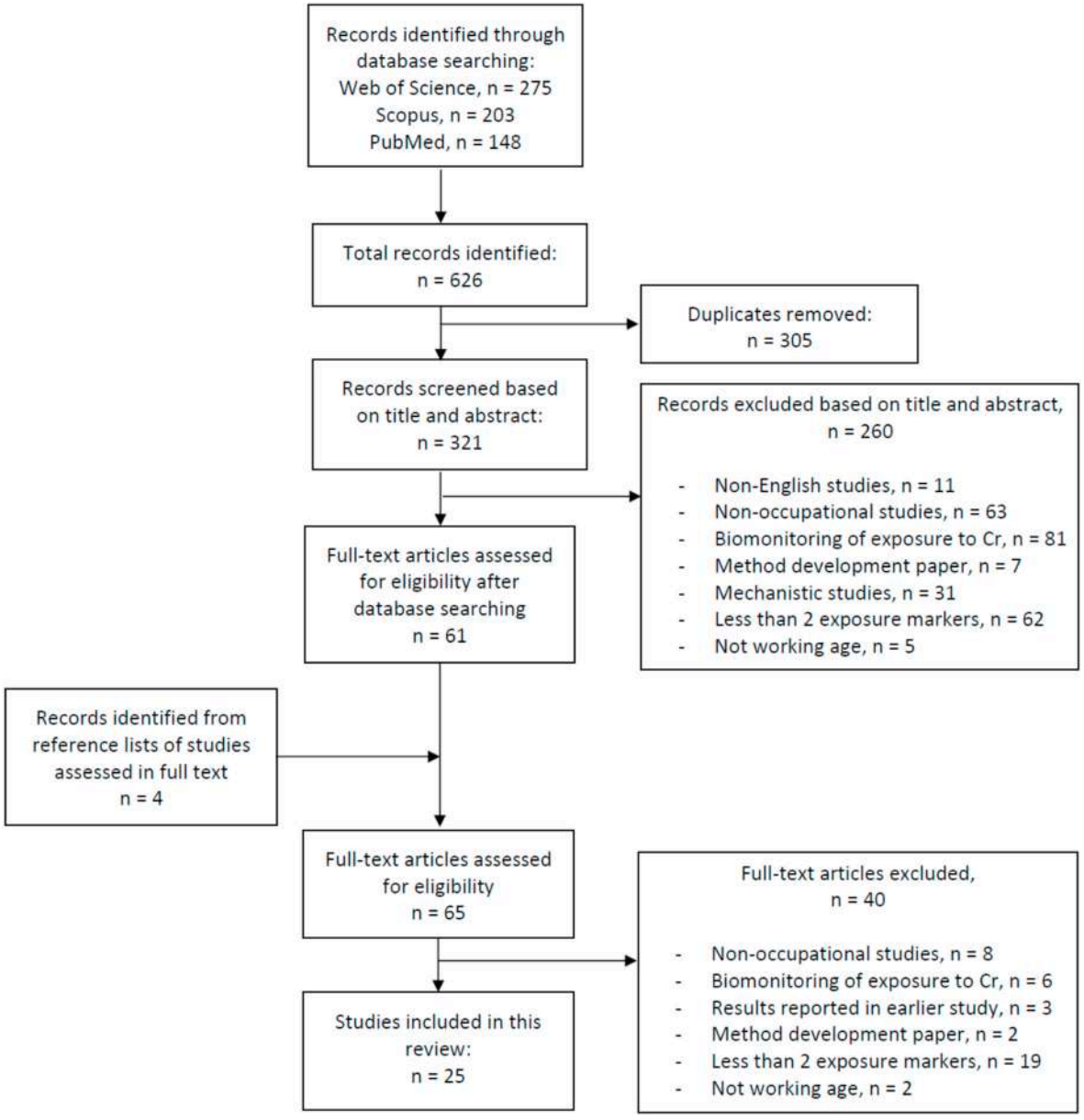

Fig. 1. PRISMA flowchart of the study inclusion process.

3.2. Published biomonitoring data: systematic literature review

Considering the observed decreasing time trend in the unpublished biomonitoring dataset, only articles published between January 1, 2010 and September 30, 2020 were included in the systematic literature search to assess current and recent biomonitoring levels. Based on our search strategy, a total of 630 articles were identified. Out of these, 626 articles were identified through database searching and 4 articles through scanning of the reference lists. After duplicate removal and an initial screening based on title and abstract, 65 studies were considered for further assessment. After full text screening, 40 studies were excluded. Out of these, 8 studies were performed in non-occupational settings (e.g. lab study and general population), 6 used biomonitoring for the assessment of exposure to total Cr (not explicitly indication of potential exposure to Cr(VI) by the authors), 3 displayed biomonitoring data of another study, 2 were considered as a method development, 19

Table 5
Summary of the studies for occupational exposure to Cr(VI).

Industry	Study Population country N° workers N° controls N° companies	Biomonitoring data - directly exposed workers Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	Key findings exposure assessment	LaKind scoring strengths and/or weaknesses	Reference
Welding	Germany 241 workers 0 controls 25 companies	**Welders (n=241)** ● Cr–U: *1.2* µg/l (P_{25}: <LOQ, P_{75}: 3.61) *<1.35* µg/g creat. (P_{25}: <0.74, P_{75}: <3.24) **High-exposure group (n=16):** ● Cr-RBC: *1.95* µg/l (P_{25}: <LOQ, P_{75}: 2.37) ● Cr–U: *13.53* µg/l (P_{25}: 5.21, P_{75}: 53.03) ca. *9.95* µg/g creat. ca. (P_{25}: 3.83, P_{75}: 38.99)	Significant positive correlation between: • Respirable Cr-Air and Cr–U ($r = 0.61$, $p < 0.0001$) High-exposure group: • gas metal arc welders with massive or flux-cored wire of stainless steel • Confined spaces or insufficient ventilation Detection frequency: • Cr-RBC was only detected in 15 out of 150 stainless-steel welders (n = 8 FCAW, n = 1 GMAW, n = 6 SMAW).	15 EQA	Weiss et al. (2013) *
	Italy 100 workers 0 controls 24 companies	● Cr-EBC: *0.08* µg/l (P_{25}: <LOD, P_{75}: 0.22) ● Cr–U: *0.74* µg/g creat. (P_{25}: 0.41, P_{75}: 1.21) ca. *1.01* µg/l ca. (P_{25}: 0.56, P_{75}: 1.65)	No correlation between: • Cr-EBC and Cr–U Detection frequency: • Cr(VI)-EBC was never detected.	22 No exclusion of contamination Stability Cr(VI)-EBC unknown	Riccelli et al. (2018)
	Iran 94 workers 25 controls Gas pipelines	**Welders (n=59)** ● Cr–U: 6.37 ± 3.74 µg/l (P_0: 1.00, P_{100}: 18.00) ca. 4.68 ± 2.75 µg/g creat. ca. (P_0: 0.73, P_{100}: 13.24) **High-exposure group (n=6):** ● Cr–U: 12.67 ± 4.50 µg/l (P_0: 6.00, P_{100}: 17.00) ca. 9.32 ± 3.31 µg/g creat. ca. (P_0: 4.41, P_{100}: 12.5)	Weak correlation between: • Cr-Air of the breathing zone and Cr–U (R^2_p=0.481, $P < 0.05$) High-exposure group: • back welders inside confined spaces	23 8h samples	Golbabaei et al. (2012)

Industry	Study Population country N° workers N° controls N° companies	Biomonitoring data - directly exposed workers Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	Key findings exposure assessment	LaKind scoring strengths and/or weaknesses	Reference
Welding	Kenya 40 workers 0 controls 1 company	Welding ● Cr–U: 24.7 ± 8.2 µg/g creat. ca. 33.6 ± 11.2 µg/l	Significant positive correlation between: • Cr-Air of the breathing zone and Cr–U in all 112 production workers ($r = 0.86$, $P < 0.01$) $PbCrO_4$ is commonly used to formulate decorative paint	18 ILC Spot morning urine	Were et al. (2013)
Paint manufacturing	Kenya 41 workers 0 controls 1 company	Paint manufacturing ● Cr–U: 9.8 ± 5.3 µg/g creat. ca. 13.3 ± 7.2 µg/l			
Leather tanning	Kenya 31 workers 0 controls 1 company	Leather tanning ● Cr–U: 35.2 ± 12.1 µg/g creat. ca. 47.9 ± 16.5 µg/l			
	India 36 workers 36 controls small-scale tanneries	● Cr–U: 2.11 ± 1.01 µg/l ca. 1.55 ± 0.74 µg/g creat.	Higher sensitivity of cytogenetic assays for human biomonitoring of an occupationally exposed population.	26 No exclusion of contamination 24h samples	Balachandar et al. (2010)
	Kenya 40 workers 40 controls 1 company	● Cr–U: 35.6 ± 7.4 µg/g creat. (P_0: 25, P_{100}: 51) ca. 48.4 ± 10.1 µg/l ca. (P_0: 34, P_{100} : 69)	Significant positive correlation between: • Cr–U and Cr-Air of the breathing zone ($R^2 = 0.76$, $P < 0.001$)	20 ILC	Were et al. (2014)
	Egypt 304 workers 304 controls 1 company	● Cr–S: 3.1 ± 2.2 µg/l (P_0: 0.01, P_{100}: 10.3) ● Cr–U: 2.6 ± 2.5 µg/l (P_0: 0.02, P_{100} : 8.6) ca. 1.9 ± 1.8 µg/g creat. ca. (P_0: 0.01, P_{100} : 6.3)	Significant positive correlations between: • Cr-Air of the breathing zone and Cr–S (regression coefficient $\beta = 0.339$; $P < 0.05$) • Cr-Air of the breathing zone and Cr–U (regression coefficient $\beta = 0.435$; $P < 0.05$) • Cr–S and duration of current employment ($r = 0.187$, $P < 0.001$) • Cr–U and duration of current employment ($r = 0.128$, $P < 0.05$)	22 No exclusion of contamination No methodology mentioned	Abdel Rasoul et al. (2017)

Industry	**Study Population** country N° workers N° controls N° companies	**Biomonitoring data - directly exposed workers** Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	**Key findings exposure assessment**	**LaKind scoring** strengths and/or weaknesses	**Reference**
Electroplating	Italy 14 workers 0 controls 1 company	Post-shift at end of 2nd working day ● Cr(VI)-EBC: *1.0* µg/l (P_0: nd, P_{25}: nd, P_{75} : 1.9, P_{100} : 2.9) ● Cr-EBC: *2.6* µg/l (P_0 : 0.2,	Significant positive correlations between: • Cr–P and Cr–U at the end of the working shift ($r_S = 0.77$, $p < 0.01$) • Cr-EBC and Cr-RBC ($r_S = 0.57$, $p < 0.05$) • Cr–U and Cr-RBC at the beginning of shift ($r_S = 0.86$, $p < 0.01$)	21 Stability Cr(VI)-EBC unknown Small sample size	Goldoni et al. (2010)

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Table 5 (*continued*)

Industry	Study Population country N° workers N° controls N° companies	Biomonitoring data - directly exposed workers Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	Key findings exposure assessment	LaKind scoring strengths and/or weaknesses	Reference
		P_{25} : 0.8, P_{75} : 3.2, P_{100} : 6.1) ● Cr–P: *3.0* μg/l (P_0 : 1.4, P_{25} : 2.0, P_{75} : 3.4, P_{100} : 5.3) ● Cr-RBC: *3.4* μg/l (P_0 : 1.2, P_{25} : 2.0, P_{75} : 3.8, P_{100} : 5.8) ● Cr–U: *2.8* μg/g creat. (P_0 : 1.3, P_{25} : 2.3, P_{75} : 3.3, P_{100} : 5.5) ca. *3.8* μg/l ca. (P_0 : 1.8, P_{25} : 3.1, P_{75} : 4.5, P_{100} : 7.5) Post-shift at end of working week ● Cr(VI)-EBC: *0.5* μg/l (P_0: nd, P_{25} : 0.3, P_{75} : 3.6, P_{100} : 10.1) ● Cr-EBC: *2.2* μg/l (P_0 : 1.0, P_{25} : 1.3, P_{75} : 7.7, P_{100} : 21.2) ● Cr–U: *2.4* μg/g creat. (P_0 : 1.0, P_{25} : 1.8, P_{75} : 4.3, P_{100} : 7.5) ca. *3.3* μg/l ca. (P_0 : 1.4, P_{25} : 2.4, P_{75} : 5.8, P_{100} : 10.2)	• Cr-EBC and Cr(VI)-EBC at the beginning (r_S = 0.91, $p < 0.01$) and end ($r = 0.94$, $p < 0.01$) of the working shift		
	Egypt 41 workers 41 controls 1 company	**All chromium workers (n=41):** ● Cr–S: *3.30* μg/l (P_0 : 0.09, P_{100} : 7.20) **High-exposure group (n=27):** ● Cr–S: *3.90* μg/l (P_0 : 0.09, P_{100} : 7.20)	High-exposure group: • 34.1% of the workers wore PPE. Their Cr–S ranged from 0.13 to 4.40 with a median of 2.05 μg/l 65.9% of the workers didn't wear PPE. Their Cr–S ranged from 0.09 to 7.20 with a median of 3.9 μg/l.	23 No exclusion of contamination	El Safty et al. (2018)
	China 157 workers 93 controls 20 companies	● Cr-RBC: *4.41* μg/l (P_0 : 0.93, P_{100} : 14.98)	Cr-RBC in smokers were significantly higher than that in non-smokers ($P < 0.05$).	18	Zhang et al. (2011)
Industry	**Study Population** country N° workers N° controls N° companies	**Biomonitoring data - directly exposed workers** Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	**Key findings exposure assessment**	**LaKind scoring** strengths and/or weaknesses	**Reference**
Electroplating	Great Britain 354 workers 152 controls 53 companies	**All chromium workers (n=354):** ● Cr–U: 1.2 ± 1.2 μg/g creat. ca. 1.7 ± 1.7 μg/l *1.1* μg/g creat. (P_{90}: 4.9) ca. *1.5* μg/l ca. (P_{90}: 6.6) **High-exposure group (n=180):** ● Cr–U: 1.6 ± 1.3 μg/g creat. ca. 2.1 ± 1.8 μg/l *1.5* μg/g creat. (P_{90}: 6.0) ca. *2.0* μg/l ca. (P_{90}: 8.1)	Significant positive correlations between: • Cr–U and Cr-Hand for chromium electroplaters ($r = 0.71$, $P < 0.0001$) • Cr–U and Cr-Hand for all directly exposed chromium workers ($r = 0.63$, $P < 0.0001$) • Cr–U and inhalable Cr(VI)-Air exposure for chromium workers ($r = 0.62$, $P < 0.0001$) • Cr–U and inhalable Cr-Air exposure for chromium workers ($r = 0.52$, $P = 0.03$) High-exposure group: • chromium electroplaters	16 EQA ILC	Beattie et al. (2017)
	Taiwan 105 workers 125 controls 16 companies	● Cr–U: 2.3 ± 1.8 μg/g creat. ca. 3.1 ± 2.4 μg/l ● Cr-Hair: 7.2 ± 4.7 μg/g ● Cr-Fingernails: 12.7 ± 4.5 μg/g	Significant positive correlations between: • Cr–U and Cr-Air • Cr–U and Cr(VI)-Air • Cr–U and Cr-Hair • Cr–U and Cr-Fingernails	21	Pan et al. (2018)
	China 162 workers 87 controls Several companies	● Cr–B: *6.37* μg/l (P_0: 0.04, P_{100}: 58.92) ● Cr–U: *1.66* μg/l (P_0: 0.42, P_{100}: 94.27) ca. *1.02* μg/g creat. ca. (P_0: 0.26, P_{100}: 57.83)	Significant positive correlation between: • Cr–U and Cr–B ($r_S = 0.211$, $P < 0.01$) High-exposure group: • Two subgroups were identified using Cr–B and Cr–U. The cut-off values were 6.37 μg/l for Cr–B and 1.61 μg/l for Cr–U.	21	Xia et al. (2019)
Industry	Study Population country N° workers N° controls N° companies	Biomonitoring data - directly exposed workers Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	Key findings exposure assessment	LaKind scoring strengths and/or weaknesses	Reference
Electroplating	China 66 workers	● Cr–B: *7.81* μg/l (P_{25}: 4.69, P_{75}: 16.66)	Cr–U, exposure duration, and age were the major risk factors	20	Jia et al. (2020)

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Table 5 (*continued*)

Industry	Study Population country N° workers N° controls N° companies	Biomonitoring data - directly exposed workers Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	Key findings exposure assessment	LaKind scoring strengths and/or weaknesses	Reference
	66 controls Several companies	● Cr–U: *6*.87 μg/l (P_{25}: 2.33, P_{75}: 14.47) ca. *5*.05 μg/g creat. ca. (P_{25}: 1.71, P_{75}: 10.64)			
	Brazil 50 workers 50 controls 2 companies	● Cr–B: 2.02 ± 0.20 μg/l ● Cr–U: 10.65 ± 5.26 μg/g creat. ca. 14.48 ± 7.15 μg/l	Although internal exposure levels are below the exposure level recommended by ACGIH, a significantly increase in the effect biomarkers was observed.	21	Muller et al. (2020)
Mixed	Great Britain 58 workers 22 controls Several companies	● Cr(III)-EBC: *0*.54 μg/l (P_0 : 0.14, P_{90}: 4.21, P_{100} : 11.03) ● Cr(VI)-EBC: *0*.72 μg/l (P_0 : 0.01, P_{90}: 2.45, P_{100} : 27.3) ● Cr–U: *1*.5 μg/g creat. (P_0 : 0.3, P_{90}: 6.3, P_{100} : 17.1) ca. *2*.1 μg/l ca. (P_0 : 0.4, P_{90}: 8.6, P_{100} : 23.2)	Weak moderate correlation between: • post working week Cr–U and Cr(VI)-EBC (r = 0.33, p = 0.01)	16 EQA	Leese et al. (2017)
Aviation industry (assembling)	Italy 43 workers 23 controls 1 company (small & large hangar)	● Cr–U: 3.14 ± 0.69 μg/l ca. 2.31 ± 0.51 μg/g creat.	Pre-shift Cr–U at the beginning of the working week were higher than references from controls.	18	Genovese et al. (2015)
Printing	Thailand 75 workers 75 controls 16 companies	Cr–S: 1.24 ± 1.13 μg/l (P_0 : 0.1, P_{100} : 4.21) Cr–U: 6.86 ± 1.93 μg/g creat. (P_0 : 0.1, P_{100} : 9.5) ca. 9.33 ± 2.62 μg/l ca. (P_0 : 0.1, P_{100} : 12.9)	Significant positive correlations between: • Cr-Air of the breathing zone and Cr–U (r = 0.247, p = 0.032) • Cr-Air of the breathing zone and Cr–S (r = 0.166, p = 0.158)	19 ILC Spot morning urine	Decharat (2015)
Chromate production	China 100 workers 80 controls 1 company	● Cr–B: *15*.68 μg/l	Significant positive correlation between: • Cr–B and Cr-Air of the workplaces (r = 0.568, P < 0.001)	19 data is only presented graphically	Song et al. (2012)
Industry	**Study Population** country N° workers N° controls N° companies	**Biomonitoring data - directly exposed workers** Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	**Key findings exposure assessment**	**LaKind scoring** strengths and/or weaknesses	**Reference**
Chromate production	China 87 workers 30 controls 1 company	● Cr–B: 8.5 ± 1.3 μg/l *6*.4 μg/l (IQR: 7.2)	High-exposure group: • Two subgroups were identified using Cr–B. The cut-off value was 9.10 μg/l.	20 No exclusion of contamination	Hu et al. (2018)
	China 115 $workers_{2006}$ 60 $controls_{2006}$ 63 $workers_{2008}$ 45 $controls_{2008}$ 84 $workers_{2011}$ 30 $controls_{2011}$ 2 companies	● $Cr–B_{2006}$: *15*.6 μg/l (IQR: 13.7) ● $Cr–B_{2008}$: *7*.2 μg/l (IQR: 6.9) ● $Cr–B_{2011}$: *6*.6 μg/l (IQR: 6.8)	Significant positive correlation between: • Cr-Air of the workplaces and Cr–B in chromium exposed groups in the years 2006 and 2008 (r_{2006} = 0.60, r_{2008} = 0.35). High-exposure group: • Two subgroups were identified using Cr–B. The cut-off value was 20 μg/l.	20	Li et al. (2016)
	China 79 workers 112 controls 1 company 5 workshops	● Cr–B: 9.19 μg/l 6.90 μg/l (P_0: 1.17, P_{100}: 51.88) ● Cr–U: 17.03 μg/g creat. ca. 23.16 μg/l *12*.47 μg/g creat. (P_0: 2.78, P_{100}: 97.23) ca. *16*.96 μg/l ca. (P_0: 3.78, P_{100}: 123.23)	Significant positive correlation between: • Cr-Air of the breathing zone and Cr–B (r = 0.472, P < 0.001)	20 ILC	Xiaohua et al. (2012)
	China 115 workers 60 controls 1 company ** Extra contextual information and results reported in (Wang et al., 2011b) and (Wang et al., 2012)	● Cr-RBC: 12.45 ± 20.28 μg/l (P_0: 0.96, P_{100}: 115.01) ● Cr–U: 17.41 ± 14.67 μg/g creat. (P_0: 0.20, P_{100}: 83.30) ca. 23.68 ± 19.95 μg/l ca. (P_0: 0.27, P_{100}: 113.29) ● Cr–B: 23.49 μg/l *15*.52 μg/l (P_0: 5.95, P_{100}: 207.15)	Significant positive correlations between: • Cr-RBC and Cr–U in workers exposed to chromate (r = 0.205, p = 0.049) • Cr-Air of the workplaces and Cr–U in both workers and controls (r = 0.816, p < 0.000) • Cr-Air of the workplaces and Cr–B in both workers and controls (r = 0.842, p < 0.000) • Cr–U and Cr–B in both workers and controls (r = 0.824, p < 0.000)	19	Wang et al. (2011a)**

* Extra contextual information and results reported in (Pesch et al., 2015a).

Abbreviations: Cr-Air, Chromium level in air; Cr–B, Chromium level in whole blood; Cr-EBC, Chromium level in exhaled breath condensate; Cr(III)-EBC, trivalent chromium levels in exhaled breath condensate; Cr(VI)-EBC, hexavalent chromium levels in exhaled breath condensate; Cr-Fingernails, Chromium level in fingernails;

Cr-Hair, Chromium level in hair; Cr-Hand, Chromium level on hands; Cr–P, Chromium level in plasma; Cr-RBC, Chromium level in red blood cells; Cr–S, Chromium level in serum; Cr-Surface, Chromium level on surface; Cr–U, Chromium level in urine; Creat., creatinine; EQA, external quality assurance; ILC, interlaboratory comparison; IQR, interquartile range; N°, number of; P_0, 0^{th} percentile; P_{25}, 25th percentile; P_{75}, 75th percentile; P_{90}, 90th percentile; P_{100}, 100th percentile; r_s, Spearman's rank correlation coefficient; SD, standard deviation;

used less than 2 exposure markers and 2 did not fit within the age limit. Finally, 25 studies were included in this review. An overview of the steps in the literature search is given in Fig. 1.

3.2.1. Data extraction

Table 5 shows the studies included in this review. More information about other extracted data is provided in the Supplementary Material S5–S10. Twenty out of twenty-five studies report a total amount of Cr in urine for the biomonitoring of Cr(VI) exposure. The other five studies report a total amount of Cr in serum (El Safty et al., 2018), red blood cells (RBC) (Zhang et al., 2011) or whole blood (Li et al., 2016; Hu et al., 2018; Song et al., 2012). The different biomarkers (including sampling times) used in each study are summarized in Supplementary Material S5. Since the first elimination half-live of Cr(VI) in urine is relatively short (7–8h), urine samples are mostly collected at the end of the work shift. The presented results focus on post-shift urine samples (when this data is available) to allow comparison between studies. In order to compare creatinine-adjusted and non-adjusted urinary Cr concentrations, a typical creatinine urine concentration of 1.36 g creatinine/l is assumed (Cocker et al., 2011). An approximated urinary concentration is indicated by *circa* (i.e. 'ca.').

3.2.2. Reported biomonitoring levels across industries

As mentioned before, 25 studies were included in this review. Two out of these 25 studies, namely Were et al. (2013) and Leese et al. (2017), reported exposure levels for more than one industrial setting. Were et al. (2013) was performed in the welding, paint manufacturing and leather tanning industry. Leese et al. (2017) reported exposure levels for workers potentially exposed to occupational Cr(VI) compounds in various occupational settings. No detailed information about the number of workers recruited from each occupational setting was given by the authors. Therefore, the industrial setting in Leese et al. (2017) is considered as one 'mixed' occupational setting. Overall, the HBM studies included in this review have been performed in chromate production industries (n = 5), welding (n = 6), electroplating (n = 8), leather tanning (n = 4), paint manufacturing (n = 1), assembling (n = 1), printing (n = 1) and mixed occupational settings (n = 1). More details of the type of industry (e.g. welding on stainless steel or mild steel, hard or decorative chrome plating) is provided in the Supplementary Material S9 (Column "Study Characteristics"). Eight studies (Pesch et al., 2018; Stanislawska et al., 2020; Weiss et al., 2013; Riccelli et al., 2018; Goldoni et al., 2010; Beattie et al., 2017; Leese et al., 2017; Genovese et al., 2015) were performed in European countries, twelve studies (Golbabaei et al., 2012; Balachandar et al., 2010; Pan et al., 2018; Zhang et al., 2011; Xia et al., 2019; Jia et al., 2020; Song et al., 2012; Decharat, 2015; Hu et al., 2018; Li et al., 2016; Xiaohua et al., 2012; Wang et al., 2011a) in Asian countries, four studies (Were et al., 2013, 2014; Abdel Rasoul et al., 2017; El Safty et al., 2018) in African countries and one study (Muller et al., 2020) in a South-American country. Overall, the reported median or mean urinary Cr levels (20 out of 25 articles) were lower in European countries (ranging from 0.96 μg/l to ca. 5.81 μg/l) compared to non-European countries (ranging from 1.66 μg/l to 48.4 μg/l). More information about the published current and recent urinary Cr levels (creatinine-corrected and -uncorrected) in relation to the limit values is provided in Fig. 2. This figure shows that the studies included in this review reported a wide range of exposure levels (Fig. 2A and B). The reported exposure levels varies across countries (Fig. 2C and D) and across industries (Fig. 2E and F).

3.2.3. Reported biomonitoring levels across studies within each industry

3.2.3.1. Welding (n = 6). Four of the studies in the welding industry were conducted in European countries, more specifically in Germany (Weiss et al., 2013; Pesch et al., 2018), in Italy (Riccelli et al., 2018) and in Poland (Stanislawska et al., 2020). Riccelli et al. (2018) and Pesch et al. (2018) report urinary Cr levels that were in reasonable agreement. The median urinary Cr levels were respectively 0.96 μg/l and ca. 1.01 μg/l. Weiss et al. (2013) observed slightly higher urinary Cr levels with a median of 1.2 μg/l and a 75th percentile of 3.61 μg/l. The other European study (Stanislawska et al., 2020) reported higher Cr levels with a median value of 5.18 μg/l compared to the three European studies discussed before.

The non-European studies were performed in Iran (Golbabaei et al., 2012) and Kenya (Were et al., 2013). Golbabaei et al. (2012) observed a similar range of urinary Cr levels as those reported by Stanislawska et al. (2020). Were et al. (2013) reported a higher mean urinary Cr level (ca. 33.6 μg/l).

3.2.3.2. Electroplating (n = 8). Two of the studies in the electroplating industry were conducted in European countries, more specifically in Great Britain (Beattie et al., 2017) and in Italy (Goldoni et al., 2010), and report similar ranges of urinary Cr levels, despite the small sample size (n = 14) used in Goldoni et al. (2010) that may hamper the interpretation of the results. The median urinary Cr levels were respectively ca. 2.0 μg/l and 3.3 μg/l, with maximum levels up to ca. 10.2 μg/l.

The other 6 studies in the electroplating industry were performed in China (Xia et al., 2019; Zhang et al., 2011; Jia et al., 2020), Taiwan (Pan et al., 2018), Brazil (Muller et al., 2020) and Egypt (El Safty et al., 2018). Out of these 6 non-European studies, four studies (Pan et al., 2018; Muller et al., 2020; Xia et al., 2019; Jia et al., 2020) report a total amount of Cr in urine for the biomonitoring of Cr(VI) exposure. The other two non-European studies report a total amount of Cr in serum (El Safty et al., 2018) and RBC (Zhang et al., 2011). The median (or mean) urinary Cr levels ranged from 1.66 to ca. 14.48 μg/l. For Cr levels in serum, El Safty et al. (2018) observed a median value of 3.30 μg/l. For Cr levels in RBC, Zhang et al. (2011) observed a median value of 4.41 μg/l.

3.2.3.3. Leather tanning (n = 4). In the leather tanning industry, Cr(III) salts are mainly used as tanning agent (China et al., 2020). Under certain conditions (e.g. oxidizing agents, high T, pH) Cr (III) can oxidise to Cr (VI) (Zhao and Chen, 2019). The studies in the leather tanning industry are only included in this review if the authors explicitly indicated the presence of Cr(VI) exposure in the workplace (e.g. environmental air Cr (VI) measurements or raw products containing Cr(VI)). This is an inclusion criterion for all studies in this review.

All four studies in the leather tanning industry were performed in non-European countries, namely in Kenya (Were et al., 2013, 2014), in India (Balachandar et al., 2010) and in Egypt (Abdel Rasoul et al., 2017). Were et al. reported in 2013 and in 2014 high urinary Cr levels for the leather tanning industry in Kenya, respectively ca. 47.9 ± 16.5 μg/l and ca. 48.4 ± 10.1 μg/l. The two other studies, namely Balachandar et al. (2010) and Abdel Rasoul et al. (2017), report about 20 times lower urinary Cr levels.

3.2.3.4. Chromate production (n = 5). All five studies in the chromate production industry (Song et al., 2012; Hu et al., 2018; Li et al., 2016; Xiaohua et al., 2012; Wang et al., 2011a) were performed in China and report a total amount of Cr in whole blood for biomonitoring of Cr(VI) exposure. Out of these, one study (Wang et al., 2011a) used whole blood

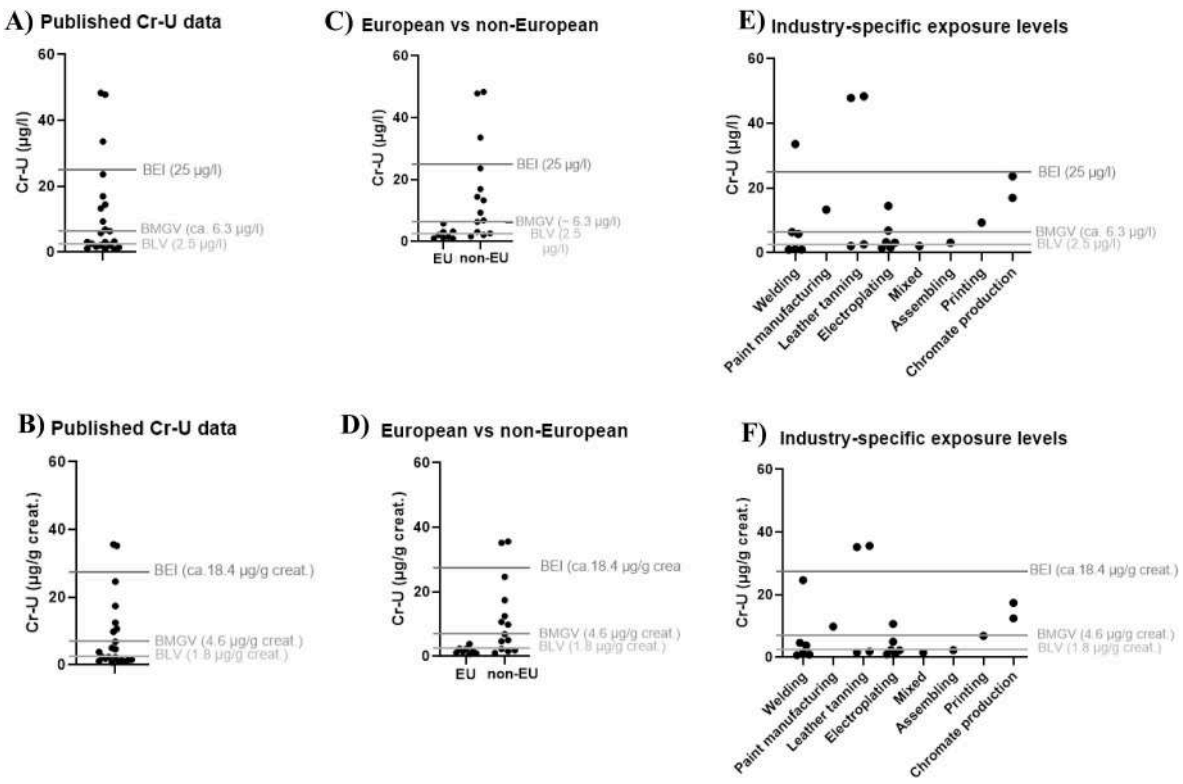

Fig. 2. Current and recent exposure levels in relation to the following occupational limit values: (i) Biological Limit Value (BLV = 2.5 µg/l or 1.8 µg/g creat.) (ANSES, 2017), (ii) Biological Monitoring Guidance Value (BMGV = ca. 6.3 µg/l or 4.6 µg/g creat.) (HSE, 2020), (iii) Biological Exposure Index (BEI = 25 µg/l or ca. 18.4 µg/g creat.) (ACGIH, 2020). Urinary Cr data (20 out of 25 articles) is shown as median or mean.

in combination with urine and RBC as exposure matrices and another study (Xiaohua et al., 2012) reported biomonitoring data for Cr whole blood and urine. The median Cr blood levels ranged from 6.4 to 15.68 µg/l. For Cr levels in RBC, Wang et al. (2011a) observed an average value of 12.45 µg/l with a standard deviation of 20.28 µg/l. For urinary Cr levels, Wang et al. (2011a) and Xiaohua et al. (2012) reported average levels of 17.41 µg/g creatinine and 17.03 µg/g creatinine.

3.2.4. Correlations between exposure markers

Studies included in this review observed significant positive correlations between exposure markers for occupational Cr(VI) exposure, as shown in Table 5 (Column "Key findings exposure assessment"). Different rules of thumb exist for interpreting the size of a correlation coefficient (SAGE, 2012; Mukaka, 2012; Overholser, Sowinski, 2008; Schober et al., 2018). The following rule of thumb is used in this review (SAGE, 2012):

- weak correlation: $-0.3 <$ correlation coefficient $< +0.3$
- moderate correlation: $-0.7 <$ correlation coefficient < -0.3 or $+0.3 <$ correlation coefficient $< +0.7$
- strong correlation: correlation coefficient < -0.7 or $+0.7 <$ correlation coefficient

3.2.4.1. Correlations between an external and an internal exposure marker. Nine studies (Pesch et al., 2018; Stanislawska et al., 2020; Weiss et al., 2013; Golbabaei et al., 2012; Abdel Rasoul et al., 2017; Decharat, 2015; Beattie et al., 2017; Were et al., 2013, 2014) observed weak to strong correlations between personal air Cr levels and urinary Cr levels. Pan et al. (2018) and Wang et al. (2011a) observed a correlation between ambient air Cr levels and urinary Cr levels as shown in Table 6a. Pan et al. (2018) did not report a value for the correlation and is therefore excluded from this table.

Four studies (Pesch et al., 2018; Stanislawska et al., 2020; Beattie et al., 2017; Pan et al., 2018) reported a correlation between air Cr(VI) levels and urinary Cr levels (Table 6b). Out of these, one study (Pan et al., 2018) used stationary air sampling and the others used personal air sampling. The correlations between airborne Cr or Cr(VI) levels and urinary Cr levels indicate that exposures to Cr(VI) may occur via inhalation. Pan et al. (2018) did not report a value for the correlation and is therefore excluded from this table.

Other studies also indicated the importance of the inhalation route by observing a correlation between air Cr levels and blood samples (Table 6c). Four studies (Song et al., 2012; Li et al., 2016; Wang et al., 2011a; Xiaohua et al., 2012) observed moderate to strong correlations between air Cr levels and Cr levels in whole blood. Out of these, one study (Xiaohua et al., 2012) used personal air sampling and the others used stationary air sampling. Three studies (Stanislawska et al., 2020; Abdel Rasoul et al., 2017; Decharat, 2015) observed moderate to strong correlations between personal air Cr levels and Cr levels in serum. Stanislawska et al. (2020) also observed a moderate correlation ($r_S = 0.67$) between personal air Cr(VI) levels and Cr levels in serum.

Beattie et al. (2017) observed a strong correlation between personal air Cr levels and urinary Cr levels ($r = 0.52$) and between Cr levels on the hands (dermal contamination) and urinary Cr levels ($r = 0.63$). This observation indicates that exposures to Cr(VI) may occur via a combination of inhalation, dermal and ingestion routes.

3.2.4.2. Correlations between internal exposure markers. Correlations were observed between urinary Cr at the end of the working week and the following exposure biomarkers: Cr(VI) levels in exhaled breath condensate (EBC) (Leese et al., 2017 [$r = 0.33$]), Cr levels in blood (Wang et al., 2011a [$r = 0.824$]; Xia et al., 2019 [$r_S = 0.211$]), Cr levels in erythrocytes (Wang et al., 2011a [$r = 0.205$]), plasma levels (Goldoni et al., 2010 [$r_s = 0.77$]) and Cr levels in hair and fingernails (Pan et al., 2018). Furthermore, Goldoni et al. (2010) observed strong correlations

Table 6a
Interpreting the size of correlation coefficient between air Cr levels and urinary Cr levels.

Size of correlation	Study: reported correlation	Industrial setting	Country	Type of air sample	Airborne dust fraction
Strong	Were et al. (2014): $R^2 = 0.76$	Leather tanning	Kenya	Personal	Inhalable
	Were et al. (2013): $r = 0.86$	Welding, paint manufacturing and leather tanning	Kenya	Personal	Inhalable
	Wang et al. (2011a): $r = 0.816$	Chromate production	China	Ambient	NM
Moderate	Stanislawska et al. (2020): $r_s = 0.59$	Welding	Poland	Personal	Inhalable
	Weiss et al. (2013): $r = 0.61$	Welding	Germany	Personal	Respirable
	Golbabaei et al. (2012): $R_p^2 = 0.481$	Welding	Iran	Personal	Inhalable
	Beattie et al. (2017): $r = 0.52$	Electroplating	Great Britain	Personal	Inhalable
	Pesch et al. (2018): $r_s = 0.44$	Welding	Germany	Personal	Respirable
	Abdel Rasoul et al. (2017): $\beta = 0.435$	Leather tanning	Egypt	Personal	NM
Weak	Decharat (2015): $r = 0.247$	Printing	Thailand	Personal	Inhalable

Abbreviation: NM: not mentioned.

Table 6b
Interpreting the size of correlation coefficient between air Cr(VI) levels and urinary Cr levels.

Size of correlation	Study: reported correlation	Industrial setting	Country	Type of air sample	Airborne dust fraction
Moderate	Stanislawska et al. (2020): $r_S = 0.58$	Welding	Poland	Personal	Inhalable
	Beattie et al. (2017): $r = 0.62$	Electroplating	Great Britain	Personal	Inhalable
Weak	Pesch et al. (2018): $r_S = 0.25$	Welding	Germany	Personal	Respirable

Abbreviation: NM: not mentioned.

between Cr levels in EBC and Cr levels in RBC [$r_S = 0.57$] and between Cr levels in EBC and Cr(VI) levels in EBC [$r = 0.94$].

3.2.5. *Quality scoring*

Supplementary Material S11 displays the quality scoring according to the adapted LaKind criteria for occupational exposure to Cr(VI). As mentioned before, each assessment component is assessed by giving a score from 1 (Tier 1 = highest quality) to 3 (Tier 3 = lowest quality). The justification for each classification is provided in Table 5 and in the Supplementary Material (S5–S10). Overall, the studies with the highest quality are those of Pesch et al. (2018), Weiss et al. (2013), Beattie et al. (2017) and Leese et al. (2017).

The adapted LaKind criteria are used in this review to consider the following quality issues:

- **Population size**

Small sample sizes may hamper the interpretation of the results. Only 5 studies (Balachandar et al., 2010; Goldoni et al., 2010; El Safty et al., 2018; Genovese et al., 2015; Were et al., 2014) recruited less than 50 occupationally exposed individuals (Tier-2 categorization). Three of these 4 studies had a sample size between 40 and 50 (cut-off value). One study (Goldoni et al., 2010) had a sample size of only 14 workers. The other studies were categorized as Tier 1 (≥50 occupationally exposed individuals). The justification for these classifications can be found in Table 5 (column "Study Population").

- **Study participants**

Another important criterion related to the study design of a HBM study is the selection of the study participants. Inclusion or exclusion criteria and recruitment strategies may influence the final biomonitoring results of the HBM study and may lead to selection bias. Only 3 studies (Beattie et al., 2017; Genovese et al., 2015; Goldoni et al., 2010) used surveillance measurements through an unbiased selection protocol with a high response rate (Tier-1 categorization). The remaining studies used a method of sample selection through considering exclusion criteria or through conduction special purpose or risk measurements (Tier-3 categorization). The justification for these classifications can be found in Supplementary Material S11.

- **Biomarker specificity and biomarker stability**

Although the measurement of Cr in urine is not specific for Cr(VI) exposure (Tier-3 categorization), the total amount of Cr in urine is the most used biomarker in this review (20 out of 25 studies). In contrast, Cr (VI) in EBC is considered a specific biomarker of Cr(VI) exposure (Tier-1 categorization). Three studies (Riccelli et al., 2018; Goldoni et al., 2010; Leese et al., 2017) report exposure levels for this specific biomarker. One of these three studies, namely Leese et al. (2017), documented the stability issues related to Cr(VI) in EBC (Tier-1 categorization). The justification for these classifications can be found in Supplementary Material S7 (column "Method characteristics - Stability history").

Another specific biomarker is Cr in RBC. This is considered as a specific biomarker, because Cr(VI) can easily enter the RBC through a nonspecific anion channel (EPA, 1998). In contrast, Cr(III) is taken up by the RBC with a very low efficiency (EPA, 1998). Five studies (Weiss et al., 2013; Goldoni et al., 2010; Zhang et al., 2011; Wang et al., 2011a;

Table 6c
Interpreting the size of correlation coefficient between airborne Cr levels and Cr levels in blood.

Size of correlation	Study: reported correlation	Industrial setting	Country	Type of air sample	Airborne dust fraction	Blood fraction
Strong	Wang et al. (2011a): $r = 0.842$	Chromate production	China	Ambient	NM	Whole blood
Moderate	Song et al. (2012): $r = 0.568$	Chromate production	China	Ambient	NM	Whole blood
	Li et al. (2016): $r_{2006} = 0.60/r_{2006} = 0.35$	Chromate production	China	Ambient	Inhalable	Whole blood
	Xiaohua et al. (2012): $r = 0.472$	Chromate production	China	Personal	NM	Whole blood
	Stanislawska et al. (2020): $r_S = 0.68$	Welding	Poland	Personal	Inhalable	Serum
	Abdel Rasoul et al. (2017): $\beta = 0.339$	Leather tanning	Egypt	Personal	NM	Serum
Weak	Decharat (2015): $r = 0.166$	Printing	Thailand	Personal	Inhalable	Serum

Abbreviation: NM: not mentioned.

Stanislawska et al., 2020) report levels for this specific biomarker (Tier-1 categorization). The justification for these classifications can be found in Supplementary Material S5 (column "Exposure matrix").

- **Technique and method sensitivity**

All the studies included in this review reported an acceptable LOQ (below 1 μg/l) and were therefore categorized as a Tier-1 study for the LaKind criteria method sensitivity. Only 4 studies mentioned how the authors calculated the LOD or LOQ. The justification for these classifications can be found in Supplementary Material S7 (columns "Technique" and "Method characteristics: LOD, LOQ).

- **Sample contamination**

A Tier-3 categorization might not always be a problem depending on the user's intent for the study data (e.g. not reporting background levels when the user is interested in correlations between exposure markers), but it would indicate a study of low utility in some cases such as the issue of contamination (LaKind et al., 2014). Low Cr levels in biological matrices must be interpreted with caution due to the ubiquitous presence of Cr (ATSDR, 2012). Twenty-one out of the twenty-five studies included in this review documented procedures to avoid contamination of the samples (Tier-1 categorization) while the other 4 studies (Riccelli et al., 2018; Balachandar et al., 2010; Abdel Rasoul et al., 2017; El Safty et al., 2018) did not report these procedures (Tier-2 categorization). The justification for these classifications can be found in Supplementary Material S7 (column "Method characteristics - Contamination-free").

- **Quality assurance**

Eleven studies (Abdel Rasoul et al., 2017; Balachandar et al., 2010; El Safty et al., 2018; Golbabaei et al., 2012; Hu et al., 2018; Li et al., 2016; Muller et al., 2020; Pan et al., 2018; Riccelli et al., 2018; Wang et al., 2011a; Zhang et al., 2011) did not use nor report procedures for quality assurance (Tier-3 categorization). Five studies (Pesch et al., 2018; Stanislawska et al., 2020; Weiss et al., 2013; Beattie et al., 2017; Leese et al., 2017) participated in an external quality scheme and five studies (Xiaohua et al., 2012; Were et al., 2013, 2014; Decharat, 2015; Beattie et al., 2017) participated in an interlaboratory comparison (Tier-1 categorization). The remaining studies used internal quality control (Tier-2 categorization). The justification for these classifications can be found in Supplementary Material S7 (column "Quality assurance").

- **Matrix adjustment, study records and background levels**

When restricted to the studies using Cr in urine for the biomonitoring of Cr(VI) exposure (n = 20), only 2 studies (Pesch et al., 2018; Weiss et al., 2013) include results for adjusted and non-adjusted urinary Cr concentrations (Tier-1 categorization). This might hamper the comparison of the biomonitoring results between studies. The justification for these classifications can be found in Supplementary Material S5 (column "Matrix adjustment and sampling strategy").

To help interpret the biomonitoring levels, detailed information about the subject's job and specific tasks in combination with exposure measurements might help. Only 5 studies (Pesch et al., 2018; Weiss et al., 2013; Were et al., 2013, 2014; Beattie et al., 2017) reported sufficient (according to OECD guidelines) study records (Tier-1 categorization). The justification for these classifications can be found in Supplementary Material S6 (columns "Task with higher exposure/variables influencing exposure" and "RMMs in place/to be applied") and Supplementary Material S9 (column "Study characteristics").

Reporting of background levels might also help to interpret the biomonitoring levels. Only 4 studies (Li et al., 2016; Beattie et al., 2017; Leese et al., 2017; Genovese et al., 2015) reported background levels for controls included in the study population and reference values for the general population, if available (Tier-1 categorization). The justification for these classifications can be found in Supplementary Material S7 (columns "Biomonitoring data - Controls" and "Reference values - General population").

4. Discussion

The goal of this review was fourfold: (1) to assess current and recent biomonitoring levels in workers occupationally exposed to Cr(VI) with a focus on urinary Cr levels at the end of a working week, (2) to identify variables influencing these biomonitoring levels, (3) to identify how urinary Cr levels correlate with other Cr(VI) exposure markers and (4) to identify gaps in the current research. Each of these goals is discussed in turn. Furthermore, the quality of the unpublished and the published biomonitoring dataset is discussed.

4.1. Current and recent biomonitoring levels in workers' occupational exposure to Cr(VI)

Although assessment of total urinary Cr is not specific for Cr(VI) exposure, it is the traditional biomarker for routine biomonitoring of occupational exposure to Cr(VI). The results of the analysis of the unpublished biomonitoring data from Belgian workers showed a decreasing time trend of 30% in urinary Cr levels between 1998 and 2018. Longer time span would increase the robustness of the time trend estimation. Analysis of the median or mean urinary Cr data reported in literature (20 out of 25 articles) from workers occupationally exposed to Cr(VI) showed that 3 median (or mean) values exceeded the BEI of 25 μg/l, recommended by ACGIH and 10 median (or mean) values exceeded the BMGV of ca. 6.3 μg/l, recommended by HSE. All of these 10 studies were performed in non-European countries. With regard to the most stringent BLV of 2.5 μg/l set in France and the Netherlands, 3 European and 11 non-European median (or mean) exposure levels exceeded this limit value. More information about the published current and recent urinary Cr levels (creatinine-corrected and -uncorrected) in relation to the limit values was already shown in Fig. 2.

The studies included in this review reported a wide range of exposure levels, namely in various countries and industries. This resulted in a heterogeneous biomonitoring dataset. Therefore there is a need for more measurement campaigns to establish current exposure levels in these industrial settings and countries.

4.2. Variability of biomonitoring levels

4.2.1. Variability of reported biomonitoring levels across industries

Overall, the reported median or mean urinary Cr levels (20 out of 25 articles) were lower in European countries (ranging from 0.96 μg/l to ca. 5.81 μg/l) compared to non-European countries (ranging from 1.66 μg/l to 48.4 μg/l). This also applies for the other internal exposure levels.

4.2.2. Variability of reported biomonitoring levels across studies within each industry

4.2.2.1. Variability of reported biomonitoring levels across studies (n = 6) within welding industry

4.2.2.1.1. European studies (n = 4). Riccelli et al. (2018) and Pesch et al. (2018) report urinary Cr levels for welders that were in reasonable agreement. The median urinary Cr levels were respectively 0.96 μg/l and ca. 1.01 μg/l. On one hand, Weiss et al. (2013) observed similar a median urinary Cr level, namely 1.2 μg/l. On the other hand, Weiss et al. (2013) observed higher urinary Cr levels for a sub-population of welders and reported a 75th percentile of 3.61 μg/l for urinary Cr levels. The higher levels detected in Weiss et al. (2013) could be explained by the different welding techniques in combination with the type of welding material included in the study population. Riccelli et al. (2018)

investigated the exposure to Cr(VI) in 100 stainless steel tungsten inert gas (TIG) welders and Pesch et al. (2018) in a study population consisting of 50 welders using TIG, gas metal arc welding (GMAW) and other welding techniques with GMAW predominantly applied to mild steel and TIG to stainless steel. Weiss et al. (2013) investigated a larger study population (n = 241), who used a larger variety of welding techniques. The welders used GMAW, TIG, shielded metal arc welding (SMAW), flux-cored arc welding (FCAW) and miscellaneous welding techniques during the shift. When restricted to stainless steel, Weiss et al. (2013) observed the following pattern for urinary Cr levels: FCAW > GMAW > SMAW > TIG. More information about the welding techniques and the type of welding material is provided in the Supplemental Information S9 (Column "Study Characteristics").

The other European study (Stanislawska et al., 2020) reported higher Cr levels with a median value of 5.18 μg/l compared to the three European studies discussed before. An explanation for the higher urinary Cr levels might be the higher air Cr levels observed in Stanislawska et al. (2020) in combination with the efficacy of local exhaust ventilation (LEV) and the use of respiratory protection equipment (RPE). Stanislawska et al. (2020) did not report anything about the efficiently use of LEV and no RPE was available for the welders, while the other European studies were equipped with efficiently LEV, a range of RPE was used and workers were aware of the importance of these preventive measures. More information about the air levels and the presence of RMM (LEV and RPE) is provided respectively in Supplementary Material S10 and in Supplementary Material S6.

4.2.2.1.2. Non-European studies (n = 2). Golbabaei et al. (2012) observed a similar range of urinary Cr levels as those reported by the European study of Stanislawska et al. (2020). There was a lack of RPE in both studies (Supplementary material S6, column "RMMs in place/to be applied"). Similar to the European study of Weiss et al. (2013), Golbabaei et al. (2012) identified welding in confined spaces as a high-exposure scenario for welders.

Were et al. (2013) reported a higher mean urinary Cr level (ca. 33.6 μg/l). A plausible explanation for these higher Cr levels compared to the Cr levels reported in the previously described studies, is the lack of PPE in combination with inadequate working conditions, no training and inadequate personal hygiene (Supplementary material S6, column "RMMs in place/to be applied").

4.2.2.2. Variability of reported biomonitoring levels across studies (n = 8) within electroplating industry

4.2.2.2.1. European studies (n = 2). Beattie et al. (2017) and Goldoni et al. (2010) report similar ranges of urinary Cr levels, despite the small sample size (n = 14) used in Goldoni et al. (2010) that may hamper the interpretation of the results. The median urinary Cr levels were respectively ca. 2.0 μg/l and 3.3 μg/l, with maximum levels up to ca. 10.2 μg/l.

4.2.2.2.2. Non-European studies (n = 6). Out of thee 6 non-European studies conducted in the electroplating industry, four studies (Pan et al., 2018; Muller et al., 2020; Xia et al., 2019; Jia et al., 2020) report a total amount of Cr in urine for the biomonitoring of Cr(VI) exposure. The other two non-European studies report a total amount of Cr in serum (El Safty et al., 2018) and RBC (Zhang et al., 2011). The median (or mean) urinary Cr levels ranged from 1.66 to ca. 14.48 μg/l.

The influence of PPE on exposure to Cr(VI) in the electroplating industry is a plausible explanation for the observed variance in the Cr levels reported in the studies included in this review. El Safty et al. (2018) observed that not wearing PPE led to an almost doubling of the median serum level. Also Beattie et al. (2017) indicated the importance of PPE for electroplaters. Sampling was carried out at three time points over the duration of that study. Additional urine samples were requested approximately 6 and 12 months after feedback had been provided from the initial visit. A reduction of 23% was observed in the urinary Cr levels of Cr electroplaters working in a subset of companies, characterized by control deficiencies. Increased risk awareness, including the use of PPE to minimize dermal exposure, throughout the study has contributed to the decreasing trend in urinary Cr levels. The urinary Cr levels reported by Beattie et al. (2017) are lower than those reported by Pan et al. (2018) (ca. 2.1 ± 1.8 μg/l vs ca. 3.1 ± 2.4 μg/l). A smaller fraction of workers (only 14.3%) used PPE in the study of Pan et al. (2018) compared to Beattie et al. (2017). Although PPE were made available to the workers in the study of Muller et al. (2020), they reported high urinary Cr levels (ca. 14.48 ± 7.15 μg/l). The authors (Muller et al., 2020) indicated that these workers did not use the available PPE. Zhang et al. (2011) reported Cr levels in RBC up to 14.98 μg/l with a median of 4.41 μg/l 52% of the workers in this study were provided with masks. More workers used gloves (95%) and protective clothes (63%). A plausible explanation for these higher Cr levels in RBC is the high environmental air Cr levels with a median of 60 μg/m^3 observed in this study, compared to other studies. The environmental data are provided in the Supplementary Material S10. With the protection of gloves and protective clothes, inhalation is the main exposure pathway in the lack of (efficient) RPE. More information about PPE is provided in the Supplementary Material S6, column "RMMs in place/to be applied".

No information was reported by Goldoni et al. (2010), Xia et al. (2019) and Jia et al. (2020) about RMMs or variables influencing exposure.

4.2.2.3. Variability of reported biomonitoring levels across studies (n = 4) within leather tanning industry

4.2.2.3.1. Non-European studies (n = 4). Were et al. reported in 2013 and in 2014 high urinary Cr levels for the leather tanning industry in Kenya, respectively ca. 47.9 ± 16.5 μg/l and ca. 48.4 ± 10.1 μg/l. Both studies were characterized by inadequate working conditions (Supplementary Material S6, column "RMMs in place/to be applied") leading to high mean urinary levels. The authors (Were et al., 2014) indicated the lack of environmental policies and enforcement of legislation in developing countries as the cause of inadequate working conditions and consequently higher urinary Cr levels. The two other studies, namely Balachandar et al. (2010) and Abdel Rasoul et al. (2017), report about 20 times lower urinary Cr levels. Also the personal Cr air levels in Abdel Rasoul et al. (2017) were 3–7 times lower compared to Were et al. (2013, 2014) . The environmental data are provided in the Supplementary Material S10. A plausible explanation for the discrepancy in reported urinary Cr levels in the leather tanning industry might be the significant positive correlation between Cr levels in the air of the breathing zone and urinary Cr levels reported by Were et al. (2013), Were et al. (2014) and Abdel Rasoul et al. (2017), in combination with the lack of RPE (Supplementary Material S6, column "RMMs in place/to be applied").

Balachandar et al. (2010) did not report information about the lack or presence of correlations between urinary and air Cr levels and used stationary air sampling instead of personal air sampling.

4.2.2.4. Variability of reported biomonitoring levels across studies (n = 5) within chromate production industry

4.2.2.4.1. Non-European studies (n = 5). All five studies in the chromate production industry report a total amount of Cr in whole blood for biomonitoring of Cr(VI) exposure. Out of these, one study (Wang et al., 2011a) used whole blood in combination with urine and RBC as exposure matrices and another study (Xiaohua et al., 2012) reported biomonitoring data for Cr whole blood and urine. The median Cr blood levels ranged from 6.4 to 15.68 μg/l. Although at least 90% of the workers was provided with gloves and masks in all these studies (no information about the effectiveness, suitability, and use of the PPEs was given by the authors), these studies reported remarkably higher Cr levels in the biological matrices of workers (and controls) compared to other European occupational studies in literature. Namely, Gil et al. (2011) observed a median value of 0.78 μg/l with a 95th percentile of 4.04 μg/l

for Cr levels in whole blood of 278 workers in the Italian iron and steel industry and Julander et al. (2014) reported a median blood Cr concentration of 1.4 μg/l with a maximum value of 5.0 μg/l for recycling workers in the Swedish e-waste industry. For residents of areas with a high density of industry, Bonberg et al. (2017) observed a median Cr blood level of 1.51 μg/l with a 95th percentile of 2.09 μg/l in 2821 residents of the German Ruhr area. Three other studies included in this review (Xia et al., 2019; Jia et al., 2020; Muller et al., 2020) also used whole blood for biomonitoring of exposure to Cr(VI) and were performed in the electroplating industry. Similar to the five studies in the chromate production industry, Xia et al. (2019) and Jia et al. (2020) also reported higher median values of 6.37 μg/l and 7.81 μg/l for workers in the Chinese electroplating industry. No information about the provision or use of PPEs was given by the authors of both studies. Muller et al. (2020) observed a lower mean blood Cr level of 2.02 μg/l for Cr electroplaters in Brazilian electroplating industry. As mentioned before, 2 out of 5 studies (Wang et al., 2011a; Xiaohua et al., 2012) conducted in the chromate production industry used whole blood in combination with another biological exposure matrices. The reported Cr levels for these matrices were also remarkably higher compared to other European occupational studies included in this review. Wang et al. (2011a) observed an average value of 12.45 μg/l with a standard deviation of 20.28 μg/l for Cr levels in RBC, while European occupational studies included in this review reported median levels of 3.4 μg/l, 0.09 μg/g Hb and 1.95 μg/l for respectively Italian Electroplating industry, Polish and German welding industry (Goldoni et al., 2010; Stanislawska et al., 2020; Weiss et al., 2013). Furthermore, Wang et al. (2011a) and Xiaohua et al. (2012) reported average urinary Cr levels of 17.41 μg/g creatinine and 17.03 μg/g creatinine, while lower median urinary Cr levels of 2.8 μg/g creatinine, 3.81 μg/g creatinine and <1.35 μg/g creatinine were reported by respectively Goldoni et al. (2010), Stanislawska et al. (2020) and Weiss et al. (2013).

In addition to biomonitoring data of Cr levels in whole blood for directly exposed workers in the Chinese chromate production industry, the 5 studies that used whole blood as exposure matrix also reported biomonitoring data for a control group. The background levels of controls ranged from 2.9 to 3.9 μg/l (Supplementary material S9, column "Biomonitoring data Controls") and also these values are remarkably higher compared to European reference values reported in literature. Minoia et al. (1990) observed a mean blood Cr level of 0.23 μg/l with a maximum value of 0.75 μg/l in 519 Italians subjects, Llobet et al. (1998) reported a mean value of 0.2 μg/l with a maximum value of 1.1 μg/l in 144 Spain subjects and Nisse et al. (2017) observed a mean value of 0.60 μg/l with a 95th percentile of 1.26 μg/l in 1130 French subjects. For the general population in China, Ding et al. (2012) observed a median value of 1.19 μg/l with a 95th percentile of 5.59 μg/l.

Depending on how well-controlled the occupational exposure is within the factory and on the selection criteria of the controls, elevated Cr levels for both workers and controls might be caused by (pre-) analytical factors, occupational exposure and environmental exposure. Each of these factors are briefly considered in turn:

- Though (pre-)analytical factors (e.g. needles, blood stoppers, anti-coagulants/additives) may be a contribution factor to the elevated Cr Levels in whole blood (Wood et al., 2010; Penny and Overgaard, 2010; Hodnett et al., 2012), exogenous contamination from Cr in pre-analytical factors was not always observed in literature (Bro et al., 1988; Sommer et al., 2021). Nevertheless, they all recommend to consider precautionary procedures (e.g. contamination control, testing sampling protocol, storage stability) to minimize or eliminate these sources of errors. One of the studies in this review with elevated Cr levels for controls, namely Song et al. (2012), indicated the difference in anti-coagulants as possible explanation for the elevated blood Cr levels in controls. Hu et al. (2018) used the same protocol as Song et al. (2012). More information about the method characteristics is provided in the Supplementary Material S7.
- Four studies (Wang et al., 2011a; Xiaohua et al., 2012; Li et al., 2016; Song et al., 2012) selected controls (farmers, salesmen, …) outside (>20 km) the factory. Hu et al. (2018) included controls from the administrative personnel. The authors explicitly indicated that the controls were not (directly) exposed to Cr. Therefore, it is unlikely that occupational exposure is the major relative contributing factor for the elevated background levels in all these 5 studies. More information about the selection of the controls is provided in the Supplementary Material S9 (column "Study characteristics").
- With respect to the relative contribution of environmental exposure, the median air Cr levels for controls ranged from 0.06 to 0.2 μg/m^3 in these 5 studies (Supplementary Material S10 - Environmental monitoring data). The environmental airborne Cr levels reported in these studies were higher than airborne Cr levels reported in European studies. The following ranges are reported in EU: 0–0.003 μg/m^3 (remote areas), 0.004–0.07 μg/m^3 (urban areas) and 0.005–0.2 μg/m^3 (industrial areas) (WHO, 2000). Concerning the environment policy of China, Gao and Xia (2011) indicated the presence of strict environmental regulations on Cr wastes and the presence of historical Cr contamination in some areas. Zhao et al. (2020) indicated that environmental Cr(VI) contamination in northeast China has been ongoing for over 60 years and they observed elevated urinary Cr levels with a median of 1.28 μg/l in 134 residents living in three Cr polluted villages. Therefore, environmental exposure might be a contributing factor for the elevated Cr levels in these studies.

4.2.3. *Variables influencing current and recent urinary Cr levels*

Repeating biological monitoring over time could drive sustainable improvements in exposure control, as suggested by the linear mixed effect model results of the unpublished urinary Cr data. Improved working conditions, better exposure control, feedback to workers and increased risk awareness over years (Beattie et al., 2017; Xiaohua et al., 2012; Li et al., 2016; El Safty et al., 2018; Riccelli et al., 2018) were all reported by authors of the included review articles to have decreased Cr levels in biological matrices.

Worker or workplace variability might also account for the variability in urinary Cr levels. The linear mixed effect model applied at sub-major ISCO levels (three-digit) showed that about 15% of the total variance in Cr data was explained by the variance between jobs (three-digit ISCO) and the industries. In the literature review, significant associations were found between urinary Cr levels and job characteristics such as work tasks and work duration (Decharat, 2015; Were et al., 2014). Some of the studies (Weiss et al., 2013; Pesch et al., 2018; Genovese et al., 2015; Decharat, 2015; Were et al., 2013) assessed for this review, included a (sub)group which multitasked a combination of different processes or (welding) techniques. Therefore, it was not always possible to assign workers to specific work tasks. Moreover, urinary Cr levels can also be affected by other individual variables such as hobbies, diet and individual capacity to reduce Cr(VI) (NIOSH, 2013).

The identification of high-exposure groups in the literature review showed the importance of the hierarchy of controls in different occupational settings to reduce exposure levels (El Safty et al., 2018; Golbabaei et al., 2012; Beattie et al., 2017). This highlights the need for (re) new(ed) sector-specific information, instruction and training targeted to this issue. Furthermore, one German (Weiss et al., 2013) and one Iranian study (Golbabaei et al., 2012) showed that welding in confined spaces is indicative of Cr(VI) exposure. If workers weld in confined spaces, airborne Cr(VI) levels can easily accumulate in the absence of precautionary measures. Currently, there is no specific limit value for welding in confined spaces, only a general limit value for welding fumes. Therefore, more attention is needed for welding in confined spaces.

4.3. *Correlation of urinary Cr levels with other Cr(VI) exposure markers*

The main limitation of the traditional biomonitoring method used for biomonitoring of exposure to Cr(V), namely urinary Cr levels, is that it

may overestimate the exposure to Cr(VI) since it measure the exposure to both Cr(III) and Cr(VI). Correlations between airborne Cr(VI) levels, Cr(VI) levels in EBC, Cr levels in RBC and urinary Cr levels allow to study the reduction of Cr(VI) to Cr(III) in the body. If the use of specific biomarkers is validated and correlations between these specific biomarkers and urinary Cr exist, the internal exposure to Cr(VI) may be calculated from urinary Cr levels.

4.3.1. Correlation of urinary Cr levels with air levels

The correlations between airborne Cr or Cr(VI) levels and urinary Cr levels indicate that exposures to Cr(VI) may occur via inhalation. Were et al. (2013) conducted two measurement campaigns one year apart and observed that personal air Cr levels were strongly correlated with urinary Cr levels in both of these (R^2 = 0.86 and 0.76 for the first and second campaign, respectively). A plausible explanation for these strong correlations might be the lack of RPE in combination with inadequate working conditions (Supplementary Material S6, column "RMMs in place/to be applied"). Wang et al. (2011a) also indicated inhalation exposure as a significant contributor to urinary levels, with a strong correlation (r = 0.816) being observed between Cr air levels of the workplaces and urinary levels in both workers and controls. Weak to moderate correlations between urinary and personal air Cr levels were observed by Pesch et al. (2018), Weiss et al. (2013), Golbabaei et al. (2012), Abdel Rasoul et al. (2017), Decharat (2015) and Beattie et al. (2017). Pan et al. (2018) observed a positive correlation between urinary and ambient air Cr levels. The association between Cr levels in the air and Cr levels in urine may be influenced by the physical and chemical properties of the inhaled Cr(VI) compounds. Properties such as size and solubility affect the behaviour of Cr(VI) deposited in the respiratory tract (Cena et al., 2014a, 2014b; Brand et al., 2013). These properties will probably account for the differences in absorption, retention and excretion. Other factors influencing these correlations might be individual variability of the workers, varying occupational settings, methodological and sampling factors (Balachandar et al., 2010).

Only three studies reported a correlation between Cr(VI) levels in air of the breathing zone and Cr levels in urine. Pesch et al. (2018) observed a weak correlation (r_S = 0.25) between respirable Cr(VI) levels and urinary levels for welders. Beattie et al. (2017) and Stanislawska et al. (2020) found a moderate correlation (r = 0.62 and r = 0.58) between inhalable Cr(VI) levels and urinary levels for chrome electroplaters. These differences in reported correlations are to be expected because of differences between the three studies in the type of exposure (welding vs electroplating vs "mixed"), the accompanying difference in physical and chemical properties of the inhaled Cr(VI) compounds and the different type of measurement to characterize the Cr(VI) levels in the air of the breathing zone (respirable vs inhalable). Furthermore, the differences in reported correlations may be due to the inaccuracies of assessing exposure to Cr(VI) resulting from the instability of Cr(VI) such as the difficulties in sampling and analysis of Cr(VI) (Unceta et al., 2010; KHADEM et al., 2017).

As mentioned before, total urinary Cr is the most common biomarker for routine biomonitoring of occupational exposure to Cr(VI). A validated equation showing a correlation between urinary Cr levels and airborne Cr(VI) levels allows a possible conversion of urinary Cr levels into corresponding airborne Cr(VI) levels. Such equations reported in literature are mainly derived from Cr plating activities (Lindberg and Vesterberg, 1983; Tola et al., 1977). The applicability of these equations to other exposure scenarios is questionable. For example, stainless steel welders may be exposed to different oxidation states of Cr. This is supported by Pesch et al. (2018) who concluded that airborne Cr(VI) levels cannot be precisely estimated from urinary Cr levels. Furthermore, the possibility to transform urinary Cr levels into airborne Cr(VI) levels depend on the quality of these equations and the experimental conditions (sampling time of urine sample, sampling duration of air measurements, number of workers, …). These equations are often derived from occupational studies with a limited number of workers and various experimental conditions such as collecting urine samples at the end of the 2nd working day (Lindberg and Vesterberg, 1983) or at the end of the working week (Tola et al., 1977).

4.3.2. Correlation of urinary Cr levels with other exposure biomarkers

Five studies included in this review observed correlations between urinary Cr at the end of the working week and the following exposure biomarkers: Cr(VI) levels in EBC (n = 1), Cr levels in blood (n = 2), Cr levels in erythrocytes (n = 2), plasma levels (n = 1) and Cr levels in hair and fingernails (n = 1). 13 out of 25 studies only used 1 internal exposure matrix (Supplementary Material S5 (Column "Exposure Matrix").

Even though urinary Cr levels are not specific for occupational exposure to Cr(VI), the sampling and analysing process for urine Cr levels is simpler than the potentially specific biomarkers Cr in RBC and Cr(VI) in EBC. Therefore, total urinary Cr is often used for routine biomonitoring of occupational Cr(VI) exposure. As mentioned before, the possible existence of correlations between validated specific biomarkers and urinary Cr allow to convert urinary Cr levels into internal Cr(VI) exposure levels. Therefore further measurement campaigns need to consider multiple internal exposure matrices in order to study the reduction of Cr(VI) to Cr(III) in the human body and to assess correlation between the different exposure biomarkers.

4.4. Gaps in the current research

4.4.1. Specific biomarkers

As mentioned before, the main limitation of using urinary Cr levels as biomonitoring method is that it cannot differentiate the exposure to Cr(VI) from the exposure to Cr(III). Furthermore, the most stringent BLV of 2.5 μg/l, set in France and the Netherlands, is close to the background level of the general population (e.g. BRV = 0.65 μg/l). Therefore, there is a need to validate specific biomarkers for exposure to Cr(VI) such as Cr(VI) in EBC and Cr in RBC.

Three studies (Goldoni et al., 2010; Leese et al., 2017; Riccelli et al., 2018) reported exposure levels for Cr(VI) in EBC. All three studies were conducted in European countries. Overall, low Cr levels or low number of detects were reported, which is in line with environmental measures and risk awareness. In the study of Riccelli et al. (2018), Cr(VI) was never detected (LOD = 0.2 μg/l) in the EBC samples, which is in line with the type of welding (TIG welding) and the risk awareness of the workers. The median levels of Cr(VI) in EBC reported by Goldoni et al. (2010) and Leese et al. (2017) were 1.0 μg/l and 0.72 μg/l, respectively. Both studies consist of a (sub)group of Cr electroplaters, for whom higher exposure levels are reported in literature, compared to TIG welders in European countries. As mentioned before, Cr(VI)-containing products are used during electroplating processes, whereas Cr(VI) is formed as a by-product during welding processes. Therefore, it is likely to expect lower levels for welding processes.

Five studies (Goldoni et al., 2010; Zhang et al., 2011; Wang et al., 2011a; Weiss et al., 2013; Stanislawska et al., 2020) included in this review report levels for Cr in RBC. Three studies (Weiss et al., 2013; Goldoni et al., 2010: Stanislawska et al., 2020) were conducted in European countries. Weiss et al. (2013) reported low number of detects (15 out of 150; LOQ = 1.5 μg/l), which is in line with the type of occupational setting, namely welding, and risk awareness (Supplementary Material S9, column "Study Characteristics"). Stanislawska et al. (2020) also observed low levels for Cr in RBC of welders. Goldoni et al. (2010) reported higher Cr levels in RBC for Cr electroplaters, which is in line with the type of occupational setting. The non-European studies (Zhang et al., 2011; Wang et al., 2011a) reported elevated Cr levels in RBC for both workers and controls in the electroplating and chromate production industry (Supplementary Material S9, column "Biomonitoring data - Controls"). The discrepancy between elevated levels for both workers and controls may be due to (pre-)analytical factors, occupational and environmental exposure.

Although urinary Cr levels are not specific for Cr(VI) exposure, a

limited number of studies included in this review used specific biomarkers to assess exposure to Cr(VI). In addition, these studies were conducted in distinct industries (welding, electroplating, chromate production and "mixed"). The difference in exposure scenarios limits the possibility to aggregate the biomonitoring data of the specific biomarkers and to make a general conclusion about the use of specific biomarkers. Nevertheless, the use of total urinary Cr is supposed to be appropriate in work tasks in which Cr(VI) is used as start-product and in which co-exposure to other oxidation states of Cr is negligible, like chrome plating. This may not be the case in work tasks in which co-exposure to different oxidation states of Cr may occur, like stainless steel welding. Therefore there is a need for further measurement campaigns to assess the appropriateness of specific biomarkers in distinct industries and tasks.

4.4.2. Occupational settings

From the literature review it is apparent that the available data do not report all the occupational settings where exposure to Cr(VI) can occur, such as wood preserving and spraying of Cr(VI)-containing paints. Furthermore, exposure to Cr(VI) is not limited solely to workers operating directly in areas of the workplace where Cr processes occur (Leese et al., 2017; Beattie et al., 2017; Balachandar et al., 2010; Genovese et al., 2015). Therefore there is a need for further measurement campaigns to establish current exposure levels in these settings (including the use of specific biomarkers) and to compare these with relevant limit values.

4.4.3. Exposure routes

Goldoni et al. (2010), Were et al. (2014), Beattie et al. (2017) and Leese et al. (2017) indicated that exposures to Cr(VI) may occur via a combination of inhalation, dermal and ingestion routes. The measurement of Cr in urine captures total exposure by all these routes. As mentioned before, 9 studies observed weak to strong correlations between personal air Cr levels and urinary Cr levels. Out of these 9 studies, only one study (Beattie et al., 2017) investigated the correlation between dermal and urinary Cr levels. Further investigation of the contribution of the different exposure routes is needed, so that better guidance on the use of control measures can be provided.

4.5. Quality of unpublished biomonitoring dataset

A low between-jobs variation of 15% can be related to the grouping strategy of jobs utilised in exposure assessment. When exposure is linked to individual ISCO codes, the workers merged under the same class are assumed to have the same level of exposure which results in a heterogeneous classification. This is an innate limitation of utilisation of ISCO classification system in exposure assessment studies. In order to obtain exposure classification closer to real life scenarios, tasks can be taken into consideration (Pesch et al., 2015a). Increasing resolution of ISCO codes would also provide more homogenous classification with regards to occupation. Nevertheless, this approach results in decrease in sample size per 4-digit ISCO code as study sample is allocated to a greater number of job classes (Sauvé et al., 2020).

The main limitation of our binary expert assessment method is the absence of validity and interrater reliability evaluation. Validity refers to how likely exposure categorization corresponds to real exposure classification and is statistically evaluated by sensitivity and specificity analysis estimated from gold standard. Reliability refers to the consistency of agreement among raters (assessors) or methods. The extent of the agreement is evaluated by the Kappa statistics (K). An association between K, sensitivity and specificity exists and true prevalence of exposure is further needed to calculate sensitivity and specificity. Testing for the credibility of an exposure assessment method is crucial as errors in exposure groupings generate bias in odds ratios or regression coefficients in studies investigating the link between exposure and disease. Validity assessment have been study of interest in occupational epidemiology because gold standard or information on actual prevalence of exposure is often lacking. In order to tackle the issue of missing validity information, Burstyn et al. has proposed a simulation based method to calculate the sensitivity and specificity by using K and exposure prevalence obtained through different exposure assessment methods (Burstyn et al., 2013). However, since a reliability analysis was not applied to our expert assessment approach, we were not able to determine validity through this approach. On the other hand, chromium exposure decisions of experts are based on risk assessment of the workplace they are responsible for and on periodical examinations of workers, thus assumed to be reflective of a first-hand knowledge of the worksite involved.

4.6. Quality of published biomonitoring dataset

In this review, adapted LaKind scoring criteria for exposure to Cr(VI) are used to consider study quality issues in a systematic way. The LaKind score gives an indication of the overall quality of the study. As mentioned before, a Tier-3 categorization might not always be problematic depending on the user's intent for the study data (e.g. not reporting background levels when the user is interested in correlations between exposure markers). However, it would indicate a study of low utility in some cases (e.g. inability to demonstrate samples were free of contamination). Hence only considering the overall LaKind score is insufficient to differentiate the quality of studies.

The quality assessment of the studies included in this review is based on the information in the article (and any supplemental information provided). The consequence of this is that if a study participated in an external quality scheme and did not report this, it would be categorized as Tier-3 instead of Tier-1. Only 4 studies (Hu et al., 2018; Pesch et al., 2018; Pan et al., 2018; Jia et al., 2020) included in this review provided supplemental information which was needed for a better interpretation of the results. Therefore, it is recommended that authors publishing biomonitoring data make more use of journals' supplemental information options to provide additional essential information, in order to allow a more thorough interpretation of biomonitoring results.

Besides insufficient reporting, there are other factors affecting the direct comparison and interpretation of data published in literature, such as different study characteristics (target population), different sampling years, different sampling strategies (morning, spot, 8h and 24h), different units (creatinine-adjusted or non-adjusted urinary Cr concentrations), different statistical parameters and analysis (mean, median, 25th-75th percentiles, range, LOD definition, representation of values below LOD, …). Therefore, this review reinforces the call (Berman et al., 2017; Latshaw et al., 2017; Kromerová and Bencko, 2019; Nakayama et al., 2019; Scholten et al., 2020; Fréry et al., 2020) for more harmonization in conducting HBM in future research and highlights the importance of the recent and current efforts (e.g. IPCHEM, DEMOCOPHES, COPHES, HBM4EU) to harmonize biomonitoring data across Europe. The chromates study protocol (Santonen et al., 2019) developed under HBM4EU presents harmonized methodologies for the collection and analysis of occupational hygiene and HBM samples and this protocol will contribute undoubtedly to the harmonization of biomonitoring data on occupational exposure to Cr(VI).

5. Conclusion

Due to required authorization of Cr(VI) compounds and the potential binding limit value for Cr(VI) compounds, it is important to gather existing occupational biomonitoring data concerning Cr(VI). We combined the findings from a linear mixed effect model, applied on unpublished biomonitoring data and a systematic literature review to investigate the Cr(VI) exposure levels. Specifically, our research focused on urinary biomonitoring data. Overall, the results showed a decreasing time trend in urinary Cr levels and reinforce the importance of preventive measures such as control the use of PPE, increased risk

awareness, improved working conditions, controlling Cr(VI) air levels at the workplace and minimizing exposure to Cr(VI) compounds during work shift (e.g. rotating jobs or maximum 8h shift duration). With regard to the most stringent BLV of 2.5 μg/l, which is close to the background level of the general population, set in France and the Netherlands, 3 European studies and 11 non-European studies exceeded this limit value. These results support the need for more specific Cr(VI) biomarkers. Furthermore, this review reinforces the call for more harmonization in conducting future HBM research. We identified the following gaps in current literature, which need to be further investigated: i) the available data do not report all the occupational settings where exposure to Cr(VI) can occur, ii) workers' exposure via dermal contact needs to be further investigated in occupational biomonitoring studies and iii) the specific biomarkers for Cr(VI) exposure were only used in a limited number of studies and need to be further validated in more occupational settings.

Disclaimer

The contents, including any opinions and/or conclusions expressed of this manuscript, are those of the authors alone and do not necessarily reflect the opinions or policy of the organisations to which they are employed.

Declaration of competing interest

No conflicts of interest are declared.

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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.2021.113799.

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The health effects of wearing facemasks on cardiopulmonary system of healthy young adults: A double-blinded, randomized crossover trial

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ABSTRACT

Background: Facemask had increasingly been utilized as a personal protective measure to reduce exposure to ambient particulate matter (PM) during heavily-polluted days and routine life. However, evidence on the potential effects on cardiovascular system by wearing particulate-filtering facemask was limited.

Methods: We conducted a double-blinded randomized crossover trial (RCT) to evaluate the effects of wearing N95 facemasks on the molecular responses of cardiopulmonary system among 52 healthy college students in Beijing, China. We measured cardiopulmonary health indicators and collected biological samples before and after (up to 5 h at multiple time points) a 2-h walk to examine the changes in lung function, biomarkers of respiratory and systemic oxidative stress/inflammation. We applied linear mixed-effect models to evaluate the effect of the facemask-intervention on the health of cardio-pulmonary system.

Results: In the trial wearing real facemasks, FEV_1 increased by 2.05% (95% CI: 0.27%–3.87%), 2.80% (95% CI: 1.00%–4.63%), and 2.87% (95% CI: 1.07%–4.70%) at V1 (30-min), V2 (3-h), and V3 (5-h) after the 2-h walk outsides, respectively. Compared with participants wearing the sham mask, the percentage change of nitrate in EBC was lower among those wearing the real mask. After the 2-h exposure, urinary MDA levels increased compared to the baseline in both trials. Real trial was lower than sham trial for 6 cytokines (i.e., IL-6, IL-10, IL-13, IL-17A, IFN-γ and TNF-α) in serum at 5-h post-exposure. Wearing facemasks on polluted days produced better improvement, however, on cleaner days, the improvement was weaker.

Conclusions: Short-term use of N95 facemasks appeared to effectively reduce the levels of lung function declines, the respiratory oxidative stress, and the systemic inflammation/oxidative stress which may be induced by short-term exposure to PM. Wearing facemasks on polluted days ($PM_{2.5} > 75$ μg/m^3) presented larger beneficial effects on the cardiopulmonary health than in clean days ($PM_{2.5} < 75$ μg/m^3).

1. Introduction

Ambient particulate matter (PM) had been well recognized as one of the leading risk factors of human health (Cohen et al., 2017). Currently, the concentrations of $PM_{2.5}$ (particles with aerodynamic diameters less than 2.5 μm) in most regions of the world exceed the guideline of the World Health Organization, especially in developing countries and regions. Therefore, it is very common to carry out personal protections to reduce the PM exposure. Wearing facemasks had been widely recommended by academic researchers and the department of public health as one of the practical solutions to minimize the adverse effects of air pollution (Allen and Barn, 2020; Cai and He, 2016; Carlsten et al., 2020; Rajagopalan Sanjay et al., 2020). While much attention had been put on the particle-removing efficiency of facemasks, the health effects of using facemasks on the cardiopulmonary health have not been fully evaluated.

There was evidence that wearing a N95 facemask for a few hours to days in real-world condition might improve the cardiopulmonary health, particularly in highly polluted circumstances. However, the results were inconsistent across different studies and only a few health outcomes have been investigated (Faridi et al., 2021; Guan et al., 2018;

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Langrish et al, 2009, 2012; Laumbach et al., 2014; Shakya et al., 2016; Shi et al., 2017; Yang et al., 2018). For example, two studies reported that wearing N95 facemasks for 48 h (worn as much as possible) was associated with reduced systolic blood pressure (SBP) (Langrish et al., 2009; Shi et al., 2017), but another study found no effect on SBP by wearing N95 facemasks for five days and 4 h per day (Yang et al., 2018). Only one interventional study on traffic policemen found that wearing a N95 facemask could offset potential decline in lung function caused by ambient air pollution exposure (Shakya et al., 2016). More evidence is needed regarding the effects on lung function of wearing facemasks, as consistent research reported a significant association between PM and lung function.

Inflammation and oxidative stress have been well recognized as the mechanisms that particles affected cardiopulmonary health, but the effects of wearing facemasks on pulmonary or systemic inflammation and oxidative stress were not fully investigated. In a double-blinded randomized crossover study, researchers found that wearing N95 facemasks for 2 h during peak traffic resulted in reductions in exhaled nitric oxide, multiple inflammatory cytokines in exhaled breath condensate (EBC), but no clear beneficial effects on endothelial function or systemic oxidative stress (Guan et al., 2018). Little evidence was provided about the effect of facemask-wearing on the systemic inflammation or oxidative stress.

Besides the beneficial effects of wearing facemasks during pollution days, there were some concerns about the potential adverse stress induced by wearing a facemask on cardiopulmonary system (Rajagopalan Sanjay et al., 2020). On one side, the available data to date suggested that N95 facemasks can effectively reduce $PM_{2.5}$ exposure in a manner that translates into cardiopulmonary health benefits. On the other side, wearing facemasks has some problems, including inducing heat, poor adherence, CO_2 build-up, discomfort, and respiratory resistance, resulting in some harmful impacts on cardiopulmonary health (Huang and Morawska, 2019). Study participants rated the N95 as the most protective due to sturdiness and fit, but also as uncomfortable and difficult to breathe through (Steinle et al., 2018). A study found that the N95 filtering facepiece respirator (FFR) dead-space partial pressure carbon dioxide levels were elevated (Roberge et al., 2010). Carbon dioxide can build up for a long time and cause drowsiness (Johnson, 2016). In addition, arguments had been made that these masks might worsen overall exposure by engendering a false sense of security (Huang and Morawska, 2019). Therefore, more data on the health responses deriving from the short-term facemask wearing are desiderated.

To comprehensively understand the health effects of facemasks on cardiopulmonary system, we conducted a double-blinded, randomized crossover trial (RCT) in a group of healthy young adults in Beijing China. We investigated the differences in the respiratory inflammation and oxidative stress, lung function, systemic inflammation and oxidative stress responses between participants wearing real and sham facemasks.

2. Methods

2.1. Study design and participants

The study was conducted at the campus of Peking University (PKU) in Beijing, China from December 2018 to April 2019. Fifty-two healthy, non-smoking volunteers were enrolled into the study. All subjects declared that they had no history of alcohol addiction nor diagnosed chronic cardiopulmonary diseases (including asthma, bronchitis, and rhinitis, etc.). None of them was on regular medication usage nor had symptoms of upper airway infection within 3 months. The volunteers lived in dormitories of two universities which were within 5 km. Thirty-one volunteers were from University of Science and Technology Beijing (USTB) and the others were from Peking University (PKU). The study was approved by the Institutional Review Board of the Ethics Committee of Peking University Health Sciences Center (IRB00001052-18071), and registered on Chinese Clinical Trial Registry (ChiCTR1800018628). Written informed consent was obtained from each of the participants during the enrollment.

To ensure the double-blinded and crossover design, each participant was asked to attend the study twice, with one time randomized to wear a reusable facemask (Respirator 3200; 3M, USA) installing a N95 filter (real trial) or not (sham trial). In the second trial, they wore a facemask with the filter installment opposite to the first trial (Guan et al., 2018). The facemask we chose consisted of a replaceable filter (3701CN; 3M, USA) which made the double-blind design feasible, and its filtration efficiency (92% for $PM_{2.5}$) had been tested in another study which was sufficient for our purpose. The body of the facemask was composed of soft silicone padding and louver-type enclosure that did not affect ventilation. The real facemask embedded filter membrane in the middle whereas the real facemask did not. The participants were instructed on how to wear the facemask in order to make the facemasks fitted their faces closely and comfortably. The appearance of the respirator and the material of the filter membrane were recorded in the supplementary material (Supplementary material: Fig. S8).

Fig. 1 showed the scheme of our study design. The trial visits were conducted in the Hospital of PKU, which was located where we conducted the 2-h outdoor exposure experiment. During each visit, the participants first went to the clinic for baseline measurement and biological sample collection (V0). Then, our investigators led them to the campus to walk slowly on a pre-defined (on-campus) road for 2 h wearing the customized facemask (real or sham). After the walking exposure, subjects returned to the clinic, took off the facemasks, and underwent the following three tests in 30-min (V1), 3-h (V2), and 5-h (V3) after the exposure (Fig. 1). All the clinical visits were implemented in a quiet exam room in the Hospital of PKU, and participants were not allowed to leave the room until all four visits had been completed. After a 2-week washout duration, each subject needed to go through the same study procedure but wore the facemask opposite to the first trial.

2.2. Air pollution measurement

Darta for air pollution and meteorological parameters were obtained from the Peking University Urban Atmosphere Environment Monitoring Station during the study period. The monitoring station was located on the roof of a six-story building on the campus of PKU where we conducted the exposure study. The station provided 1-min online data of $PM_{2.5}$ (TEOM, Model 1400, Thermo), sulfur dioxide (SO_2) (Model 43i-TL; Thermo), temperature, and relative humidity (Met One Instruments Inc., Grants Pass, OR, USA).

2.3. Lung function measurement

Lung function was measured using a hand-held portable spirometer (Spirolab New, MIR, Italy) following the ATS recommendation (Graham et al., 2019). The lung function parameters of interest for this study were forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC). Participants were instructed to inhale to maximum capacity (total lung capacity) in the standing position with nose clips, and then to exhale as fast and as long as possible. Calibrated reusable turbines and disposable mouthpieces were used for each participant to get the required parameters. Each participants competed at least five tests. The reproducibility criterion was that the difference between the two highest values was $\leq$150 mL for FEV_1 and $\leq$150 mL for FVC. We calculated the average values of eligible results for statistical analysis.

2.4. Biological samples collection

Exhaled breath condensate (EBC) was collected at all the four visits by using a commercially available device from RTube™ (Respiratory Research, Inc., US) following the recommended instruction (Horváth et al., 2005). Prior to each clinical visit, the aluminum cooling sleeves

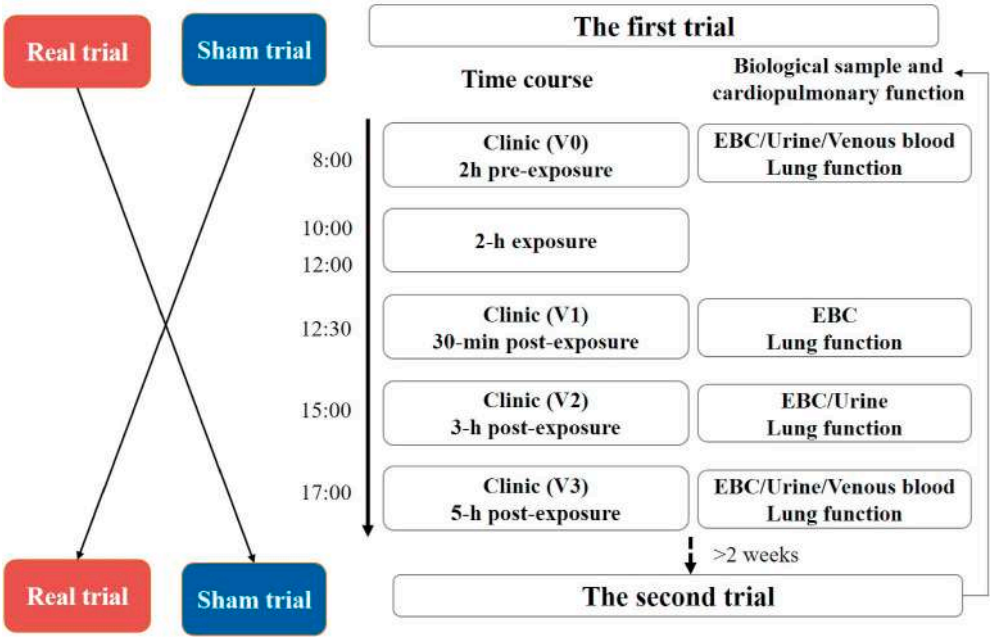

Fig. 1. Time course of health outcomes measurement.

were kept in a sealing bag and frozen in a −80 °C freezer for at least 2 h before transported to the hospital with dry ice in an insulation box. Subjects were asked to gargle for 3 times and wear nose clips before collection. During collection, the cooling sleeve was placed over the collection chamber and the subject was instructed to breath normally through the mouthpiece for 10 min. After each collection, around 1.5 mL EBC sample was collected using the plunger and sub-packed into centrifuge tubes instantly. At the start (V0) and the end (V3) of each trial, peripheral venous blood samples were drawn by a nurse using 6 mL evacuated and promoting coagulating tubes, and then centrifuged at 4000 r/min for 10 min to obtain serum samples after standing for 30-min. The serum samples were collected into 1.5 mL centrifuge tubes and stored at −80 °C within 30 min to minimize the in vitro changes. Urine samples (fractional) were collected in three times, i.e., V0, V2 and V3, and were stored at −80 °C for the future analysis.

2.5. Biomarkers analysis

EBC pH and nitrite/nitrate The pH value of EBC was measured on site using a pH meter (SevenCompact™, Mettler Toledo Inc.) after degassing the sample with argon stream. And the concentrations of nitrite in EBC were detected by an HPLC-UV (Model e2695, Waters, USA) with ION PAK Anion HC HPLC column (4.6*150 mm, WAT026770, Waters, US). Briefly, after the equilibration of the HPLC system with the Borate/Boric mobile phase (7.5 mM: 7.5 mM, pH 9.1), the concentrations of nitrite from the standards and the real EBC samples were detected at the wavelength of 214 nm by Photodiode Array (PDA) detector (Waters 2998, USA) with the flow rate of 0.8 mL/min. Besides, the concentrations of nitrate in EBC were determined by Ion Chromatography (Thermo Fisher, ICS-1100).

Urinary malondialdehyde (MDA) As the product of lipid peroxidation, we measured malondialdehyde from the urine samples as a biomarker of systemic oxidative stress. Malondialdehyde (MDA), released from its bound form(s) in urine by acid treatment, was measured as Thiobarbituric Acid derivative, using a High Performance Liquid Chromatography-UV detector (Model e2695, Waters, USA) according to a previously published paper (Lee et al., 2006). Briefly, 150 μL urine, 450 μL Thiobarbituric Acid (TBA) solutions, and 900 μL 0.5 mol/L phosphorous acid were added into a 1.5-mL centrifuge tube. The mixtures were incubated at 95 °C for 1-h, cooled in ice water for 5 min, followed by 5-min centrifugation (5000 g/min). The chromatographic column Nova-Pak (C18, 4 μm, 3.9*150 mm, Waters) was used to separate MDA in the mixtures, and the mobile phase was phosphate buffer (pH = 6.8) and methanol (60:40, V/V) with the flow rate of 0.8 mL/min. MDA was detected at the wavelength of 532 nm by Photodiode Array (PDA) detector (Waters 2998, USA). Concentrations of MDA were corrected by creatinine due to its highly potential influence by metabolism. Urinary creatinine levels were measured by a commercial kit (Jiancheng Bioengineering Institute, Nanjing, China).

Serum cytokines We used a commercially available analyzing kit (Human Cytokine/Chemokine Magnetic Bead Panel, Millipore Corporation, MA, USA) to detect 10 cytokines in serum samples (Knatten et al., 2014a). In short, 50 μl serum was firstly centrifuged at 13,000 g for 10 min. 25 μl of supernatant was collected to measure the concentrations of interleukin (IL)-1β, interleukin (IL)-2, interleukin (IL)-4, interleukin (IL)-6, interleukin (IL)-8, interleukin (IL)-10, interleukin (IL)-13, interleukin (IL)-17A, IFN-γ and TNF-α using Flex MAP 3D™ (Merck Millipore). Milliplex Analyst software (Merck Millipore, USA, version 5.1) was used to analyze median fluorescence intensity for each sample, and 3-parameter logistic regression and standard curve fitting methods were used to calculate the concentrations of cytokines in the samples (Hu et al., 2020; Knatten et al., 2014a). The limits of detection (LOD) for IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17A, IFN-γ and TNF-α were 0.8, 1.0, 4.5, 0.9, 0.4, 1.1, 1.3, 0.7, 0.8 and 0.7 pg/mL, respectively. For each cytokine, non-detectable values were replaced with half of the LOD. The detection rates for IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17A, IFN-γ and TNF-α were 10%, 15%, 70%, 59%, 99%, 98%, 94%, 99%, 99%. Due to the low detection rate for IL-1β and IL-2, they were excluded from the analysis.

2.6. Statistical analysis

We used linear mixed-effect model to examine the effects of N95 facemask on cardiopulmonary responses. The measured levels of all the health outcomes were logarithmic transformed due the skewed distribution. We used linear mixed-effects models to estimate the changes in health outcomes after the 2-h exposure at different post-exposure time points for two different trials (real and sham). We included in the model an interaction term between an indicator variable for wearing facemask ("1" for real mask and "0" for sham mask) and an indicator variable for

clinical visit ("V0", "V1", "V2", "V3"). Participants' age, sex, and body mass index were introduced as fixed effects. To account for the potential influence of clinical visit days, we also controlled for the 2-h average temperature and relative humidity. We included the urinary creatinine as a covariate when fitting the model for MDA to account for the dilution effect (Gong et al., 2013). Random intercepts for participants were included to account for the potential correlation in the repeated measurements of each participant. Based on the estimated coefficients of the interaction term, we calculated the percentage change (%) and 95% confidence intervals (CIs) from the baseline ("V0") at each of the following visit. We further evaluated the statistical significance of the difference, using the method of examining overlap, between the percentage (%) change at each visit in health outcome among those wearing real and sham masks (Schenker and Gentleman, 2001).

To examine whether the effects of wearing facemasks on cardiopulmonary responses depends on ambient air pollution, we classified all the person-visits into two trials based on the average concentration of $PM_{2.5}$ with a threshold of 75 μg/m^3, which is the 24-h mean concentration regulating level for $PM_{2.5}$ in China. We then conducted a sensitivity analysis using the same model to estimate the percent changes in health outcomes during polluted days ($PM_{2.5}$ > 75 μg/m^3) and clean days ($PM_{2.5}$ < 75 μg/m^3). All the analyses were conducted using R (Version 3.6.1; R Development Core Team).

3. Results

3.1. Characteristic information of the study participants

The characteristic information of the volunteers was shown in Table 1. We recruited 31 females and 21 males with a mean age of 20 ± 2 years. The overall average body mass index was 20 kg/m^2. According to the self-administrated questionnaire, all the participants remained healthy throughout the study period, and stayed within the central urban area of Beijing during the washout period. 48 out of the 52 participants completed two trials, and 4 participants finishing one trail (real trial). Therefore, we had 400 lung function measurements, 400 EBC samples, 300 urine samples, 200 blood samples.

3.2. Air pollution concentrations in all clinical visiting days

Fig. 2 showed the average air pollutant concentrations and meteorological parameters during the 2-h walks in all the clinic visiting days and in polluted and clean days. The average $PM_{2.5}$ concentrations were 75.8 μg/m^3 throughout the entire study period. The levels of $PM_{2.5}$ ranged from 4.9 to 254.8 μg/m^3. There were 8 days when the 2-h concentrations of $PM_{2.5}$ exceeded 75 μg/m^3 with an average of 127.7 ± 62.5 μg/m^3, and 9 days when the 2-h concentrations of $PM_{2.5}$ were below 75 μg/m^3 with an average of 21.70 ± 16.12 μg/m^3. During the whole study period, the mean environmental temperature and relative humidity were 9.84 °C and 26.14%, respectively, while in the 'polluted' days, the mean RH was 32.73% which was higher than that in the level of 'clean' days. The $PM_{2.5}$ and SO_2 concentrations 24 h before each visit were similar to those in the 2-h intervention-period (Supplementary material: Table S5). We conducted a paired *t*-test and found there was no significant differences in the concentrations of air pollutants between the 2-h period and the 24-h period in the previous day.

Table 1
Characteristics of the 52 study participants.

Gender	N	former smokers	Mean ± SD			
			Age (years)	Height (cm)	Weight (kg)	BMI(kg/m^2)
Male	21	1	19.71 ± 1.31	175.76 ± 4.65	67.33 ± 10.02	21.77 ± 2.95
Female	31	0	21.25 ± 2.34	162.23 ± 5.38	52.06 ± 6.43	19.75 ± 1.96
Total	52	1	20.63 ± 2.11	167.69 ± 8.39	58.23 ± 10.99	20.57 ± 2.58

3.3. Descriptive statistics of health outcomes

Table 2 showed the health endpoint levels of the four tests by different types of intervention, i.e., real trial versus sham. The trend of EBC nitrate was similar in both trials, increasing at 30-min post-exposure and then gradually decreasing to lower levels at 3-h, and 5-h post-exposure. Compared to the trial wearing sham facemasks, the mean concentration of nitrite was lower at 30-min, 3-h post-exposure in the trial of wearing real masks (120.37 vs 113.26, 172.62 vs 148.91, respectively). There were similar decreases at 5-h post-exposure in EBC pH values in two trials. There were slight increases in the levels of FVC and FEV_1 after 2-h walking. Compared to the baseline levels, the mean concentrations of serum IL-10, IL-13, IL-4, IL-17A became decreased at 5-h post-exposure in the trial wearing real masks, whereas the mean concentrations of those cytokines were higher in the trial wearing sham masks. The mean concentrations of IL-6, IL-8, TNF-α, IL-1β decreased at 5-h post-exposure in both trials.

3.4. Effects of facemasks on lung function

In the main analysis, we estimated the changes in each health endpoint after the 2-h walk at different post-exposure times by the intervention method (real vs sham). We found that FEV_1 was increased after the 2-h walk when wearing real facemasks, with increments by 2.05% (95%CI:0.27%–3.87%), 2.80% (95%CI:1.00%–4.63%) and 2.87% (95%CI: 1.07%–4.70%) at V1 (30-min), V2 (3-h) and V3 (5-h) after the exposure (Fig. 3). In contrast, there was no appreciable change in FEV_1 after the 2-h walk for the trial of wearing sham facemasks. It was notable that the increases in FEV_1 for the real-facemask trial at the three post-exposure times were larger than the changes for the sham one. Similar results were observed for FVC, i.e., FVC was increased from baseline by 2.40% (95%CI: 0.27%–4.58%), 3.21% (95%CI: 1.07%–5.4%) and 2.68% (95%CI: 0.55%–4.86%) in the real-facemask trial, whereas there were no notable differences in the percentage changes between the two trials at all the three post-exposure times.

We also performed the sensitivity analysis by dividing the observations into two trials, i.e., the 'polluted" days ($PM_{2.5}$ >75 μg/m^3) and the 'clean' days ($PM_{2.5}$ <75 μg/m^3). During 'clean' days, the improvements in FEV_1 were still appreciable at 30-min, 3-h, 5-h post-exposure in the trail wearing real facemasks, but the differences did not differ between two trials at 5-h post-exposure. (Supplementary material: Fig. S5). And during the 'polluted' days, there appeared to be different in the percentage changes in FVC between the two trials after the exposure (Supplementary material: Fig. S2).

3.5. Effects of facemasks on respiratory and systemic oxidative stress biomarkers

As shown in Fig. 4, EBC nitrate showed a downward trend at post-exposure compared to the baseline level in the trial wearing real facemasks, whereas EBC nitrate was gradually increased in the trial wearing sham facemasks. Differences in the changes of EBC nitrate were observed at 1-h post-exposure (−12.41%, 95%CI: −23.39% to −1.43%), 3-h post-exposure (−20.64%, 95%CI: −31.83% to −9.46%) and at 5-h post-exposure (−26.31%, 95%CI: −37.53% to −15.11%) between two trials. Regarding EBC nitrite and pH, there seemed no appreciable differences in their changes at three post-exposure times between the two trials (Fig. 4). After the 2-h exposure, the concentrations of urinary MDA increased compared to the baseline in both trials, but did not differ between the two trials (Fig. 4). During the 'polluted' days, we found that the increases in MDA in urinary from the baseline were higher in the real-facemask trial than the sham one at 3-h and 5-h post exposure

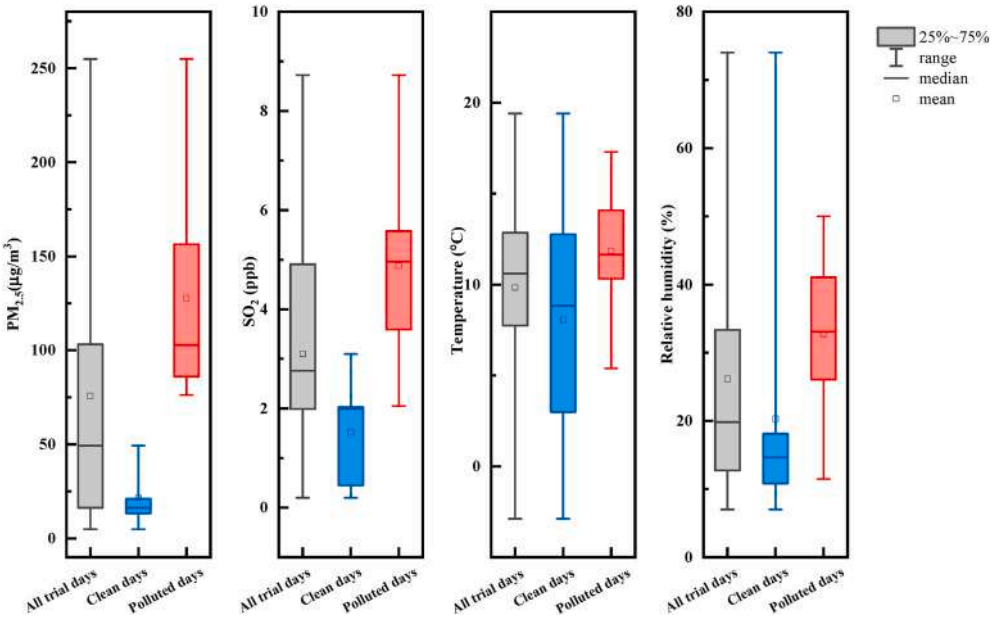

Fig. 2. Distribution of environmental $PM_{2.5}$, SO_2, temperature, and relative humidity during the 2-h exposure.

(Supplementary material: Fig. S3). And during the 'clean' days, the differences between the two trials were reversed, with the trial wearing sham facemasks showing higher increases from baseline (Supplementary material: Fig. S6).

3.6. Effects of facemasks on serum cytokines

At 5 h (V3) after the 2-h walk, we observed a clear decreasing trend in serum cytokines for the trial of wearing real facemasks, with eight of them showing reductions, namely, IL-6 (−18.96%, 95% CI: −34.28% to −0.06%), IL-8 (−7.08%, 95% CI: −16.31% to −3.17%), IL-10 (−27.61%, 95% CI: −37.30% to −16.43%), IL-13 (−12.29%, 95% CI: −22.11% to −1.22%), IL-17A (−17.12%, 95% CI: −23.17% to −10.6%) and TNF-α (−22.77%, 95% CI: −27.05% to −18.25%). In contrast, the levels of IL-13, IFN-γ for the group of wearing sham facemasks increased after the exposure. By comparing the changes in cytokines between the two trials, we found there were differences in percentage changes in 6 of the cytokines, including IL-6, IL-10, IL-13, IL-17A, IFN-γ and TNF-α (Fig. 5). In the sensitivity analysis of 'polluted' days, real trial was lower than sham trial for 6 cytokines (i.e., IL-6, IL-10, IL-13, IL-17A, TNF-α and IL-8) in serum at 5-h post-exposure and there was no notable difference of IFN-γ between two trials (Supplementary material: Table S2). The percentage change of IL-8 was higher in the trial wearing sham facemasks and there was no appreciable difference of IL-1β between two trials in the 'clean' days (Supplementary material: Table S3).

4. Discussion

A double-blinded randomized crossover trial was conducted to estimate the effects of wearing N95 facemasks on cardiopulmonary system of healthy young adults in China. After the 2-h walk wearing facemasks with real or sham filters, lung function parameters, and biomarkers for respiratory and systemic inflammation/oxidative stress have quadrupled from the baseline in 5-h after exposure. We observed differences in all the health outcomes between the two trials wearing sham and real facemasks.

We found notable increases in FEV_1 and FVC after 2-h walk wearing the real facemask, and differences in FEV_1 between the real and sham trials. Walking for 2 h with real facemasks was associated with an improvement in FEV_1 and FVC sustained up to 5 h. By contrast, in the trial wearing sham masks, there was no change in either parameter of lung function from the baseline. Although both trials experienced the beneficial effect of walking in terms of an improvement in FVC, the increase in the trial of wearing sham facemasks was lower than that in the real trial. This finding was consistent with another two RCT studies that were conducted in London. One reported attenuated increases in FEV_1 and FVC after walking along a busy street for 2 h compared to walking in the park for healthy elderly (Sinharay et al., 2018), and the other one reported reductions in FEV_1 by up to 6.1% and FVC by up to 5.4% after a 2-h walk on busy street (higher exposure), with reductions after walking in a park (lower exposure) smaller for the asthmatic subjects (McCreanor et al., 2007). In another interventional study, it was found that cyclists' FVC increased during riding in a low-traffic site (low level of air pollution), but decreased during riding in a high-traffic site (high level of air pollution) (Park et al., 2017). Another study conducted among children and adults demonstrated reducing indoor $PM_{2.5}$ may contribute to improved lung function by using an electrostatic air filter for 1 week (Weichenthal et al., 2013). The results mentioned above may suggest a beneficial effect on lung function induced by the reduction of particles exposed in a microenvironment, e.g., walking in a cleaner circumstance or using an indoor air purifier, while our study added more evidence to the beneficial effects on lung function when the inhaled PM was reduced by wearing N95 facemasks.

In addition, the beneficial effects associated with facemask intervention were strengthened when analyzing observations in the polluted days (Supplementary material: Figs. S2–S4). We found that the changes in FVC and FEV_1 in the trial of wearing sham facemasks at 30-min, 3-h and 5-h post-exposure were substantially attenuated, whereas the percentage change of FEV_1 in the trial wearing real facemask still increased at 30-min and 3-h post-exposure (Supplementary material: Fig. S2). Moreover, difference in the changes of FVC was observed between the two trials. We also found that, in days with the 2-h mean concentration of $PM_{2.5}$ smaller than 75 μg/m^3, the difference in lung function between the real facemask wearing trial and the sham facemask wearing trial narrowed, with differences in FEV_1 only occurring at 1-h and 5-h post-exposure (Supplementary material: Fig. S5). The results may suggest that a more beneficial effects on the lung function from the facemask wearing during heavily polluted days.

We observed differences in EBC nitrate, a biomarker of respiratory oxidative stress between the trials of sham and real facemask interventions. After the 2-h walk, the subjects wearing real facemasks showed a decreasing trend in EBC nitrate, while the subjects wearing

Table 2
Summary of health endpoints (mean ± SD) in sham-facemask group and real-facemask group during the intervention periods.

Endpoints	Trial	V0	V1	V2	V3
Respiratory inflammation					
EBC_nitrate (ng/mL)	Sham	358.99 ± 172.68	378.95 ± 374.94	332.54 ± 268.57	308.13 ± 239.91
	Real	328.17 ± 182.94	532.30 ± 951.70	439.18 ± 669.34	360.42 ± 292.35
EBC_nitrite (ng/mL)	Sham	123.32 ± 32.95	120.37 ± 54.22	172.62 ± 98.09	132.54 ± 42.66
	Real	127.65 ± 50.82	113.26 ± 34.06	148.91 ± 71.92	144.12 ± 72.62
EBC_pH	Sham	7.60 ± 0.48	7.49 ± 0.54	7.65 ± 0.43	7.79 ± 0.42
	Real	7.57 ± 0.50	7.56 ± 0.47	7.55 ± 0.51	7.74 ± 0.42
Lung function					
FVC (L)	Sham	3.63 ± 0.92	3.62 ± 0.84	3.66 ± 0.84	3.65 ± 0.82
	Real	3.56 ± 0.86	3.65 ± 0.87	3.67 ± 0.86	3.64 ± 0.83
FEV_1 (L)	Sham	3.25 ± 0.71	3.22 ± 0.66	3.26 ± 0.68	3.27 ± 0.65
	Real	3.17 ± 0.64	3.23 ± 0.66	3.25 ± 0.66	3.26 ± 0.66
Oxidative damage					
Urinary_MDA (μmol/L)	Sham	0.49 ± 0.30	–	0.41 ± 0.27	0.48 ± 0.41
	Real	0.50 ± 0.39	–	0.41 ± 0.29	0.39 ± 0.31
Creatinine (mol/L)	Sham	17.30 ± 9.04	–	8.25 ± 5.18	6.87 ± 5.66
	Real	15.08 ± 9.72	–	7.38 ± 4.87	5.40 ± 4.16
Blood Cytokines					
IFN-γ (pg/mL)	Sham	23.59 ± 14.59	–	–	28.10 ± 15.38
	Real	24.87 ± 15.49	–	–	25.85 ± 15.11
IL-10 (pg/mL)	Sham	4.98 ± 3.39	–	–	6.14 ± 0.80
	Real	7.56 ± 5.71	–	–	6.14 ± 0.80
IL-13 (pg/mL)	Sham	6.89 ± 9.95	–	–	8.20 ± 11.55
	Real	8.03 ± 10.86	–	–	7.62 ± 11.38
IL-17A (pg/mL)	Sham	6.87 ± 6.52	–	–	6.94 ± 6.72
	Real	7.95 ± 6.03	–	–	6.48 ± 4.47
IL-1β (pg/mL)	Sham	0.53 ± 0.52	–	–	0.43 ± 0.29
	Real	0.57 ± 0.45	–	–	0.43 ± 0.29
IL-2 (pg/mL)	Sham	0.47 ± 0.30	–	–	0.61 ± 0.35
	Real	0.99 ± 1.12	–	–	0.50 ± 0.32
IL-4 (pg/mL)	Sham	12.69 ± 14.17	–	–	12.95 ± 15.66
	Real	14.86 ± 21.59	–	–	12.95 ± 15.66
IL-6 (pg/mL)	Sham	4.63 ± 13.31	–	–	4.17 ± 11.51
	Real	4.99 ± 12.34	–	–	4.78 ± 12.97
IL-8 (pg/mL)	Sham	8.57 ± 6.13	–	–	7.64 ± 4.69
	Real	8.31 ± 5.25	–	–	7.87 ± 6.12
TNF-α(pg/mL)	Sham	4.41 ± 1.16	–	–	4.20 ± 1.18
	Real	4.95 ± 1.63	–	–	3.87 ± 1.3

Abbreviations:EBC_nitrite: nitrite in Exhaled Breath Condensate; EBC_nitrate: nitrate in Exhaled Breath Condensate; FVC: Forced Vital Capacity; FEV_1: Forced Expiratory Volume in 1s; Urinary_MDA: malondialdehyde in urinary.
V0: pre-exposure; V1: 30-min post-exposure; V2: 3-h post-exposure; V3: 5-h post-exposure.

sham facemasks showed an increasing trend (Fig. 4). Even though the changes in both trials at three post-exposure time points were not different from zero at $\alpha = 0.05$, we found there were notable differences between the two trials at 3- and 5-h post-exposure, with a higher level of EBC nitrate in the trial of wearing sham facemasks. These results may indicate that wearing a facemask while walking may be beneficial to human's respiratory oxidative stress condition since EBC nitrate has been treated as a relatively stable biomarker of oxidative stress, which had been positively associated with levels of air pollution in healthy adults (Zhang et al., 2013) and reflected the oxidative stress states of asthma and COPD patients (Corradi et al., 2003; Gessner et al., 2007). Unfortunately, we did not find appreciable difference in EBC nitrite between the two trials at either of the three post-exposure time points, even though we found the increases at the 3- and 5-h post-exposure points in both trials increased. The inconsistency in the results of EBC nitrate and nitrite may reflect the complexity of the health effects induced by the facemask. A study of subjects with and without tracheostomy showed that oropharyngeal bacteria chemically reduce salivary nitrates to nitrite, which contributes significantly to the concentration of nitrite in EBC collected by oral respiration (Marteus et al., 2005; Zetterquist et al., 2009). In our study, subjects fasted at baseline and before post-exposure EBC collections, and then all subjects were asked to eat a similar diet during the post-fasting meal. Controlling for food consumption and RCT designs may have mitigated any effects of oropharyngeal contamination, which may make our results more credible.

Results of the urinary MDA were consistent with EBC nitrate, which confirmed that wearing facemasks during walking would help to attenuate the oxidative stress induced by PM exposure. For all the visits after the intervention, we observed increases in urinary MDA from the baseline for both trials, but no notable differences between them. However, if we only considered the 'polluted' days ($PM_{2.5}$ >75 μg/m^3), there were differences between the two trial, with subjects wearing sham facemasks showed a higher level of urinary MDA increase. Notably, the increases in MDA post-exposure were higher for the real-intervention trial than the sham one during the 'clean' days ($PM_{2.5}$ <75 μg/m^3), which may suggest that the protective effect of the masks may be prominent on 'polluted' days as hinted in the results of lung function. This assumption may make sense since the particles exposed in the heavily 'polluted' days would generate considerable health effects, and if we take a facemask in these days, the removal of the particles would also be considerable enough to make health improvement to some extent. However, during a 'clean' day, since there were fewer effects induced by particle exposure, the removal of them would generate a less amount of benefit acquisition than in the 'polluted' days.

The current study also provided us evidence on the beneficial effects of wearing facemasks on the inflammatory response of cardiovascular system. We found that at 5-h after the 2-h walk outdoors, the serum levels of IL-13, TNF-α and IFN-γ in the trial of wearing sham facemasks increased, while IL-13, IL-6, IL-8, IL-10, IL-17A and TNF-α decreased for the trial of wearing real facemasks. And the changes in, IL-6, IL-10, IL-13, IL-17A, IFN-γand TNF-α were different between the two trials (Fig. 5). Similar results were observed in both 'polluted' and 'clean' days (Supplementary material: Fig. S2 -S7). Systemic inflammation has been proposed to be mainly responsible for the biological mechanism of $PM_{2.5}$ exposure on the cardiovascular health. Particles inhaled into the lung would cause pulmonary inflammatory response, produce a variety of cytokines and oxidative stress products into the circulatory system and trigger systemic inflammatory and oxidative stress (Brook Robert D. et al., 2010). And it was possible that the decreases in serum cytokines in the real-facemask trial and the differences between the two trials in the

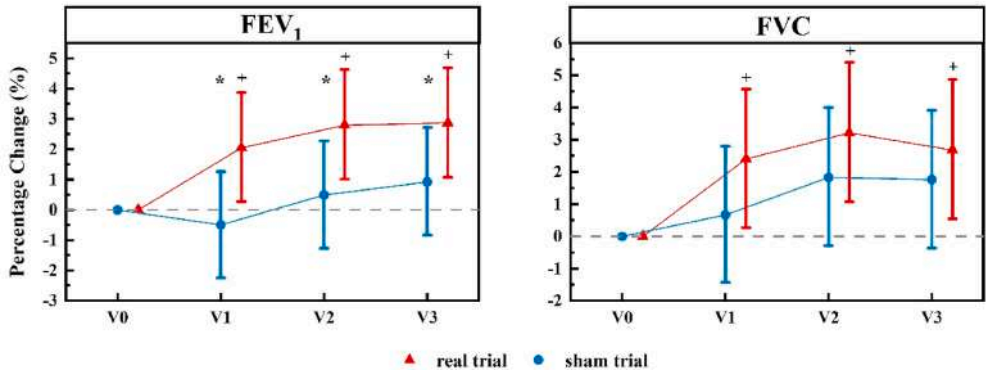

Fig. 3. Percentage Change in Lung Function from the Baseline at 30-min, 3-h and 5-h Post-exposure in the Sham Trial or Real Trial. +: Significantly different from baseline; *: Significantly different between the trial wearing real and sham facemasks.

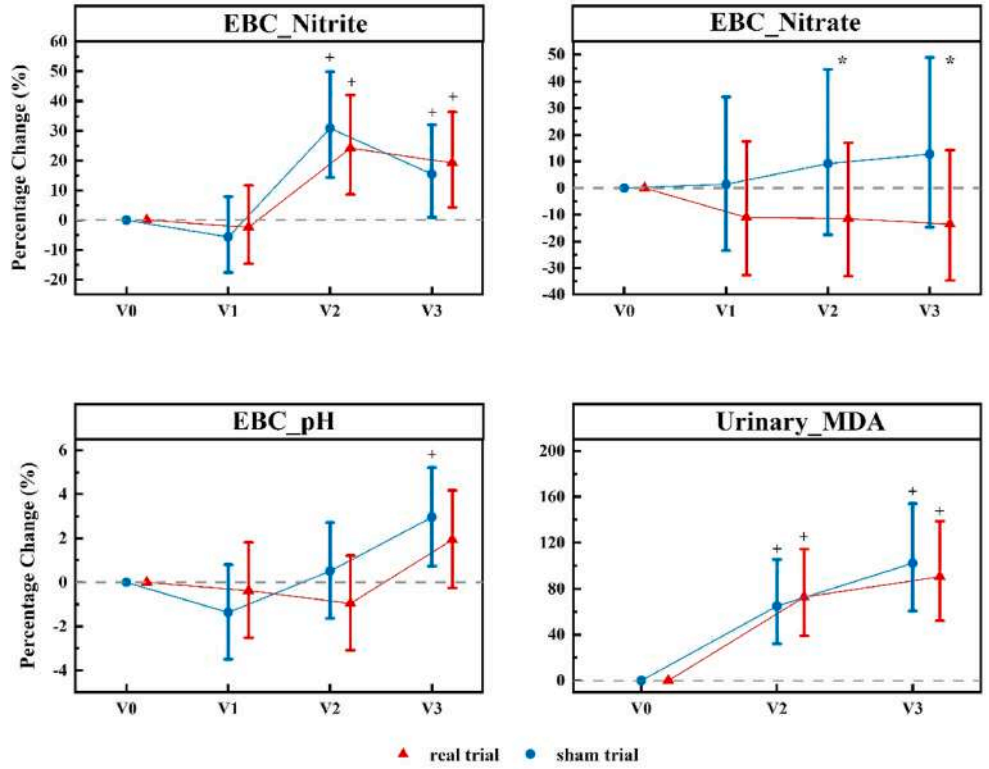

Fig. 4. Percentage Change in Respiratory and Systemic Oxidative Stress Biomarkers from the Baseline at 30-min, 3-h and 5-h Post-exposure in the Sham Trial or Real Trial, +: Significantly different from baseline; *: Significantly different between the trial wearing real and sham facemasks.

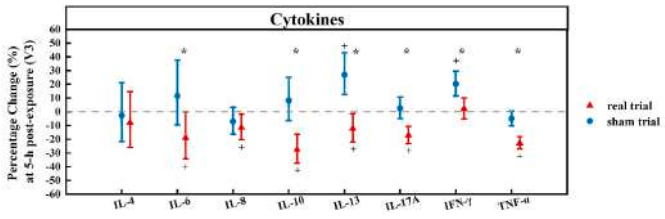

Fig. 5. Percentage Change in Serum Cytokines from the Baseline at 5-h post-exposure in the Sham Trial or Real Trial, +: Significantly different from baseline; *: Significantly different between the trial wearing real and sham facemasks.

current study were due to, at least partially, the intervention of facemask. Immunity and inflammation are closely related. Immunoregulation can be divided into pro-inflammatory regulation by Th17 cells and anti-inflammatory regulation by Treg cells, which play a role of secreting pro-inflammatory factors and anti-inflammatory factors respectively (Lane et al., 2010). Multiple inflammatory factors are needed to characterize the association between $PM_{2.5}$ exposure and inflammation. Many reports had shown that short-term exposure to PM increased the levels of pro-inflammatory cytokines including IL-6, TNF-α, IL-γ, and IL-8, IL-1, IL-4 and IL-6 in bronchial fluid, EBC, and blood (Corradi et al., 2010; Hu et al., 2020; Knatten et al., 2014b). Given the complex and intertwined nature of the inflammatory network (Sammour et al., 2010), the higher IL-10 and IL-13 (both anti-inflammatory cytokines) levels may be interpreted as increased immunosuppression or increased inflammatory activation by PM and facemask wearing. In another RCT study of facemask intervention, the

researchers observed that wearing N95 facemasks for 2 h during peak traffic resulted in reductions of multiple cytokines in exhaled breath condensate (Guan et al., 2018). Some evidence suggests that cytokine levels change only after days or weeks, not hours (Thompson et al., 2010; Törnqvist et al., 2007; van Eeden et al., 2001). Taken together, the current study provided evidence that a short-term wearing of a N95 facemask could generate beneficial effects on the systemic inflammation for at least 5-h after the exposure.

In summary, the current study utilized a double-blinded RCT design to provide molecular-level evidence on the health effects of wearing facemasks on cardiopulmonary system of young healthy adults in China. We may conclude that the facemasks appeared to reduce short-term PM-induced respiratory oxidative stress, the decline of lung function, and systemic inflammation/oxidative stress, and these beneficial effects of wearing facemasks on heavily 'polluted' days ($PM_{2.5}$ >75 μg/m^3) appeared to be more pronounced than in 'clean' days on lung function, systemic oxidative stress, and respiratory inflammation. Taken together, results from this interventional study demonstrated clear, albeit modest, cardiopulmonary benefits of short-term using facemasks in healthy adults. The use of facemasks offers individuals a feasible and affordable way to reduce exposure to hazardous air pollution in a highly-polluted circumstance, leading to significant public health benefits. Because our study participants were healthy young adults, one could reasonably expect different or even smaller cardiopulmonary benefits of air filtration among vulnerable populations, such as young children or elderly people. In addition, it is plausible that increased respiratory resistance and discomfort due to the wearing of facemasks might mitigate the potential health benefits resulting from the filtration of particles. Furthermore, the potential benefits from a longer intervention period could be expected and should be investigated. Additional trials are warranted to confirm or refute the protective effect of wearing facemasks, and healthcare providers may need to be cautious when recommending N95 facemasks to some susceptible subjects.

Some limitations of our study should be discussed. First, we did not consider potential face-seal leaks of the facemasks, which could be a penetration pathway for aerosol particles. Second, in part due to our participants were young healthy adults, the observed changes in certain biomarkers were fairly small. Thus, our results may not be applicable to other populations with different ages or disease status. More studies are needed to further evaluate potential health effects associated with wearing a personal protective equipment.

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CRediT authorship contribution statement

Meijie Jiang: Writing – original draft, Formal analysis, Investigation, Software, Visualization. **Xueling Meng:** Visualization. **Liang Qi:** Project administration. **Xinyan Hu:** Investigation. **Ruiwei Xu:** Investigation. **Meilin Yan:** Software. **Yunxiu Shi:** Investigation. **Xin Meng:** Investigation. **Weiju Li:** Project administration. **Yifan Xu:** Investigation. **Shiyi Chen:** Investigation. **Tong Zhu:** Conceptualization, Methodology. **Jicheng Gong:** Conceptualization, Methodology, Supervision, Project administration, Writing – review & editing.

Declaration of competing interest

All the authors declared no conflict of 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.2021.113806.

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Thyroid hormones in relation to polybrominated diphenyl ether and metals exposure among rural adult residents along the Yangtze River, China

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ABSTRACT

Although several studies indicate that exposure to polybrominated diphenyl ethers (PBDEs) and metals may influence thyroid function, the evidence is limited and inconsistent in general population. The current study was conducted to determine the levels of plasma PBDEs and urinary metals and evaluate the associations of co-exposure to both with thyroid hormones (THs) among rural adult residents along the Yangtze River, China. A total of 329 subjects were included in current analyses, and 8 PBDEs congeners and 14 urinary metals were measured to reflect the levels of environmental exposure. Multiple linear regression models were used to evaluate the association between PBDEs, metals and THs levels. Bayesian Kernel Machine Regression (BKMR) was used to examine PBDEs and metals mixtures in relation to THs. The geometric mean (GM) and 95% confidence interval (CI) of total measured PBDEs was 65.10 (59.96, 70.68) ng/g lipid weights (lw). BDE-209 was the most abundant congener, with a GM (95% CI) of 47.91 (42.95, 53.26) ng/g lw, accounting for 73.6% of the total PBDEs. Free thyroxine (FT4) was significantly negatively associated with BDE-28, 47, 99, 100, 154, and 183, and urinary strontium [β (95% CI): −0.04 (−0.07, −0.02)], but positively associated with selenium [β (95% CI): 0.04 (0.02, 0.06)]. Free triiodothyronine (FT3) was negatively associated with BDE-28 [β (95% CI): −0.03 (−0.05, −0.01)] and urinary arsenic [β (95% CI): −0.01 (−0.02, −0.001)]. The current study did not observe a statistically significant association of thyroid-stimulating hormone (TSH) with PBDEs and urinary metals. BKMR analyses showed similar trends when these chemicals were taken into consideration simultaneously. We found no significant interaction in the association between individual chemical at the 25th versus 75th percentiles and THs estimates, comparing the results when other chemicals were set at their 10th, 50th, and 90th percentile levels. Further study is required to confirm these findings and determine potential mechanisms.

1. Introduction

In hypothalamus–pituitary–thyroid (HPT) axis, the neuroendocrine cells of hypothalamus produce thyrotropin releasing hormone, and then promote the release and synthesis of thyroid stimulating hormone (TSH) and THs (i.e., thyroxine [T4] and triiodothyronine [T3]). Free thyroxine (FT4) and free triiodothyronine (FT3) are the free form of T4 and T3 respectively, accounting for less than 1% of T4 and T3, which have been used to assess thyroid function. It is a complicated regulation system of negative feedback that attempts to maintain the normal thyroid function and body metabolic homeostasis, including many proteins, enzymes, and carbohydrates metabolism (Foster et al., 2021; O'Kane et al., 2018). The disruption of HPT axis may result in various clinical or subclinical manifestations (Cooper and Biondi, 2012). It is clear that the HPT axis can be disrupted by exogenous environmental factors such as some persistent organic pollutants (e.g., polychlorinated biphenyls) and heavy metals (e.g., mercury) (Chen et al., 2013; Maervoet et al., 2007).

Polybrominated diphenyl ethers (PBDEs) are a class of synthetic

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flame retardants with similar chemical structure and properties to polychlorinated biphenyls, which were often used in various kinds of furniture, textiles, electronic products, and construction material. Historically, three PBDEs commercial products (i.e., penta-, octa- and deca-BDE) have been manufactured as major flame retardant mixtures (Bloom et al., 2008). Since they are semi-volatile and are not covalently bound to the consumer products in which they were incorporated, PBDEs were easily released into the surrounding environment. PBDEs are strongly lipophilic, previous studies showed that PBDEs were persistent and widely distributed in aquatic environment and bioaccumulated in various fish species (Kang et al., 2017). In addition, PBDEs may be a global pollutant that can be measured in samples taken from multiple sources such as indoor and outdoor air, marine sediments, human serum, urine, hair, nail, and breast milk (Ding et al., 2016; Kelly et al., 2008; Shi et al., 2013; Zhao et al., 2020a). Given that the potential environmental pollution and health effects of PBDEs, penta- and octa-BDE have been removed from the market. As for deca-BDE, dominated by BDE-209, its production and use remain fairly common in China. Unavoidably, because of the physical properties of the chemical, no matter how quickly PBDEs are phased out of use, PBDEs will continue to exist in the living animals and the environment for some time into the future.

The long term health effects of PBDEs exposure are an important potential concern. Several studies have investigated the potential association between PBDEs exposure and thyroid hormones (THs) during pregnancy, but there was no consistent association between THs and any BDE exposures (Chevrier et al., 2010; Makey et al., 2016; Stapleton et al., 2011; Vuong et al., 2015). Chen et al. suggested that BDE-209 exposure was positively associated with an increase in total thyroxine ($r = 0.270$, $p = 0.029$) among occupational workers from deca-BDE manufacturing plant (Chen et al., 2018). The result also showed that a 10-fold increase in the BDE-209 levels was related to an increase of 8.63 nmol/L (95% confidence interval: 0.93–16.30) in total thyroxine. However, a cross-sectional study conducted in 85 Alaska Native people found no significant association between serum PBDEs (including BDE-28/33, 47, 99, 100, 153, and 209) and either free or total thyroxine (Byrne et al., 2018). Another study conducted in 36 New York anglers, who represent a population with potentially increased dietary exposure to PBDEs, showed that there was no significant relationship of PBDEs (including BDE-28, 47, 66, 85, 99, 100, 138, 153, and 154) and thyroid function (including TSH, FT4, total thyroxine, and total triiodothyronine) (Bloom et al., 2008). However, Bloom et al. suggested that the sum of these PBDEs might be positively associated with FT4 when there was an approximately ninefold increase in sample size. It is still unclear whether PBDEs cause thyroid function disorders among general population, including those living in surrounding areas of the aquatic environment.

On the other hand, with the dramatic economic growth and increased industrialization in China, metal pollution has gradually become a health issue of great concern (Han et al., 2021; Li et al., 2013). Metals are ubiquitously dispersed in all natural environmental media, and its exposure will likely continue for a long time. People are exposed to metals mainly via intake of contaminated water and food, inhalation of polluted air, and direct contact with the skin. Cadmium and mercury have been extensively studied, because they are widely distributed in the environment and have been shown to be environmental endocrine disruptors (Castiello et al., 2020; Chen et al., 2013; Chung et al., 2019; Kim et al., 2021). However, the available evidence about the association between other metals and THs in general population is sparse and inconsistent, including arsenic and lead. For example, in National Health and Nutrition Examination Survey, there was no association between blood lead and TSH (Krieg, 2019). But another study included 5628 Chinese general population indicated that blood lead level was associated with increased TSH and hypothyroid status only in women (Nie et al., 2017). The potential mechanisms of THs inhibition might involve inhibiting the deiodination of T4, or competitive inhibition of binding of the hormones to their carrier protein (Rana, 2014). Of especial complexity with regard to metals is that their toxicity is not only related to the dose, but also related to their ability to change between their different chemical valency. Because the different metals are frequently found together in the environment, it is necessary to further investigate the potential impact of co-exposure to multiple metals on thyroid function.

PBDEs and metals are the industrial and technical grade products, whose pollution may coexist in the same environment. Nevertheless, whether simultaneous exposure to PBDEs and metals has a joint effect on thyroid function is unclear. In this study, we simultaneously investigated the association of PBDEs and metals exposure with THs in the general population living in rural areas along the Yangtze River, because they have more possibilities to be exposed to these chemicals bioaccumulated in aquatic products. Another aim was to investigate the interactions of PBDEs and metals exposure on THs levels.

2. Materials and methods

2.1. Study population

This study population forms part of the Wanjiang Cohort, which is located in sectional rural area along the Yangtze River. This cohort is designed to investigate the effect of long-term or chronic exposure to environmental pollutants on the health of adult residents along the Yangtze River (Cui et al., 2017). All participants were randomly recruited by local village doctors over the phone in 2019. All participants were required to be free of thyroid disease and thyroid medication use (based on self-report). The participants with a history of severe diseases in liver, kidney and gastrointestinal system were also excluded from current study. We also exclude those women that were in pregnancy and lactation because they had a higher basal metabolic rate and higher basal concentrations of THs during this period.

All study participants were invited to complete a face-to-face questionnaire once they agreed to participate in this study. The questionnaire information including demographic characteristics, lifestyle and behavioral habits (including smoking status, current alcohol-drinking, physical activity), personal disease history (including thyroid gland disease, head and neck tumors) and other medical history. The height and weight of participants was measured by a trained investigator and who was asked to report the weight of each participant to one decimal place. Then, all subjects were asked to provide a fasting blood and urine specimens in the second morning (between 7:00 a.m. and 8:30 a.m.) to minimize the influence of daily fluctuations in THs secretion. Finally, 329 participants were included in present analyses. This study was approved by the Ethics Committee of Anhui Medical University. All participants gave their informed consent.

2.2. Sample collection

All subjects were asked to fast for at least 8 h prior to collecting blood and urine specimens. All fasting samples were collected by medical staff from the local village clinic. Approximately 5 mL of blood was collected using two vacuum tubes (containing dipotassium ethylene diamine tetraacetate (EDTA-K_2) and anticoagulant-free), respectively. The serum and plasma were immediately separated after centrifugation. Approximately 0.5 mL of serum was sent to the laboratory within 1h for blood glucose and lipid testing. First morning urine specimen was collected from each participant by using a clean polyethylene centrifuge tube (approximately 50 mL). All biological specimens were stored at −80 °C within 3 h of collection until further analysis.

2.3. Plasma PBDEs measurement

The plasma specimens were measured for 8 PBDEs congeners (BDE-28, 47, 99, 100, 153, 154, 183 and 209), which are the flame retardant

mixtures mainly used commercially, using gas chromatograph-mass spectrometer at Anhui Medical University. The detailed detection method has been introduced elsewhere (Zhang et al., 2021). Briefly, we used internal standard substances (BDE-77 and 13C12-BDE-209, obtained respectively from Accustandard, USA and Cambridge Isotope Laboratories) and the mixture of formic acid and acetonitrile to pre-process plasma specimens. After the protein in the plasma had been denatured, this liquid system was extracted through a solid phase extraction column (Oasis© HLB-columns, 60mg/3 cc, Waters Corporation, USA) to remove the remaining lipids and other impurities. The PBDEs extracted from the column was eluted with dichloromethane, dried with nitrogen, and reconstituted with n-hexane. Finally, a quantified 50 μL of reconstituted solution containing PBDEs was prepared for instrumental testing.

All performance parameters of the instrumental testing had been successfully established in the preliminary test, including ion source, column flow, temperature programming, and scanning time. We established a standard substance (obtained from Accustandard, USA) curve in each experiment, and measured a blank specimen every 15 specimens, and used a spiked recovery method to ensure the stability of the experiment performance. The spiked recovery values ranged from 85.3% to 114.3%. The limit of detection (LOD) for PBDEs ranged from 0.03 mg/L (BDE-47) to 0.75 mg/L (BDE-209). The total lipid levels were calculated as described previous study for standardizing PBDEs concentrations in circulating blood (Guo et al., 2018b).

2.4. Urinary metals measurement

In this study, we measured 14 urinary metals levels and used them as predictive variables. Urinary levels of 13 metals [i.e., arsenic (As), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), lead (Pb), selenium (Se), strontium (Sr), zinc (Zn)] were determined using an inductively coupled plasma optical emission spectrometry (PerkinElmer Optima 7000DV, USA) at Anhui Medical University. The measurements of 13 metals were based on accordingly 13 sets of calibration standards in nitric acid (HNO_3) with internal standards. In order to carry out the chemical analysis on the urine samples, 3 mL of urine from each participant was diluted with 9 mL of 5% HNO_3 (guarantee reagent, GR). Following mixing, the resulting solution was microwave-digested at 90 °C for 1 h. After digestion, the resulting solution was centrifuged with 4500 r/min for 8 min and the liquid supernatant was extracted for subsequent analysis. Metallic cadmium (Cd) level was measured using a graphite furnace atomic absorption spectrometry (GFAAS, Analytik Jena AG ZEEnit®700P, Germany). The HNO_3 was added to raw urine specimens in order to acidify the sample. 1% diammonium hydrogen phosphate was added to the urine sample as a matrix modifier. In the conduct of test, one reference standard was used for every 10 test samples analyzed and the spiked recovery method was used to ensure the instrument performance. The spiked recovery values for 14 metals ranged from 92.4% to 105.2%. The LOD for 14 metals ranged from 0.04 μg/L (Mg) to 4 μg/L(Se). Urinary creatinine concentrations were measured using alkaline picric acid spectrophotometric for standardizing urinary metals. The detection reagent was using a commercial kit (Jiancheng 135 Bioengineering Ltd. Nanjing, China).

2.5. Serum thyroid hormones and urinary iodine measurement

Serum concentrations of FT3, FT4, and TSH were determined at Anhui Medical University using Roche Cobas e 411 analyzer and its manufacturer's reagents and calibrators (Roche Diagnostics GmbH, Mannheim, Germany). This was a fully automated electrochemiluminescence immunoassay process. It was required to establish an accurate standard curve and quality control testing based on the kit instructions before specimen measurement. The assay reference ranges of normal value for FT3, FT4, and TSH were (3.1–6.8) pmol/L, (12–22) pmol/L, and (0.27–4.20) uIU/mL, respectively. In addition, we also determined the urinary iodine levels because it is an important covariate in affecting thyroid function. Urinary iodine was determined by As^{3+}–Ce^{4+} catalytic spectrophotometry using a commercial kit (Wuhan Zhongsheng Biochemical Technique Co., Ltd, Wuhan, China).

2.6. Statistical analysis

Descriptive statistics were used to present the characteristics of study population, individual PBDE congeners and urinary metals, THs concentration. The specimen concentration below the LOD was assigned a value of LOD divided by the square root of two. Σ_8PBDEs was defined as the sum of eight congeners of PBDEs. The concentrations of PBDEs congeners, urinary metals and THs were natural-log (ln) transformed to decrease the effect of outliers and meet normal distribution. Spearman rank-order correlation was used to evaluate the correlations among PBDEs, urinary metals, and THs.

Multiple linear regression models were used to evaluate the association of THs concentrations with PBDEs and urinary metals, adjusted for covariates of age, gender, body mass index (BMI), education, smoking status, current alcohol-drinking, and urinary iodine/creatinine ratio, in which urinary iodine levels were natural-log (ln) transformed. For metals exposure, each metal was included in the single-metal models separately. We additionally conducted multiple-metal models considering all statistically significant metal in single-metal models to explore the simultaneous effects of co-exposure to multiple metals on THs. Partial correlation analyses for relationships of THs with PBDEs and metals were performed with the same covariates used in multiple linear regression models. In order to explore the potential threshold for effect or dose response relationship, we categorized exposures into tertiles, with first tertile as the referent group, to conduct a linear regression. Based on the β-coefficient in regression models, we calculated the percent change in THs levels between different tertiles by [exp (β)-1] × 100%. According to the normal reference ranges of TSH, logistic regression was used to evaluate odds ratios (OR) and 95% confidence intervals (CI) for high TSH (>4.20 uIU/mL) in relation with PBDEs and metals exposure. The trend test was performed in regression models using exposure tertiles as continuous variables.

Further, we used Bayesian Kernel Machine Regression (BKMR) to estimate the association of PBDEs and metals mixtures with THs, exploring the potential relationships and interactions between mixtures components (Bobb et al., 2015, 2018). BKMR facilitates exposure–response functions by using Bayesian variable selection and improves inference of correlated chemical mixture, and permits the visualization of exposure–response association. To avoid the data might not be able to distinguish among multiple chemicals in the mixture that were highly correlated, we included those PBDEs and metals with significant *p*-values in single models into BKMR models. In the analyses, the PBDEs and metals levels were natural-log (ln) transformed and then z-score normalized. Exposure–response functions were applied to examine the association between each individual chemical concentration and THs while holding other chemicals at median levels. Potential interactions within mixtures through evaluating the change in FT3 and FT4 estimate comparing individual chemical at its 25th to 75th percentiles levels, while setting other chemical at their 10th, 50th or 90th percentile levels.

In subgroup analysis, we ran the models of correlation analysis among men and women respectively. Given that thyroid function may be influenced by menopausal status among women, we additionally adjusted the menopausal status in the analysis of female subgroup. All analyses were performed using R software (version 3.3.1). All *p* values were tested in two-sided, $p < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of study participants

The characteristics of 329 study participants were shown in Table 1. The age of all participants ranged from 18 to 83, with mean age of 52.5 years. Among these participants, the majority of them were female (78.7%), were low educational level (66.3%), and were not smoking (85.1%) and not drinking (79.6%). Based on the calculation formula of total lipid, the median of total lipid was 5.5 g/L, with the interquartile range of 2.0 g/L. According to the reference ranges of normal value, there were 43 participants had abnormal concentrations of FT4 (6 participants with FT4 < 12 pmol/L and 37 participants with FT4 > 22 pmol/L), but only one participants had FT3 concentrations > 6.8 pmol/L, and 79 participants had TSH concentrations > 4.20 uIU/mL. The comparison of thyroid hormone levels according to general characteristics was showed in Supplementary Table S1.

3.2. Plasma PBDEs, urinary metals, and THs concentrations

The concentrations of plasma PBDEs, urinary metals, and THs were presented in Table 2. The geometric mean (GM) and 95% CI of $\sum_8$PBDEs was 65.10 (59.96, 70.68) ng/g lipid, where BDE-209 accounted for 73.6%. The congener with highest concentration in plasma was BDE-209 [GM (95% CI): 47.91 (42.95, 53.26) ng/g lipid]. Current PBDEs congeners were significantly correlated with each other, and the Spearman rank-order correlation was presented in Supplementary Table S2. The weakly significant correlation was observed between 14 urinary metals, correlation coefficients ranged from 0.115 to 0.480 (Supplementary Table S3). In addition, FT3 was statistically significantly associated with BMI (r = 0.115), education (r = 0.174) and alcohol-drinking (r = 0.152). FT4 was statistically significantly associated with education (r = 0.138) and alcohol-drinking (r = 0.160).

Table 1
Description of general characteristics for study population.

Variables	Mean ± SD or n (%) or IQR
Age, years	52.5 ± 12.3
Gender	
Male	70 (21.3)
Female	259 (78.7)
Body mass index, kg/m^2	
<24.0	173 (52.6)
24.0–27.9	120 (36.5)
≥28.0	36 (10.9)
Education	
Primary school	218 (66.3)
Junior high school	84 (25.5)
High school or above	27 (8.2)
Average annual household income, RMB	
< 30000	121 (36.8)
30000-59999	118 (35.9)
≥ 60000	90 (27.4)
Sleeping time, hours/day	
< 6	39 (11.9)
6-8	156 (47.4)
≥ 8	134 (40.7)
Smoking status	
Never	280 (85.1)
Ever	49 (14.9)
Current alcohol-drinking	
No	262 (79.6)
Yes	67 (20.4)
Physical activity	
Inactive	233 (70.8)
Active	96 (29.2)
Fasting blood-glucose, mmol/L	5.2 (4.8–5.6)
Total cholesterol, mmol/L	4.1 (2.4–5.0)
Triglycerides, mmol/L	2.0 (1.2–4.0)
Urinary iodine/creatinine ratio, μg/g	227.1 (153.9–309.5)

SD, standard deviation; IQR, inter-quartile range.

Table 2
The content of PBDEs and urinary metals and thyroid hormone levels from study population.

	Geometric mean (95% CI)	Percentile				
		5th	25th	50th	75th	95th
PBDEs (ng/g lipid)						
BDE-28	1.49 (1.39, 1.60)	0.47	0.98	1.55	2.24	4.08
BDE-47	0.96 (0.85, 1.07)	0.13	0.54	1.13	1.95	3.40
BDE-99	1.16 (1.05, 1.29)	0.27	0.59	1.03	1.90	6.76
BDE-100	2.04 (1.89, 2.20)	0.54	1.42	2.21	3.25	5.25
BDE-153	1.20 (1.11, 1.31)	0.35	0.75	1.19	1.82	4.69
BDE-154	1.73 (1.56, 1.93)	0.25	0.89	1.80	3.81	7.02
BDE-183	2.15 (2.00, 2.32)	0.65	1.39	2.07	3.42	6.87
BDE-209	47.91 (42.95, 53.26)	6.14	29.66	55.03	95.14	201.96
$\sum_8$PBDEs[a]	65.10 (59.96, 70.68)	16.52	40.39	66.50	111.40	228.92
Metals[b]						
As	12.62 (10.38, 15.35)	0.94	2.09	22.43	43.75	166.71
Cd	1.62 (1.45, 1.81)	0.21	0.94	1.91	2.98	6.45
Co	1.79 (1.43, 2.25)	0.09	0.23	2.05	10.76	32.37
Cr	35.06 (30.05, 40.91)	1.32	28.14	48.79	65.78	125.55
Cu	9.17 (8.38, 10.04)	1.74	6.78	11.07	13.72	23.62
Fe	24.89 (19.15, 32.37)	0.08	23.32	50.20	93.97	299.71
Li	14.56 (13.31, 15.93)	2.22	12.11	17.83	20.89	37.96
Mg	39.69 (30.37, 51.89)	0.04	39.91	78.14	134.66	270.47
Mn	1.38 (1.16, 1.65)	0.07	0.50	2.10	4.35	13.52
Mo	95.34 (85.41, 106.42)	23.35	56.41	106.13	158.77	433.28
Pb	22.38 (18.77, 26.68)	0.79	10.92	36.84	65.56	148.65
Se	111.03 (99.73, 123.61)	12.23	83.67	139.38	195.54	374.28
Sr	138.90 (125.96, 152.78)	35.87	92.97	165.48	198.92	493.40
Zn	184.93 (143.37, 238.56)	0.20	231.07	380.10	523.32	924.92
Thyroid hormones						
FT3, pmol/L	5.09 (5.02, 5.17)	4.19	4.64	5.05	5.56	6.56
FT4, pmol/L	17.46 (17.11, 17.83)	13.19	15.44	17.16	19.26	25.41
TSH, μIU/mL	2.78 (2.61, 2.96)	1.10	1.90	2.81	4.09	7.19

CI, confidence interval.
[a] Sum of 8 congeners of PBDEs, including BDE-28, -47, −99, −100, −153, −154, −183, and −209.
[b] The concentration of Mg was presented as mg/g creatinine and others were presented as ug/g creatinine.

3.3. Chemical exposures and thyroid hormones

3.3.1. Plasma PBDEs and thyroid hormones

As shown in Supplementary Table S4, the inverse associations were

observed between FT3 and BDE-28, as well as between FT4 and BDE-28, 47, 99, 100, and 183. There was no significant association between TSH and PBDEs. In multiple linear regression analyses, after adjusting for age, gender, BMI, education, smoking status, current alcohol-drinking, and urinary iodine/creatinine ratio, only BDE-28 was still negatively associated with FT3 [β (95% CI): −0.03 (−0.05, −0.01)] (Table 3). The reduced FT4 was associated with increased BDE-28, 47, 99, 100, and 183. In another dose-response relationship pattern, the decreased percent changes in FT3 and FT4 still had significant linear trend based on increased levels of these PBDEs congeners (all $p_{-trend} < 0.05$) (Fig. 1). According to the normal reference ranges of TSH, logistic regression was used to estimate the OR (95% CI) for high TSH (>4.20 uIU/mL) in relation with PBDEs and metals exposure. However, there was still no significant association between plasma PBDEs and TSH levels (Supplementary Table S5).

3.3.2. Urinary metals and thyroid hormones

In single-metal models, we observed an inverse association of FT3 with metal As, Cr, Fe, and Sr levels, while no association between urinary metals and TSH (Table 4). The result of partial correlation analyses showed similar trends (Supplementary Table S4). In multiple-metal models, we only observed As was associated with decreased FT3 [adjusted β (95% CI): −0.01 (−0.02, −0.001)] in all study population (Table 5). FT4 was positively associated with Se levels [adjusted β (95% CI): 0.04 (0.02, 0.06)] and negatively associated with Sr levels [adjusted β (95% CI): −0.04 (−0.07, −0.02)]. Similarly, a significantly reduced percent changes in FT3 and FT4 were presented between FT3 and the highest tertile of As [percent changes (95% CI): −3.92% (−7.60%, −0.20%), $p_{-trend} = 0.023$]; and between FT4 and the highest tertile of Sr [percent changes (95% CI): −8.33% (−12.98%, −3.54%), $p_{-trend} = 0.001$] (Fig. 1). However, there was still no significant association between urinary metals and TSH levels in logistic regression model (Supplementary Table S5).

3.4. Interaction and subgroup analysis

In BKMR analyses, univariate exposure–response function still observed an inverse association of FT3 with As and BDE-28 (Supplementary Fig. S1), the relationships of FT4 with Se, Sr, and several PBDEs were presented in Supplementary Fig. S2. In exploring the figures for potential interactions, bivariate exposure-response functions for every two chemicals showed no apparent differences in relationships of analyzed chemical with FT3 and FT4 estimates, where all of the other chemicals were fixed at median levels (Supplementary Fig. S3 and Supplementary Fig. S4). Similarly, no significant interactions were observed for any individual chemical with the remaining chemicals on FT3 and FT4 estimates (Fig. 2).

In subgroup analysis, there still were significant associations between the presence of some PBDEs congeners and FT3 and FT4 among women (Supplementary Fig. S5). Men in the highest tertiles of BDE-99 and BDE-100 had significantly reduced percent changes in FT3 [percent changes (95% CI): −6.67% (−12.37%, −0.70%), $p_{-trend} = 0.030$] and FT4 [percent changes (95% CI): −11.57% (−19.83%, −2.37%), $p_{-trend} = 0.017$] compared to men in first tertile, respectively (data not shown).

For urinary metals analysis, male FT3 and FT4 were inversely associated with metal Mn [Tertile 3 (T3) *vs*. Tertile 1 (T1), percent changes (95% CI): −8.79% (−14.96%, −2.18%), $p_{-trend} = 0.011$] and Sr [T3 *vs*. T1, percent changes (95% CI): −12.63% (−20.86%, −3.54%), $p_{-trend} = 0.008$), respectively. In addition, we found a significantly inverse association between metal Li and FT3 [T3 *vs*. T1, percent changes (95% CI): −4.3% (−8.06%, −0.3%), $p_{-trend} = 0.045$], and FT4 [T3 *vs*. T1, percent changes (95% CI): −7.5% (−12.8%, −1.88%), $p_{-trend} = 0.013$] among women (Supplementary Fig. S6). When we performed multiple-metal models only among female population, a significantly positive association was presented between FT3 and Se levels ($p_{-trend} = 0.001$). FT4 was negatively associated with Fe ($p_{-trend} = 0.039$) and Sr ($p_{-trend} = 0.006$), and simultaneously positively associated with Se ($p_{-trend} = 0.001$) among women (data not shown).

4. Discussion

In order to compare with other similar studies, the PBDEs congeners and urinary metals levels and its association with THs from general populations around the world were summarized in Supplementary Table S6 and Supplementary Table S7. However, in previously published studies, we only found limited information on the association of blood PBDEs and urinary metals levels with THs in the general population. A recent review presented that the concentrations of PBDEs in soil elsewhere in the world were similar to that in sectional remote areas in China (Jiang et al., 2019). This similar result was also observed in our study population. Obviously, a comparison with the study published by Huang et al. (2014) suggested that the current PBDEs concentrations in rural adult residents along the Yangtze River were likely higher than that of other general population in China during 2010–2011. This means that PBDEs pollution in China started late, but it becomes a significant concern. For metals burden, it will be a persistent health problem as long as there is industrial development. In Supplementary Table S7, we observed that the levels of Cd, As, and Pb in our study population were higher than those in other regions.

After adjusting for age, gender, BMI and so on, FT3 was found to be significantly negatively associated with As and BDE-28; and FT4 was negatively associated with Sr and most PBDEs congeners (including BDE-28, 47, 99, 100, 154, and 183), but positively associated with Se in our current study. Although these low-brominated PBDEs have been

Table 3
Associations between PBDE (ng/g lipid) and thyroid hormones levels among all study population [β (95% CI)].

PBDEs[a]	ln FT3		ln FT4		ln TSH	
	Unadjusted	Adjusted[b]	Unadjusted	Adjusted[b]	Unadjusted	Adjusted[b]
BDE-28	−0.03 (−0.05, −0.01)*	−0.03 (−0.05, −0.01)*	−0.05 (−0.08, −0.02)*	−0.05 (−0.08, −0.02)*	0.02 (−0.09,0.10)	0.01 (−0.09, 0.11)
BDE-47	−0.02 (−0.03, −0.01)*	−0.01 (−0.03, 0.01)	−0.02 (−0.04, −0.01)*	−0.02 (−0.04, −0.01)*	0.01 (−0.06,0.07)	0.01 (−0.05, 0.07)
BDE-99	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.01)	−0.02 (−0.04, 0.01)	−0.02 (−0.05, −0.01)*	−0.02 (−0.08, 0.05)	−0.04 (−0.07, 0.06)
BDE-100	−0.01 (−0.02, 0.02)	0.01 (−0.02, 0.02)	−0.03 (−0.06, −0.01)*	−0.03 (−0.06, −0.01)*	0.02 (−0.07, 0.11)	0.02 (−0.07, 0.11)
BDE-153	0.02 (−0.01, 0.04)	0.01 (−0.01, 0.03)	0.01 (−0.01, 0.04)	0.01 (−0.02, 0.03)	−0.04 (−0.12, 0.04)	−0.01 (−0.10, 0.07)
BDE-154	−0.01 (−0.03, 0.01)	−0.01 (−0.02, 0.01)	−0.02 (−0.04, 0.01)	−0.01 (−0.04, 0.01)	−0.05 (−0.11, 0.02)	−0.04 (−0.11, 0.02)
BDE-183	−0.02 (−0.04, 0.01)	−0.02 (−0.04, 0.01)	−0.03 (−0.06, 0.01)	−0.03 (−0.06, −0.01)*	−0.02 (−0.11, 0.08)	−0.01 (−0.10, 0.10)
BDE-209	−0.01 (−0.03, 0.01)	−0.01 (−0.03, 0.01)	−0.02 (−0.04, 0.01)	−0.01 (−0.04, 0.01)	−0.01 (−0.08, 0.05)	−0.02 (−0.08, 0.05)
$\sum_8$PBDEs[c]	−0.02 (−0.04, −0.01)*	−0.02 (−0.03, 0.01)	−0.03 (−0.06, −0.01)*	−0.03 (−0.05, 0.01)	−0.02 (−0.10, 0.06)	−0.02 (−0.11, 0.06)

*$p < 0.05$.
[a] PBDE concentrations were natural-log (ln) transformed.
[b] Adjusted for age, gender, body mass index, education, smoking status, current alcohol-drinking, and urinary iodine/creatinine ratio.
[c] Sum of 8 congeners of PBDEs, including BDE-28, -47, −99, −100, −153, −154, −183, and −209.

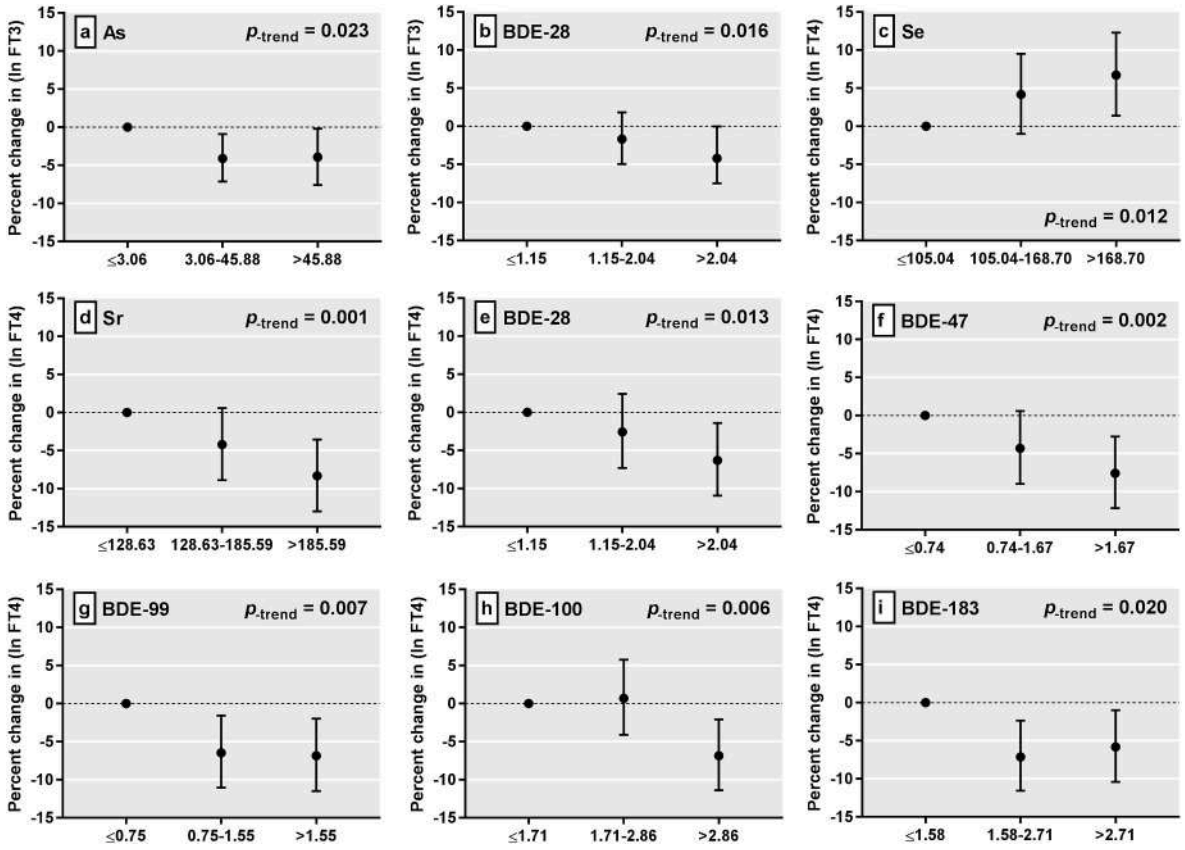

Fig. 1. Adjusted percent changes (%) and 95% confidence intervals for associations of urinary metals and PBDEs tertiles with thyroid hormones levels among all study population. The tertile of As (a), BDE-28 (b), Se (c), Sr (d), BDE-28 (e), BDE-47 (f), BDE-99 (g), BDE-100 (h), and BDE-183 (i) was presented on the x–coordinate (using the lowest tertile as reference). Units: metals (ug/g creatinine), PBDEs (ng/g lipid), FT3 (pmol/L), FT4 (pmol/L), and TSH (uIU/mL). All models adjusted for age, gender, body mass index, education, smoking status, current alcohol-drinking, and urinary iodine/creatinine ratio.

Table 4
Associations between urinary metals and thyroid hormones levels from all study population in single-metal models [β (95% CI)].

Metals[a]	ln FT3		ln FT4		ln TSH	
	Unadjusted	Adjusted[b]	Unadjusted	Adjusted[b]	Unadjusted	Adjusted[b]
As	−0.01 (−0.02, −0.003)*	−0.01 (−0.02, −0.001)*	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.01)	−0.02 (−0.05, 0.02)	−0.02 (−0.05, 0.02)
Cd	0.01 (−0.01, 0.02)	0.01 (−0.01, 0.02)	0.01 (−0.01, 0.03)	0.02 (−0.01, 0.04)	−0.03 (−0.09, 0.03)	−0.02 (−0.09, 0.04)
Co	−0.007 (−0.01, −0.01)*	−0.005 (−0.01, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.03, 0.03)	0.001 (−0.03, 0.03)
Cr	−0.004 (−0.01, 0.01)	−0.01 (−0.02, −0.001)*	−0.01 (−0.02, 0.01)	−0.01 (−0.03, 0.01)	−0.01 (−0.05, 0.04)	−0.01 (−0.06, 0.04)
Cu	−0.01 (−0.03, 0.01)	−0.01 (−0.03, 0.01)	−0.01 (−0.04, 0.01)	−0.01 (−0.04, 0.01)	0.01 (−0.06, 0.09)	0.02 (−0.06, 0.10)
Fe	−0.002 (−0.01, 0.01)	−0.006 (−0.01, −0.001)*	−0.005 (−0.01, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.03, 0.02)	−0.01 (−0.04, 0.02)
Li	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.03, 0.02)	−0.01 (−0.03, 0.02)	0.02 (−0.06, 0.09)	0.01 (−0.07, 0.09)
Mg	0.001 (−0.01, 0.01)	−0.003 (−0.01, 0.01)	−0.004 (−0.01, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.04, 0.02)	−0.01 (−0.04, 0.02)
Mn	0.002 (−0.01, 0.01)	0.001 (−0.01, 0.01)	0.003 (−0.01, 0.02)	0.003 (−0.01, 0.02)	−0.01 (−0.05, 0.03)	−0.01 (−0.05, 0.04)
Mo	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.01)	−0.02 (−0.04, 0.01)	−0.02 (−0.04, 0.01)	0.05 (−0.01, 0.11)	0.05 (−0.01, 0.12)
Pb	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.002)	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.05, 0.03)	−0.01 (−0.04, 0.04)
Se	0.02 (0.003, 0.03)*	0.01 (−0.01, 0.02)	0.03 (0.01, 0.05)*	0.03 (0.01, 0.05)*	−0.02 (−0.08, 0.05)	−0.01 (−0.07, 0.06)
Sr	−0.02 (−0.03, 0.01)	−0.02 (−0.03, −0.001)*	−0.03 (−0.05, −0.01)*	−0.03 (−0.05, −0.004)*	0.001 (−0.07, 0.07)	−0.01 (−0.09, 0.06)
Zn	0.002 (−0.01, 0.01)	−0.003 (−0.01, 0.01)	−0.003 (−0.01, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.04, 0.01)	−0.01 (−0.04, 0.02)

*$p < 0.05$.
[a] Urinary metals concentrations were natural-log (ln) transformed. The concentration of Mg was presented as mg/g creatinine and others were presented as ug/g creatinine.
[b] Adjusted for age, gender, body mass index, education, smoking status, current alcohol-drinking, and urinary iodine/creatinine ratio.

phased out, their health effects of long-term exposure in the environment continue to exist. BDE-47 and BDE-153 were reported to have a heavier body burden in various human specimens in Western countries (Bradman et al., 2007; Darrow et al., 2017). However, this burden was different from that of Chinese population, as shown in Supplementary Table S6, possibly caused by different usage patterns of PBDEs congeners. Besides, low-brominated PBDEs may be derived from deca-BDE through debromination (Law et al., 2014). For example, BDE-209, a major deca-BDE, has photochemically unstable feature which can form tetra-to nona-BDE via debromination (Su et al., 2014).Unfortunately, deca-BDE still has a broad application market in China, and those products with low-bromination PBDEs are still being widely used (Ji et al., 2017). Similarly, metals are ubiquitously dispersed in all natural environmental media and metals pollution is gradually becoming a

Table 5
Associations between urinary metals and thyroid hormones levels from all study population in multiple-metal models [β (95% CI)].

Thyroid hormones	Metals[a]	Unadjusted	Adjusted[b]
ln FT3	As	−0.01 (−0.02, −0.002)*	−0.01 (−0.02, −0.001)*
	Cr	−0.003 (−0.02, 0.01)	−0.01 (−0.02, 0.01)
	Fe	0.001 (−0.01, 0.01)	−0.003 (−0.01, 0.004)
	Sr	−0.01 (−0.03, 0.01)	−0.01 (−0.03, 0.01)
ln FT4	Se	0.05 (0.02, 0.07)*	0.04 (0.02, 0.06)*
	Sr	−0.05 (−0.07, −0.02)*	−0.04 (−0.07, −0.02)*

*$p < 0.05$.
[a] Urinary metals concentrations were natural-log (ln) transformed. The concentration of metals was presented as mg/g creatinine.
[b] Adjusted for age, gender, body mass index, education, smoking status, current alcohol-drinking, and urinary iodine/creatinine ratio.

serious health concern. Therefore, these residents living around rivers and lakes should pay more attention to the health effects of exposure to environmental pollutants, including thyroid function.

Since PBDEs congeners structurally resemble T4 and T3, it had been proven that they had thyroid toxicity and neurotoxic effects (Dong et al., 2021; Xiong et al., 2019). Oulhote et al. analyzed the data of 2007–2009 Canadian Health Measures Survey found that the elevated prevalence of hypothyroidism was significantly related to increased concentrations of BDE-47 and BDE-100 (Oulhote et al., 2016). Another study among 308 adult male sport fish consumers suggested that serum PBDEs levels were related with increased T4 (Turyk et al., 2008). In deca-BDE manufacturing workers, it had been reported that serum BDE-209 was positively associated with T4 ($r = 0.270$, $p = 0.029$), and a 10-fold increase in the BDE-209 levels was related to an increase of 8.63 nmol/L (95% CI: 0.93–16.30) in T4 (Chen et al., 2018). In addition, several studies performed in electronic and electric wastes (e-waste) dismantling areas and its surrounding areas also found similar thyroid toxicity, particularly e-waste recyclers (Guo et al., 2018b; Wang et al., 2019; Zhao et al., 2020b). China is a big economic and trade country, simultaneously numerous e-waste were imported or dumped from developed countries. It can be inferred to be the main source of PBDEs pollution in the environment (Sthiannopkao and Wong, 2013). In this study, we did not found BDE-209 was significantly associated with THs, although BDE-209 had the highest concentration in our study population. This phenomenon might be related to the properties of BDE-209, including large molecular size, extreme hydrophobicity and low bioavailability. Previous study had shown that BDE-209 mainly accumulated in adrenals, kidneys, and liver, while lipophilic tissues were the preferred sites for other congeners bioaccumulation (Linares et al., 2015). Furthermore, only a low systemic toxicity of BDE-209 could be detected in the case of long-term chronic exposure, and its NOAEL (No-Observed-Adverse-Effect-Level) values for liver and thyroid gland respectively were 1120 mg/kg b.w. and 2550 mg/kg b.w. (Fromme et al., 2014). Given the pervasive exposure to PBDEs, the persistence and bioaccumulation of it in the environment, more studies are needed to reveal its toxic dose and mechanism of action.

However, the accurate mechanism of effects of PBDEs on thyroid function still remains unclear. The potential mechanism was that PBDEs interfered with the transport and metabolism of THs. First, it has been proven that several hydroxylated PBDEs congeners could be used as THs dependent transcriptional agonists (Ren et al., 2013). In fluorescence competitive binding assay, the different levels of PBDEs congeners and its hydroxylated component (HO-PBDEs) could impede the transcription mediated by THs receptors (TR), but the activities of HO-PBDEs on TR (agonistic or antagonistic) depend on their geometric structure during binding (Li et al., 2010; Ren et al., 2013). Second, PBDEs congeners and its metabolites compete with binding of thyroxine transport proteins (including transthyretin and thyroxine-binding globulin) and directly interfere with the concentrations of FT3 and FT4 (Cao et al., 2010; Guo et al., 2018b). Third, PBDEs congeners and HO-PBDEs may directly deactivate the iodothyronine deiodinases and inhibite the deiodination of T4 (Ren and Guo, 2012; Roberts et al., 2015). In addition, serum PBDEs congeners, including BDE-99, 100, 154, and 209, were reportedly associated with liver impairment (Sun et al., 2020; Zhao et al., 2020b). The potential mechanisms that might be involved in the toxicity of PBDEs potentially could include abnormal metabolism of glycolipids, increased oxidative stress, impairment of mitochondrial function, as well as induction of inflammatory cytokines (Zhao et al., 2020b). Obviously, the liver impairment can easily affect the sulfotransferase bioactivity and deiodination of thyroid hormone deiodinase 1 and 2 in the liver.

The evidence about associations between urinary metals and thyroid function among general population was sparse. Most studies have assessed the concentrations of metals in blood with the focus being on workers in at risk occupations rather than in the general population. Urine samples are a convenient non-invasive biomonitoring media for body's metabolites and used more frequently in epidemiological studies. Urinary excessive metals also reflect the disruption of homeostasis (Wu et al., 2018). In current study, we observed urinary arsenic was inversely associated with FT3. This result was similar to that in Guo et al. study investigated relationship between blood metals and THs among pregnant (Guo et al., 2018a). Arsenic is ubiquitously dispersed in food, soil, water and air, and its pollution sources include natural sources and anthropogenic sources, such as volcanoes, coal power plants, incineration of waste, and pesticide use (Ciarrocca et al., 2012). Contaminated drinking water is the main route of human exposure to excessive arsenic

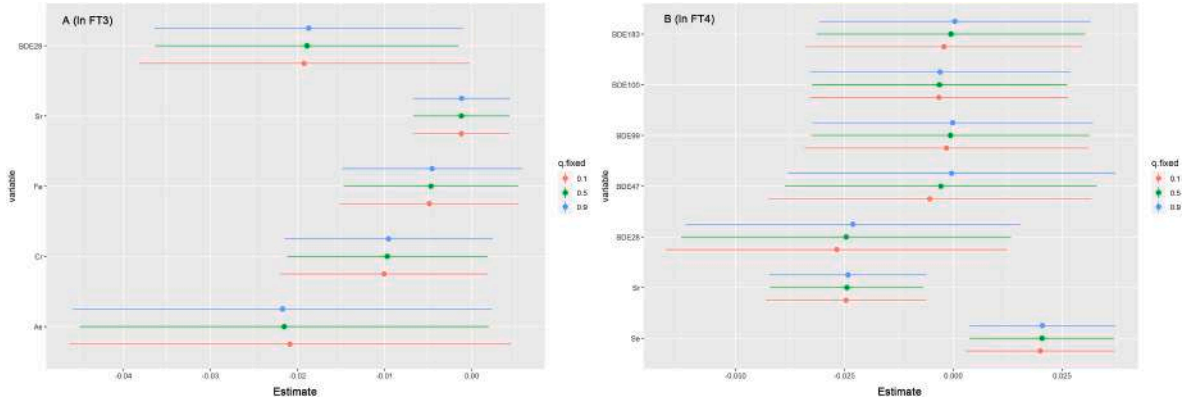

Fig. 2. Effects (estimates and 95% confidence intervals) of each chemical on FT3 (A) and FT4 (B). This figure indicated the relative effects of a single chemical at the 75th versus 25th percentile, while remaining chemicals were set at their 10th, 50th, or 90th percentile levels, respectively. All models were adjusted for age, gender, body mass index, education, smoking status, current alcohol-drinking, and urinary iodine/creatinine ratio.

(Davey et al., 2008). Arsenic was considered an endocrine disruptor and had been reported to alter some hormone receptor-mediated gene regulation, such as glucocorticoid receptor, retinoic acid receptor, and TR (Davey et al., 2008). Specifically, arsenic could disrupt THs homeostasis by acting on the transcription of TR related genes, including type I deiodinase gene (Davey et al., 2008; Sun et al., 2016a). For example, arsenic concentrations at < 150 μg/L disturbed the THs homeostasis of bighead carp larvae by increasing the thyroxine levels and reducing TR mRNA transcriptional levels (Sun et al., 2016b). In addition, the increased arsenic trioxide level was significantly associated with inhibition for vitro thyroid peroxidase activity, and the minimal dose required to inhibit this activity was between 0.1 and 1 ppm (Palazzolo and Jansen, 2008).

As we all know, selenium is an important element for synthesis of THs, and the thyroid gland has the highest selenium content in human organs (Kohrle, 2015). It has been reported that selenium supplementation (80 μg or 200 μg/day as sodium selenite or selenomethionine respectively) was effective against Hashimoto's thyroiditis and reducing thyroid peroxidase autoantibody concentration at 3 months (Toulis et al., 2010). Those pregnant women with thyroid-peroxidase-antibody positive easily suffer from post-partum hypothyroidism, but the risk of post-partum hypothyroidism were notably reduced when those women were treated 200 μg/day selenomethionine (Negro et al., 2007). However, for those with adequate-to-high selenium, excessive intake of selenium supplementation might be adversely affected, which could cause selenosis (Rayman, 2012). In current study, there was a positive association between selenium and FT4 levels. This can involve the contribution of selenium to thyroid function. Glutathione peroxidase rich in selenium has strong antioxidant properties, which is very important for resisting the damage of excess hydrogen peroxide and its reactive oxygen intermediates during THs synthesis. In addition, some selenoenzymes also could regulate the transformation of T4 to T3 (Broberg et al., 2011). Therefore, it can be assumed that selenium should have a negative correlation with T4 (Broberg et al., 2011). At this time, the thyroid gland may be stimulated by TSH to secrete more T4 due to the normal negative feedback regulation of thyroid system. Subsequently, the FT4 concentration is increasing.

For strontium, it is well known as calcium-sensing receptor (CaSR) agonists, Sr^{+2} ions seem to be able to replace Ca^{+2} ions. The experiment study suggested that strontium biased CaSR signaling toward extracellular signal-regulated kinases 1 and 2 (ERK1/2) signaling and the potency of strontium-stimulated calcitonin secretion was higher than calcium (Thomsen et al., 2012). Calcitonin secreted by the thyroid gland can regulate the body calcium balance, and this process is stimulated by the combination of T3, T4 and tyrosine during the secretion calcitonin. However, it is not clear whether strontium in turn affects thyroid function in producing T3 and T4. Although we found urinary strontium level was significantly negatively associated with FT4 in this study, this association and its potential mechanism need to be further confirmed.

To the best of our knowledge, this study is the first to evaluate the simultaneous effects of co-exposure to PBDEs and metals on THs levels in the general population and to investigate the interactions of PBDEs and metals exposure on THs. In order to acquire an exact risk association, we conducted multivariate models and subgroup analyses to adjust some common covariates, such as age, gender, smoking status, and an additional adjustment for menopausal status in the female subgroup. However, this study has several limitations. First, this study is cross-sectional study with a small sample size extracted from community cohort. This study only reveals a phenomenon and does not represent the causality. Second, the single determination of PBDEs and urinary metals may not be accurately exposure status. Multiple determinations will be beneficial to reflect long-term and chronic exposure levels. Third, the urine sample is not the preferred choice for the measurement of certain metal such as iron and lead, although urine metals levels have been used to explore the correlation with various diseases including hypertension, metabolic syndrome, cardiovascular diseases and kidney function (Domingo-Relloso et al., 2019; Wu et al., 2018; Xu et al., 2021; Yang et al., 2019). The interpretation of associations between our urinary metals and THs should be cautious, and these associations warrant further investigation.

5. Conclusions

In current cohort of rural communities along the Yangtze River, we measured the human body burden of PBDEs and metals and their associations with THs. Compared with the general population in other study areas, the burden of PBDEs and some heavy metals (i.e., Cd, As, and Pb) levels seems to be gradually accumulating among the rural residents along the Yangtze River. In addition, FT3 was significantly negatively associated with As and BDE-28; and FT4 was significantly negatively associated with Sr and multiple PBDEs congeners (i.e., BDE-28, 47, 99, 100, 154, and 183), but positively associated with Se levels. Besides, there was no statistically significant association of TSH with plasma PBDEs and urinary metals in current study. Our results indicated that the potential effects of PBDEs and metals exposure on thyroid function should raise concern, and more measures are needed to reduce the release of these pollutants in environment. However, further studies are necessary to investigate these associations and to illuminate the potential mechanisms.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Declaration of competing interest

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

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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.2021.113800.

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Urinary neonicotinoids level among pregnant women in Japan

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ABSTRACT

Neonicotinoids (NEOs) are the most important globally available class of chemical insecticides since the introduction of synthetic pyrethroids. The adverse effects of NEOs for early development have been reported via in vivo and epidemiological studies. Therefore, prenatal NEOs exposure is highly concerning. This study aimed to determine the level of NEOs exposure during daily life among pregnant women in Japan, as well as the sources of exposure. Spot urine samples were collected during the first, second, and third trimesters from 109 pregnant women who delivered their infants at obstetrics and gynecology clinics in Kumamoto city, Japan, between 2014 and 2016. Additional data were obtained from medical records and self-administered questionnaires. thiamethoxam and clothianidin (CLO) were detected in most participants (83.4% and 80.9%, respectively), and at higher concentrations than those in other areas of Japan. Multiple logistic regression analysis showed a statistical significant association of pulses in CLO (1.01 [1.00–1.02]). In conclusion, pregnant women in Japan appear to be exposed to NEOs in their daily lives, and pulses intake may be a source of NEOs exposure. These findings may further the assessment of human NEOs exposure risk.

1. Introduction

Neonicotinoids (NEOs) are the most important chemical class of insecticides introduced to the global market since the introduction of synthetic pyrethroids. NEOs are registered in more than 120 countries and are considered the most effective class of insecticides for controlling sucking insects (Jeschke et al., 2011). NEOs that have been demonstrated to provide excellent biological control while remaining safe for both humans and the environment are used extensively worldwide for crop protection, and account for approximately one-fourth of the global insecticide market (Tomizawa and Casida, 2011). Accordingly, seven NEOs, namely acetamiprid (ACE), imidacloprid (IMI), thiacloprid (THI), clothianidin (CLO), dinotefuran (DIN), thiamethoxam (TMX), and nitenpyram (NTP), are specifically used in many Japanese prefectures (Harada et al., 2016; National Institute for Environmental Studies, 2019). The total shipment amounts of these NEOs have increased by almost 7 times in 2017 relative to the amounts reported in 1996 (National Institute for Environmental Studies, 2019).

The NEOs exposure during early development is an issue of great concern. In vivo studies of mice showed that IMI exposure during the early developmental period (gestational day 4 to postnatal day 21) induces long-lasting changes in behavior and brain function (Buker et al., 2018). Consistent with this observation, studies conducted in the United States have reported associations of residential proximity to NEOs agricultural use with the incidence of tetralogy of Fallot and anencephaly (Carmichael et al., 2014; Yang et al., 2014). Furthermore,

Abbreviations: BMI, body mass index; FFQ, food frequency questionnaire; IS, internal standard; LOD, limit of detection; LOQ, limit of quantitation; SD, standard deviation; SPE, solid-phase extraction; NEOs, Neonicotinoids; ACE, acetamiprid; IMI, imidacloprid; THI, thiacloprid; CLO, clothianidin; DIN, dinotefuran; TMX, thiamethoxam; NTP, nitenpyram; nAChRs, nicotinic acetylcholine receptors; OPs, organophosphates; NACE, acetamiprid-N-desmethyl; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; PesUse, pesticide use; PesNoUse, non-pesticide use.

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proximity to agricultural sources of IMI during pregnancy have been associated with an increased risk of neural tube defects (Rull et al., 2006; Yang et al., 2014) and a reduction in IQ (Gunier et al., 2017). Ling et al. reported that first or second trimester exposure to pesticides, including IMI, or exposure to 2 or more pesticides from three chemical classes (organophosphates [OPs], pyrethroids, and carbamates), was associated with a small increase (3–7%) in the risk of preterm birth (Ling et al., 2018). It is therefore necessary to determine the human exposure levels to NEOs, especially in pregnant women. To the best of our knowledge, only one study has assessed NEOs exposure levels in pregnant women. However, the sample size and population characteristics of that study were very limited (30 pregnant women living in agricultural areas in Spain) (López-García et al., 2017). Moreover, few studies have assessed NEOs exposure levels using biological samples collected in Japan.

Previously, Ueyama et al. developed biomonitoring methods for the assessment of urinary NEOs levels, leading to the determination of these levels in some Japanese studies (Osaka et al., 2016; Ueyama et al., 2014, 2015). One such study analyzed changes in the levels of exposure to NEOs and OPs during daily life by quantifying these chemicals in urine samples collected from 95 adult women between 1994 and 2011 (Ueyama et al., 2015). Other studies also analyzed the levels of NEOs exposure among 703 healthy 3-year-old children (Osaka et al., 2016) and 52 adults (41 males and 11 females) who worked at two companies located in Aichi prefecture (Ueyama et al., 2014). However, no study has assessed NEOs exposure in pregnant women in Japan. Therefore, we aimed to determine the levels of exposure to 6 NEOs (ACE, IMI, THI, TMX, CLO, and DIN) and acetamiprid-N-desmethyl (NACE), a metabolite of ACE, during daily life among pregnant women in Japan, and to examine the sources of exposure.

2. Materials and methods

2.1. Study participants and sampling

Local residents of Kumamoto prefecture in Japan who eventually delivered their infants at obstetrics and gynecology clinics in Kumamoto city between 2014 and 2016 had been asked to donate spot urine specimens during health checkups in the first, second, and third trimesters of pregnancy. A total of 109 participants were enrolled. We could not obtain urine samples from 4 participants during the second trimester or from 9 participants during third trimester. Therefore, we analyzed a total of 314 urine samples (109, 105, and 100 during the first, second, and third trimesters, respectively). Basic maternal information (age, weight, height) was obtained from medical records and used to calculate the maternal body mass index (BMI). Information of pesticide use was obtained via a self-administered questionnaire after delivery. Food intake were assessed using a validated food frequency questionnaire (FFQ) with 138 food items, developed by National Cancer Center, Japan (Sasaki et al., 2003b, 2003c; Tsubono et al., 1996), which was administered during the second trimester of pregnancy. The demographic characteristics of the 109 pregnant women in this study are presented in Table 1.

This study was conducted after receiving approval from the Ethics Committees of Kumamoto University Faculty of Life Sciences on February 5, 2013 (Approval No. 628) and after obtaining written informed consent from all participants.

Table 1
Demographic data of the participants at the first health checkup visit (n = 109).

Variable	Mean	Standard Deviation	Min.	Max.
Age (years)	30.8	4.6	20.0	40.0
Height (cm)	158.5	4.9	148.0	175.2
Weight (kg)	52.4	8.0	39.0	80.0
BMI (kg/m^2)	20.9	3.0	15.9	33.7

BMI; body mass index.

2.2. Analytical method

The urine specimens were stored at −40 °C and later analyzed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). ACE, IMI, THI, TMX, CLO, DIN (purity >99%), formic acid (purity > 99%), acetic acid (purity > 99.5%), and 1 mol/L ammonium acetate (purity >28%) were obtained from Wako Pure Chemical Industries (Osaka, Japan). Acetamiprid-d6 (purity >99.7%) was obtained from Hayashi Pure Chemical Ind. (Osaka, Japan). Imidacloprid-d4 (purity >99.9%) and NACE (purity >99.8%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Phosphoric acid (purity >85%), methanol (purity >99.8%), acetonitrile (purity >99.8%), and ammonium solution (ultrapure reagent) were obtained from Kanto Chemical Co., Inc (Tokyo, Japan). A polymetric strong cation exchange solid-phase extraction (SPE) product, Bond Elut PCX (30 mg) (Agilent Technologies, Inc., Santa Clara, CA, USA), was used to extract NEOs from urine.

The urinary concentrations of NEOs were measured according to previously reported methods (Osaka et al., 2016; Ueyama et al., 2014, 2015). Briefly, LC-MS/MS was performed on an Agilent 1200 Infinity LC coupled with an Agilent 6470 Triple Quadrupole LC/MS System (Agilent Technologies, Inc., Santa Clara, CA, USA). The LC operating conditions were as follows: LC column, ZORBAX Eclipse Plus, C18, 2.1 × 100 mm, 1.8 μm, 600 Bar (Agilent Technologies, Inc., Santa Clara, CA, USA); mobile phase, (A) H_2O containing 17 mmol/L acetic acid and 5 mmol/L ammonium acetate, and (B) acetonitrile containing 17 mmol/L acetic acid and 5 mmol/L ammonium acetate; total flow rate of mobile phase, 0.2 ml/min; total run time including equilibration, 10 min. The initial mobile phase composition was 98% mobile phase A and 2% mobile phase B. The percentage of mobile phase B was changed linearly over 2 min to 5%, and changed linearly to 70% over the next 2 min. This percentage was maintained for 3 min, after which the mobile phase composition was allowed to return to the initial conditions and equilibrate for 3 min. The injection volume was 3 μl.

MS/MS was performed using an Agilent Jet Stream electrospray ionization (AJS ESI, Agilent Technologies, Inc., Santa Clara, CA, USA) source in the positive ion mode with multiple reaction monitoring. The nebulizer gas pressure was set at 35 psi with a source temperature of 325 °C and gas flow of 10 L/min. The capillary voltage was 4000 V (positive mode). The raw chromatograph and mass spectrogram data were processed using MassHunter Workstation software (Agilent Technologies, Inc., Santa Clara, CA, USA). The peak area ratio of each NEOs to the internal standard (IS) was used for the quantitative calculation of the respective NEOs.

The recoveries of ACE, IMI, THI, TMX, CLO, and DIN (6NEOs) standards ranged from 57.3 to 147%. The recoveries of 6NEOs standards were calculated by dividing the sum of the 6NEOs standards immediately before the separation analysis by the sum of the 6NEOs standards immediately after the separation analysis step. For THI, DIN, and NACE, samples measured from days that exceeded 3 standard deviations (SD) on the x-R chart were excluded from the analysis. As a result, 254, 275, and 173 samples were analyzed for THI, DIN, and NACE, respectively. The limit of detection (LOD) were calculated as values resulting in a signal-to-noise ratio of 3, and ranged from 0.26 to 1.59 μg/L (Table 2). Limit of quantitation (LOQ) were calculated as values resulting in a single-to-noise ratio of 10, and ranged from 0.86 to 5.31 μg/L (Table 2). Since prior studies in Japan showed > LOD of urinary NEOs concentration (Osaka et al., 2016; Ueyama et al., 2014, 2015), we have also shown them as comparison. For quality control (QC), urine samples were collected from three healthy volunteers who neither had received medication nor had been occupationally exposed to NEOs. As the concentrations of 6NEOs plus NACE (7NEOs) in the QC urine samples were very low, a standard mixture of the 7NEOs was added to yield a urinary NEOs concentration of 5 μg/L.

Table 2
Limit of quantitation, limit of detection, detection frequencies and percentiles of urinary NEOs concentrations in the study participants (n = 314 total urine samples).

NEOs	LOQ (μg/L)	LOD (μg/L)	>LOD (%)	Selected percentiles				
				Min.	25th	50th	75th	Max.
ACE (ug/g creatinine.)	0.86	0.26	0.6	<LOD	<LOD	<LOD	<LOD	4.79
IMI (ug/g creatinine.)	1.27	0.38	0.6	<LOD	<LOD	<LOD	<LOD	5.44
THI [a] (ug/g creatinine.)	3.85	1.16	0.0	<LOD	<LOD	<LOD	<LOD	<LOD
TMX (ug/g creatinine.)	1.42	0.43	83.4	<LOD	5.06	7.40	9.81	72.0
CLO (ug/g creatinine.)	3.43	1.03	80.9	<LOD	11.3	15.3	19.6	79.9
DIN [b] (ug/g creatinine.)	2.22	0.67	10.9	<LOD	<LOD	<LOD	<LOD	619
NACE [c] (ug/g creatinine.)	5.31	1.59	5.8	<LOD	<LOD	<LOD	<LOD	43.7
ttl4NEOs (mol/g creatinine.)				40.6	76.7	99.9	127	580

LOQ; limit of quantitation, LOD; limit of detection, ACE; acetamiprid, IMI; imidacloprid, THI; thiacloprid, TMX; thiamethoxam, CLO; clothianidin, DIN; dinotefuran, NACE; acetamiprid-N-desmethyl, ttl4NEOs; total 4NEOs (ACE, IMI, TMX and CLO). [a]; n = 254, [b]; n = 275, [c]; n = 173. ttl4NEOs include < LOD sample. For samples with levels < LOD, half the value of the LOD was recorded.

2.3. Data analysis

To determine the sources of NEOs exposure, the potential associations of the urinary NEOs concentration with seasons (n = 312), pesticide use during pregnancy (n = 39) and food intake during pregnancy (n = 93) were considered. For the seasonal analysis of urine samples, March, April, and May were classified as spring; June, July, and August as summer; September, October, and November as autumn; and December, January, and February as winter according to the Japan Meteorological Agency (Japan Meteorological Agency, 2019). To assess pesticide use, the following question was asked: "Have you used pesticides during your pregnancy? Or has anyone used pesticides around you during your pregnancy?" The response options were "yes" or "no." Intake of each food group (g/day) was calculated using the validated FFQ and total energy was adjusted using the method of residuals. Food groups included cereals, potatoes and starches, sugar, confectionaries, fats and oils, nuts and seeds, pulses, fish and shellfish, meats, eggs, milk and daily products, vegetables, pickled vegetables, green and yellow vegetables, other vegetables, fruits, fungi, algae, alcoholic beverages, nonalcoholic beverages, and seasonings and spices. According to Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF), although they were lower than the maximum residue limit, more than one of the NEOs were detected from 14 vegetables such as shungiku, leek and broccoli, and 5 fruits such as mandarins, persimmons and pears in 2014, 2015 and 2016 (Ministry of Agriculture, Forestry and Fisheries, 2016; Ministry of Agriculture, Forestry and Fisheries, 2018). Therefore, in addition to FFQ food groups, we grouped those NEOs detected vegetables and fruits as total leaf vegetables (Leek, shungiku, broccoli, spinach, komatsuna, Chinese cabbage and lettuce), total vegetables (leek, shungiku, broccoli, spinach, komatsuna, Chinese cabbage, lettuce, eggplant, green pepper and green beans), total fruits (mandarins, persimmons, melon, pears) and total vegetables and fruits (total vegetables and total fruits), and association with urinary NEOs concentration was analyzed. The energy intake <500 kcal/day or >5000 kcal/day were defined as outside of energy intake limit and it was referred to previous study which targeted pregnant women in Japan (Takahashi et al., 2016). Subjects with energy intakes outside of the predefined limits was not observed in this study.

The detection frequencies (>LOD) of ACE, IMI, THI, DIN and NACE were very low in this study (Table 2), therefore only TMX, CLO and total of 4 NEOs (ttl4NEOs: ACE, IMI, TMX and CLO) were statistically analyzed. For samples with concentrations below the LOD, the statistical analysis was performed using half the value of the LOD. Friedman test was performed to identify potential associations of seasons, Mann-Whitney *U* test was performed to pesticide use during pregnancy. To identify association of food intake amounts during pregnancy with exposure to NEOs, Mann-Whitney *U* test was performed as first step and multiple logistic regression analysis with stepwise variable selection method was conducted as second step with included variables $p < 0.200$ in Mann-Whitney *U* test, maternal age and maternal BMI before pregnancy. Because tap water could be a potential risk of IMI exposure (Klarich et al., 2017), tap water intake was also included as independent variables for multiple logistic regression analysis. As a result of Friedman test, there was no significant difference observed in any urinary NEOs between first, second and third trimesters. Therefore, average value of urinary NEOs in these 3 trimesters was used for analysis of pesticide use during pregnancy and food intake amount during pregnancy. Statistical analyses were conducted using the IBM® SPSS® statistics 24 (Mac® client version, IBM Co., Armonk, NY, USA). A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Urinary NEOs levels among pregnant women

The urinary NEOs concentrations and distributions in this study are summarized in Table 2. The > LOD of TMX and CLO were high, at 83.4% and 80.9%, respectively. In contrast, ACE, IMI, DIN and NACE were only detected in a few participants (0.6%, 0.6%, 10.9 and 5.8%, respectively). THI was not detected in any analyzed urinary samples. The median and maximum levels of the ttl4NEOs were 40.6 and 580 nmol/g creatinine, respectively.

3.2. Sources of NEOs exposure

The median NEOs concentrations were compared to the seasons and any significant difference was found between four seasons. (spring, summer, autumn, winter; TMX: 7.62, 6.74, 6.69, 7.07 μg/g creatinine, p = 0.186; CLO: 15.6, 16.1, 13.9, 14.3 μg/g creatinine, p = 0.463; ttl4NEOs: 102, 97.8, 97.0, 93.3 nmol/g creatinine, p = 0.481).

To determine the effect of pesticide use during pregnancy on the urinary NEOs concentration, we compared the median concentrations of NEOs between the "pesticide use" (PesUse, n = 20) and "non-pesticide use" (PesNoUse, n = 19) groups. However, no significant associations in the median values were observed (TMX: 7.54 vs 9.20 μg/g creatinine, p = 0.214; CLO: 14.9 vs 17.0 μg/g creatinine, p = 0.322; ttl4NEOs: 97.6 vs 113 nmol/g creatinine, p = 0.258).

To determine the associations between NEOs and food intake, TMX, CLO and tt4NEOs were divided into 2 groups, namely "below median (< median)" and "greater than or equal to median (≥ median)." We put these 2 groups of urinary NEOs as dependent variable and food groups that associated with any NEOs of $p < 0.200$ by Mann-Whitney *U* test (sugar, confectionaries, fats and oils, pulses, fish and shellfish, meats, milk and daily products, fruits, alcoholic beverages), tap water, maternal age and maternal BMI before pregnancy as independent variables to perform multiple logistic regression analysis. Table 3 showed variable $p < 0.05$ in the multiple logistic regression analysis. As a result of the analysis, slightly but significant association of pulses in CLO (1.01 [1.00–1.02]) was observed (Table 3). We also statistically analyzed between NEOs and vegetables and fruits that reported NEOs detection

Table 3
Multiple logistic regression analysis for food intake based on urinary NEOs concentration.

NEOs	Food group	adjusted OR (95%CI)	*P*-value
CLO	pulses	1.01(1.00-1.02)	0.015[a]

[a] ; <0.05, NEOs; neonicotinoids, CLO; clothianidin, OR; odds ratio, CI; confidence interval. n = 93 (n = 46 for < median, n = 47 for ≥ median). Dependent variable: CLO, TMX and ttl4NEOs (AEC, IMI, TMX and CLO). Independent variables: sugar, confectionaries, fats and oils, pulses, fish and shellfish, meats, milk and daily products, fruits, alcoholic beverages, tap water, age and BMI. Reference; <median.

by MAFF. However, statistical significant association were not observed between urinary NEOs and those vegetables and fruits.

4. Discussion

The present study was the first to analyze the urinary concentrations of NEOs in pregnant women in Japan. So far, only three studies have analyzed urinary NEOs concentrations in the Japanese population (Osaka et al., 2016; Ueyama et al., 2014, 2015). These studies included 52 Japanese individuals of both sexes in Aichi prefecture (Ueyama et al., 2014), 95 women in Kyoto prefecture and surrounding areas (Ueyama et al., 2015), and 703 3-year-old children in Aichi prefecture (Osaka et al., 2016). In our study, consistent with the findings of Ueyama and colleagues (Ueyama et al., 2014), high detection frequencies for TMX and CLO in urine samples were observed (83%, and 81% in our study vs 100%, and 96% in the study by Ueyama et al. (2014), respectively). Moreover, the median urinary TMX and CLO concentrations in our study were not only higher than those reported in previous studies, but also higher than the maximum value (TMX: 7.40 μg/g creatinine (median) in our study vs 0.23–0.63 μg/g creatinine (median) and 0.52–7.25 μg/g creatinine (maximum) in the previous studies; CLO: 15.3 μg/g creatinine (median) in our study vs < LOD (median) and 1.67–15.2 μg/g creatinine (maximum) in the previous studies) (Osaka et al., 2016; Ueyama et al., 2015). In this study, LOD of ACE, THI and DIN were not as low as previous studies (ACE: 0.26 μg/L in this study vs 0.01–0.03 μg/L in the previous studies; THI: 1.15 μg/L in this study vs 0.06–0.3 μg/L in the previous studies; DIN: 0.67 μg/L in this study vs 0.1–0.32 μg/L in the previous studies, respectively) (Osaka et al., 2016; Ueyama et al., 2014, 2015) and the detection frequencies of ACE, IMI, THI and DIN were low, therefore we could not compare the urinary concentration level of those NEOs to previous studies.

The greatest concern of NEOs exposure among pregnant women is the effect on fetus. Prior studies suggested the association between maternal IMI exposure during pregnancy and incidence of tetralogy of Fallot, anencephaly (Carmichael et al., 2014; Yang et al., 2014), increase risk of preterm birth (Ling et al., 2018), neural tube defects (Rull et al., 2006; Yang et al., 2014) and reduction in IQ (Gunier et al., 2017) of infant. Furthermore, Ohno et al. suggested CLO through the placental barrier in vivo study (Ohno et al., 2020) and Ichikawa et al. reported DIN and NACE were detected from urine sample of infant within 48 h after birth (Ichikawa et al., 2019). These previous studies suggest that maternal NEOs exposure during pregnancy may result in fetal NEOs exposure and may adversely affect the postnatal health of their offspring. In our study, CLO was detected from most of the subjects (80.9%) and this may imply a number of fetus are exposed to CLO. However, research on the effects of NEOs on the fetus, including in vivo study is limited. Therefore, further in vivo and epidemiological research is needed.

The total shipments of NEOs have increased by almost 7 times in 2017 compared to 1996 (National Institute for Environmental Studies, 2019). The NEOs detection frequencies in urine samples from Japanese women living in Kyoto prefecture and the surrounding area has also increased significantly between 1994 and 2011 (Ueyama et al., 2015). Taken together, these data suggest a potential relationship between the amounts of NEOs shipments and human exposure levels. The total shipment amounts for the 6NEOs analyzed in this study were higher in Kumamoto prefecture, where our subjects resided, than in Kyoto prefecture. In addition, the levels of four NEOs (ACE, THI, DIN, and IMI) were higher than those in Aichi prefecture, the site of previous related studies (Osaka et al., 2016; Ueyama et al., 2014). Therefore, we expected higher urinary concentration levels of these NEOs in our samples, compared to those from other areas of Japan. However, our study did not identify a correlation between the amounts of NEOs shipped to a prefecture and the urinary NEOs concentrations of residents.

The associations of urinary NEOs concentrations with seasons, pesticide use and food intake were analyzed to identify potential sources of NEOs exposure. Osaka et al. reported that urinary NEOs concentrations in 3-year-old children were significantly higher in the summer (August–September) than in the winter (February) (Osaka et al., 2016). Our study also considered urinary NEOs concentrations between four seasons, however no association was observed. The Kumamoto city branch of the Japan Agricultural Cooperatives (JA-Kumamoto) reported 17 fruits and vegetables (rice, soybeans, melon, watermelon, strawberry, eggplant, tomato, green paper, lotus root, cabbage, broccolini, mandarin orange, Japanese pear, Japanese apricot, green onion, onion, and Japanese parsley) that are the major fruits and vegetables consumed in Kumamoto city, where participants in this study resided (Japan Agricultural Cooperatives Kumamotoshi, 2013). Of these major fruits and vegetables (except Japanese parsley, no data), IMI and TMX are used as pesticide for more than half of them (81.3% for IMI and 56.3% for TMX) (Kumamoto prefectural government, 2019). These NEOs were used at various times during the growth cycle (e.g., seeding, planting, before harvesting) and may have varied depending on the type of vegetable (Kumamoto vegetable promotion association, 2019). In addition, many vegetables are cultivated throughout the year. Therefore, these may be a reason that seasonal difference was not observed in our study.

In our study, statistical association between urinary CLO and food intake was observed. The ≥ median CLO group reported slightly but significantly higher pulses intake than the <median group. Soybeans is among the most common ingredients used in foods in Japan, and is a component of many foods such as miso soup, soy sauce, tofu, and natto. Most of the soybeans eaten in Japan are imported product. According to a report by MAFF, domestic production of soybeans from 2014 to 2016, when this study was conducted, was 232,000 to 243,000 tons, and imports were 2,828,000 to 3,243,000 tons (Ministry of Agriculture, Forestry and Fisheries, 2016). In addition, the largest imports are from the United States, with 66.6–72.1% of soybeans imports from 2014 to 2016 coming from United States (Ministry of Finance, 2020). It is reported 34–44% of soybeans hectares in United States were seed-applies by NEOs in 2011 (Douglas and Tooker, 2015). Possibly, soybeans seed-applied by CLO leading to human exposure via processed soybeans food intake.

Drinking water could be a potential risk of NEOs exposure. The distributions and leaching potentials of NEOs were examined in Brazil, where IMI, THI, and CLO were found to potentially leach into groundwater (Miranda et al., 2011). In the United States, IMI was detected in tap water (Klarich et al., 2017). In Japan, NEOs contamination in tap water has not been assessed yet, but the National Institute for Environmental Studies reported that NEOs were detected in waterways throughout Japan (National Institute for Environmental Studies, 2016). Due to this background data, we also included tap water intake amount as an independent variable to multiple logistic regression analysis. However statistical significant association was not observed. Further studies are needed to determine the levels of residue and the assessment of NEOs contamination in tap water in Japan.

There are some limitations in this study. First, the use of spot urine sample to evaluate NEOs exposure level and using the FFQ to evaluate trends in food intake represents have some limitations. Overall, 24-h

urine collection may be preferable because the half-lives of urinary biomarkers are relatively short (Osaka et al., 2016). Moreover, ideally, NEOs with short half-lives would be analyzed using a duplicated method that would better reflect the relationships of these chemicals with the most recently consumed meals. In this study, quantitative data on the amount of the food that was consumed before the sampling was not taken. The FFQ refer to the general dietary habits not directly prior to sampling. Although these limitations, in this study, urine samples were collected during the three pregnancy periods, first, second and third trimester, and there was no statistical significant difference between these three urinary NEOs. Therefore, even though spot urine sample was used, we think it was reflected some daily exposure of NEOs. Furthermore, validity of FFQ is validated using 28 day dietary record and the reproducibility of FFQ is validated at 1 year interval (Sasaki et al., 2003a; 2003c). Although the correlation coefficient is not high in validation, it was reported that it can be used to rank individuals according to intakes for the food groups (Sasaki et al., 2003c). Therefore, we think that the intake amount of each food group reflect higher or lower food intake.

This study had some other limitations. The numbers of participants who answered questions regarding the use of pesticides was very small ($n = 39$), which may have reduced the statistical power. Accordingly, further studies with larger sample sizes are needed. In addition, although statistical significance was observed between urinary CLO and pulses intake in this study, it does not indicate NEOs concentration in food. Therefore, in order to clarify the causal relationship of NEOs human exposure from food intake, further research is necessary including food analysis.

5. Conclusion

In this study we determined the urinary NEOs concentrations in Japanese pregnant women and they were exposed to NEOs in their daily lives. Furthermore, the participants in our study had been exposed to TMX and CLO in levels higher than other populations in Japan. As sources of NEOs exposure, the intake of pulses was suggested as potential source of NEOs exposure. These results may facilitate further assessments of NEOs exposure risk in humans.

Declaration of competing interest

None.

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Urinary parabens and their potential sources of exposure among Korean children and adolescents: Korean National Environmental Health Survey 2015–2017

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ABSTRACT

Parabens are used as a preservative in several consumer products including cosmetics, personal care products, and medicinal products. These chemicals have been suspected for estrogenicity and potential adverse endocrine outcomes in humans. For the first time, exposure profiles and potential sources of major parabens are investigated for a nationally representative population of children and adolescents of Korea. In addition, major determinants of urinary paraben levels were identified. For this purpose, the children, and adolescents (n = 2355, 3–18 years of age) who participated in the Korean National Environmental Health Survey cycle 3 (2015–2017) were studied. Adjusted multiple linear regression models were employed to investigate the relationships of several potential demographic and behavioral determinants of exposure, with the urinary levels of three parabens; methyl, ethyl, and propyl paraben. Methyl and propyl paraben levels of the Korean children and adolescents were comparable to those of the US, but the high exposure group (95th percentile) showed much higher levels of exposure. Moreover, urinary ethyl paraben levels are always higher than those of other countries. The uses of personal care products including liquid soaps, fragrance products, nail polish, or antiseptic products were significantly associated with urinary paraben levels. In addition, dietary sources such as fast food and canned food consumption were identified as major contributors to ethyl paraben levels. For methyl and propyl parabens, the use of fever medications and ointments were identified as major determinants of the exposure, especially among the younger children of 3–5 years of age. These observations are related to the Korean regulations that permit the use of the parabens as preservatives in foods and medications. The findings demonstrate that the exposure profile of parabens among Korean children are unique, and mitigation efforts for some parabens are required in Korea. Further studies are warranted to confirm the exposure sources of parabens and to develop mitigation measures among Korean children and adolescents.

1. Introduction

Parabens are alkyl esters of 4-hydroxybenzoic acid. Since its first use in medicine in the 1920s, primarily to inhibit microbial growth and extend product shelf life, parabens have been extensively used as a preservative in numerous consumer products, including cosmetics, personal care products, and medications (Soni et al., 2005). In cosmetics, parabens are commonly used as preservatives (Rastogi et al., 1995). In personal care products such as moisturizers, deodorants, and shampoo, parabens are added for their antimicrobial functions

Abbreviations: CHMS, Canadian Health Measures Survey; CI, Confidence Intervals; Ctree, Conditional interference trees; GerES, the German Environmental Survey; GM, Geometric mean; KoNEHS, Korean National Environmental Health Survey; LOD, Limit of Detection; MOE, Ministry of Environment; NHANES, National Health and Nutrition Examination Survey; NIER, National Institute of Environmental Research.

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(Gruvberger et al., 1998; Guo et al., 2014).

Parabens are generally considered as safe to humans at the levels permitted by regulatory standards. However, contradictory evidence is increasing in both epidemiologic and experimental studies. Studies have indicated estrogenic properties of parabens with a longer aliphatic chain, although short-chain esters were observed to exert estrogenic effects (Boberg et al., 2010; Sun et al., 2016). In animals, propyl paraben was reported to affect the activity of a sex hormone like testosterone (Oishi, 2002). Parabens have also been associated with breast cancer development (Darbre et al., 2004; Darbre and Harvey, 2014). Parabens have been found to may affect various disorders in the body with endocrine-disrupting chemicals (Boberg et al., 2010; Nishihama et al., 2016; Nowak et al., 2018; Watanabe et al., 2013). Because of their extensive use, exposure to parabens through oral, dermal, and inhalation routes is widespread in daily lives (Błędzka et al., 2014; Dodge et al., 2018). Most of the parabens are excreted through urine as metabolites, e.g., *p*-hydroxyhippuric acid, sulfate-, and glucuronide-conjugated forms (Abbas et al., 2010; Soni et al., 2005).

Because of health concerns, several national scale biomonitoring programs have been conducted for parabens in countries including Canada, France, Germany, and US (CDC, 2021; Fillol et al., 2021; Health Canada, 2019; Murawski et al., 2020). However, among the Korean population, information on the current status of exposure to major parabens, especially in children and adolescents, along with potential sources of exposure, is limited.

This study determined the exposure levels of three most frequently used parabens, methyl, ethyl, and propyl paraben, in the children and adolescents who participated in the Korean National Environmental Health Survey (KoNEHS) Cycle 3 (2015–2017). In addition, potential sources of exposure among Korean children and adolescents were investigated. The results of the study will help understand high priority parabens and subpopulations of high exposure among Korean children and adolescents. Furthermore, this information will aid in developing appropriate mitigation measures for the paraben exposure among the general population of Korea.

2. Methods

2.1. Population and study design

The study population consisted of the children and adolescents (n = 2380; between 3 and 18 years of age) who participated in the KoNEHS Cycle 3 (2015–2017), conducted by the Korean Ministry of Environment. Among them, 25 subjects were excluded from the final analysis because of the insufficient volume of urine samples, and therefore the final number of the participating children and adolescents was 2355.

KoNEHS is a cross-sectional survey designed to monitor the exposure levels of environmental chemicals among representative Korean population using a multiple stage sampling method (Choi et al., 2017; Park et al., 2016). For children and adolescents, random sampling was performed following region, sex, and age stratification based on population data of Korea. The sampling units were the childcare or educational institutions, e.g., kindergarten, schools (Ha et al., 2014).

Written informed consent as well as information regarding the participants were collected through questionnaires from their parents or guardians. The questionnaire included demographic and socioeconomic information, and other factors related to environmental chemical exposure. This study was approved by the Ethical Review Board of the National Institute of Environmental Research (NIER), Korea (IRB No. NIER-2015-BR-006-01, July 20, 2015).

2.2. Measurement of urinary parabens

Once sampled, urine specimens were stored in a refrigerator and transported under cold (0–4 °C) conditions to the laboratory, where the samples were kept at −70 °C until analysis. Urine samples were hydrolyzed using β-glucuronidase/sulfatase, were acidified with formic acid, and preconcentrated using solid-phase extraction. Methyl paraben, ethyl paraben, and propyl paraben in urine were quantified employing an ultra-high performance liquid chromatograph-mass spectrometry (Agilent 6490, Agilent, Santa Clara, CA, USA) (NIER, 2018). Limits of detections (LODs) were as follows: methyl paraben: 0.108 μg/L; ethyl paraben: 0.107 μg/L; and propyl paraben: 0.082 μg/L. An internal quality control of the analysis was ensured by analyzing two quality controls (low and high) in each batch of analyses. External quality assurance including accuracy of the analytical method was conducted by participating in laboratory comparison programs, including the G-EQUAS (German External Quality Assessment Scheme).

Urinary creatinine was measured using the kinetic Jaffe reaction method and was analyzed by the ADVIA 1800 Auto Analyzer (Siemens Medical Solutions Diagnosis, USA). Internal quality control was ensured by analyzing commercial reference materials (Liquichek Urine Chemistry Control, Bio-Rad). External quality and accuracy of the analytical method were assessed by participating in interlaboratory comparison programs (College of American Pathologists).

2.3. Statistical methods

The distribution of urinary parabens was skewed; thus log-transformation was performed before statistical analyses and values below the LODs, LODs were divided by $1/\sqrt{2}$ (Hornung and Reed, 1990). The urinary paraben concentrations were adjusted with the urinary creatinine (μg chemical/g creatinine) for urine dilution (Barr et al., 2005; Uchida and Gotoh, 2002).

For the frequencies of use of personal care products, most responses were grouped into four categories, i.e., 'no use', 'less than once a week', 'once a week and more', 'within two days'; For food consumption questions, most responses were grouped into four categories as well, i.e., 'do not eat', 'eat less than once a week', 'eat once a week or more', 'eat once a day or more.' The covariates such as age, sex, monthly household income, regional area, exercise time, and indoor activity time were included as categorical variables.

The exposure risks of parabens associated with personal care products and food were calculated based on the survey weighted multiple linear regression. In addition, an unbiased recursive partitioning-based permutation test using conditional inference trees (CTree) was conducted to identify and visualize the relationship between critical exposure factors and paraben levels. CTree is a non-parametric class of regression trees embedding tree-structured regression models into a defined theory of conditional inference procedures (Hothorn et al., 2006).

Statistical significance was determined at $p < 0.05$. For statistical analysis of each group, SAS survey procedures ('surveyfreq', 'surveymean', 'surveyreg') were used, reflecting the population weight adjustment as well as the multistage sample design using SAS 9.4 (SAS Institute, Cary, NC, US). For CTree statistical analysis, R version 3.6.3 was used and a *p* value lower than 0.05 indicated statistical significance.

3. Results

3.1. Characteristics of the study population

Demographic characteristics distribution according to age, sex, BMI categories, regional area, monthly household income, exercise time, and indoor activity time are presented in Table 1. The females comprised 50.5% of the population. The median BMIs for subgroups of 3–5, 6–11, and 12–18 years of age were 16.0, 17.6, and 21.4, respectively. Urban and rural areas were divided according to classification of the administrative district (urban: -dong; rural: -eup, -myeon). Then, urbanized rural areas were divided into separate peri-urban areas (Lee and Lee, 2018). Industrial areas were designated as a neighborhood of identifiable industrial facilities. Most subjects lived in the urban area (61.0%),

Table 1
Weighted geometric mean and 95% confidence interval (μg/g creatinine) for parabens according to population group and characteristics of residential environment.

	N	(%)	Weighted N	(%)	Methyl paraben		Ethyl paraben		Propyl paraben	
					GM	95% CI	GM	95% CI	GM	95% CI
Total	2355	100	7337225	100	24.5	(21.6, 27.8)	12.2	(10.3, 14.3)	2.2	(1.9, 2.6)
Age group										
3–5 years	571	24.2	1383291	18.9	***55.1***	***(45.0, 67.5)***	***16.9***	***(12.7, 22.4)***	***5.2***	***(4.0, 6.7)***
6–11 years	884	37.6	2663460	36.3	***26.6***	***(22.8, 31.1)***	***10.5***	***(8.1, 13.7)***	***1.7***	***(1.4, 2.0)***
12–18 years	900	38.2	3290474	44.8	***16.3***	***(13.7, 19.3)***	***11.9***	***(9.1, 15.6)***	***2.0***	***(1.6, 2.5)***
Sex										
Male	1165	49.5	3835294	52.3	***20.0***	***(16.7, 24.1)***	13.1	(10.6, 16.1)	***1.7***	***(1.4, 2.0)***
Female	1190	50.5	3501931	47.7	***30.5***	***(26.8, 34.7)***	11.3	(9.4, 13.5)	***3.0***	***(2.6, 3.6)***
BMI Category [a]										
1st Quartile	590	25.1	1793452	24.4	***24.8***	***(20.1, 30.8)***	12.4	(9.9, 15.5)	***2.5***	***(2.0, 3.1)***
2nd Quartile	592	25.1	1872634	25.5	***26.9***	***(22.4, 32.4)***	12.3	(10.1, 15.1)	***2.4***	***(2.0, 3.0)***
3rd Quartile	587	24.9	1854499	25.3	***25.9***	***(21.5, 31.3)***	11.4	(8.8, 14.8)	***2.3***	***(1.8, 2.8)***
4th Quartile	586	24.9	1816640	24.8	***20.6***	***(17.0, 25.0)***	12.5	(9.8, 16.0)	***1.8***	***(1.5, 2.2)***
Regional area										
Rural area	468	19.9	843273	11.5	28.1	(20.2, 39.1)	***16.8***	***(10.5, 27.0)***	2.3	(1.8, 2.9)
Peri-urban area	124	5.2	490602	6.7	20.8	(13.2, 32.8)	***10.0***	***(5.3, 18.9)***	2.1	(1.5, 2.9)
Urban area	1436	61.0	4916386	67.0	24.6	(21.1, 28.8)	***12.5***	***(10.2, 15.4)***	2.4	(2.0, 2.8)
Industrial area	327	13.9	1086964	14.8	22.9	(16.3, 32.3)	***9.2***	***(6.6, 12.7)***	1.8	(1.2, 2.6)
Monthly household income (US dollars) [b]										
Do not know	107	4.2	329788	4.5	28.8	(18.5, 44.7)	11.5	(7.5, 17.7)	2.9	(1.7, 4.9)
<2350	218	8.9	688044	9.4	22.6	(17.8, 28.6)	12.9	(9.4, 17.8)	2.3	(1.6, 3.2)
2350–3525	341	13.5	998808	13.6	25.2	(20.4, 31.3)	14.3	(10.8, 18.9)	2.3	(1.8, 2.9)
3525–5875	833	35.2	2533437	34.5	25.9	(22.0, 30.5)	12.2	(10.0, 14.9)	2.3	(1.9, 2.8)
>5875	856	38.2	2787148	38.0	23.0	(19.1, 27.7)	11.4	(9.3, 14.0)	2.1	(1.7, 2.6)
Exercise time (min)										
Do not	1001	42.5	2907120	39.6	***29.5***	***(25.0, 34.9)***	11.9	(9.8, 14.4)	***2.6***	***(2.2, 3.2)***
<30	482	20.5	1468494	20.0	***25.8***	***(20.3, 32.7)***	12.8	(10.1, 16.3)	***2.1***	***(1.6, 2.7)***
30–60	543	23.1	1880314	25.6	***20.7***	***(16.9, 25.3)***	12.4	(9.8, 15.7)	***2.0***	***(1.6, 2.5)***
>60	329	13.9	1081297	14.8	***18.4***	***(15.1, 22.5)***	11.5	(8.4, 15.8)	***2.0***	***(1.6, 2.7)***
Indoor activity time (min)										
<600	570	24.2	2058331	28.1	***15.8***	***(13.1, 19.2)***	12.7	(9.2, 17.6)	***1.8***	***(1.4, 2.4)***
600–770	508	21.6	1532847	20.9	***21.2***	***(17.7, 25.5)***	12.6	(10.1, 15.8)	***2.0***	***(1.6, 2.4)***
770–840	686	29.1	1965768	26.8	***28.4***	***(23.6, 34.0)***	11.7	(9.2, 14.8)	***2.5***	***(1.9, 3.1)***
>840	591	25.1	1780279	24.2	***38.8***	***(32.4, 46.5)***	11.7	(9.3, 14.7)	***2.8***	***(2.2, 3.5)***

NOTE: $p < 0.05$.
[a] BMI Category 3–5 years (25%=15.0, 50% =16.0, 75% =17.1), 6–11 years (25%=15.7, 50% =17.6, 75% =20.3), 12–18 years (25%=19.5, 50% =21.4, 75% =23.7).
[b] Currency: 1175 won/dollar.

followed by the rural area (19.9%), industrial area (13.9%), and peri-urban area (5.2%). Age and exercise time were negatively correlated with two or more paraben concentrations in the urine (Table S1).

3.2. Distributions of the concentrations of parabens

Among the young Korean population, the urinary concentrations of all three parabens tended to be higher among the 3–5 years old group, and this trend was more outstanding for methyl and propyl parabens. Methyl parabens were detected at 55.1 μg/g creatinine (GM) among the 3–5 years old children, whereas it was at 26.6 μg/g creatinine among 6–11 years old, and 16.3 μg/g creatinine among the 12–18 years old adolescents. For propyl paraben, GMs of 5.2, 1.7, and 2.0 were detected for the 3–5, 6–11 and 12–18 years old group, respectively. On the other hand, ethyl parabens were detected at 16.9, 10.5, and 11.9 μg/g creatinine (GM) in the 3–5, 6–11 and 12–18 years old groups, respectively. Urinary creatinine concentrations measured for the 3–5, 6–11 and 12–18 years old groups were 0.840, 1.084 and 1.606 g/L (GM), respectively (Table S3). Compared to males, females showed higher methyl, propyl paraben concentrations, and lower ethyl paraben concentrations (Table 1).

3.3. Comparison with other national biomonitoring studies

The urinary parabens for the five countries are shown in Table 2. The national biomonitoring studies except Esteban showed the highest urinary methyl and propyl parabens concentrations in the youngest population and the 95 percentiles of the 3–5 years group had the highest concentration. Among several countries, KoNEHS had the highest concentration of methyl paraben (GM: 55.1 μg/g creatinine), followed by NHANES, CHMS and GerES (GM: 53.5, 17, 15.12 μg/g creatinine). NHANES had the highest concentration of propyl paraben (GM: 7.5 μg/g creatinine), followed by KoNEHS, CHMS and GerES (GM: 5.2, 2.0, 0.695 μg/g creatinine). Interestingly, the KoNEHS had the highest concentration of ethyl paraben (GM: 16.9 μg/g creatinine). In comparison, other countries were very low (GerES 0.930 μg/g creatinine) or not detected.

3.4. Evaluation of the paraben exposure factors

Multiple linear regression analyses showed that the urinary levels of ethyl paraben were positively associated with fast food consumption and canned food consumption, after adjusting for covariates (Table 3). No other dietary factors were associated with urinary levels of methyl and propyl parabens. Among pharmaceuticals and personal care products, several items were identified to be associated with elevated levels of urinary parabens (Tables 4 and 5). Use of liquid soaps including shower gel and shampoo was associated with ethyl paraben levels. For propyl paraben levels, uses of fragrance and antiseptic products were also significantly associated (Table 4). Among pharmaceuticals, the use of medications for fever antipyretics and dermatitis (ointments) were significantly associated with the urinary methyl and propyl parabens (Table 5).

The CTree analysis identified the use of fever medication as an initial discriminator for methyl paraben, followed by age. For children who

Table 2
Urinary concentrations of three parabens (μg/g creatinine) among Korean children and adolescents, in comparison with those reported in national biomonitoring programs.

	KoNEHS 2015–2017					NHANES 2015–2016[a]					CHMS 2016–2017[b]					GerES 2014–2017[c]				Esteban 2014–2016[d]			
	N	DR	GM	75th	95th	N	DR	GM	75th	95th	N	DR	GM	75th	95th	N	DR	GM	95th	N	DR	GM	95th
Methyl paraben																							
3–5 years	571	100	55.1	178	5365	140	97.9	53.5	198	2210	542	88.9	17	44[e]	-[f]	93	96	15.12	1130	398	94.2	5.3	311.4
6–11 years	884	100	26.6	89.8	754	415	96.1	22.2	60.2	684	531	88.4	8.7	19	-[f]	155	95	7.255	715				
12–18 years	900	100	16.3	58.7	309	405	98.3	18.8	65.4	467	531	87.5	7.2	30	190[e]	145	99	5.208	261				
Ethyl paraben																							
3–5 years	571	99.0	16.9	81.4	456	140	38.6	NC	5.00	83.0	542	35.8	NC	2.5[e]	-[f]	99	58	0.930	3.53	398	29.4	NC	17.5
6–11 years	884	98.9	10.5	56.8	440	415	35.9	NC	2.54	16.0	531	26.3	NC	NC	-[f]	166	71	0.921	6.54				
12–18 years	900	99.6	11.9	38.3	177	405	39.3	NC	1.64	12.7	531	28.2	NC	1.0	27[e]	149	76	0.912	10.1				
Propyl paraben																							
3–5 years	571	99.3	5.2	21.3	824	140	100	7.5	16.3	724	542	70.7	2.0[e]	5.8[e]	53[e]	99	27	0.695	101	398	30.9	NC	45.1
6–11 years	884	94.6	1.7	5.7	99.4	415	98.3	2.7	6.21	69.5	531	70.3	-[f]	-[f]	-[f]	166	33	0.551	40.7				
12–18 years	900	97.9	2.0	5.8	94.9	405	98.8	2.4	10.1	103	531	70.1	-[f]	3.8[e]	-[f]	149	36	0.469	10.0				

Abbreviations: KoNEHS, Korean National Environmental Health Survey; NHANES, National Health and Nutrition Examination Survey; CHMS, Canadian Health Measures Survey; GerES, the German Environmental Survey; GM, Geometric mean; DR, Detection rate; LOD, Limit of Detection; LOQ, Limit of Quantification, NC, Detection rate <60% the analysis was performed but the geometric mean was not calculated.

[a] US national biomonitoring 3–5 years, 6–11 years, 12–19 years, U.S. (CDC, 2021).

[b] Canada national biomonitoring 3–5 years, 6–11 years, 12–19 years (Health Canada, 2019).

[c] Germany national biomonitoring 3–5 years, 6–10 years, 14–17 years (Murawski et al., 2020).

[d] France national biomonitoring 6–17 years (Fillol et al., 2021).

[e] Use data with caution (CV between 16.6% and 33.3%).

[f] Data is too unreliable to be published (CV > 33.3%).

Table 3
Multiple linear regression analysis and 95% confidence intervals for parabens in dietary factors.

	N (%)	Weighted N (%)	Methyl paraben			Ethyl paraben			Propyl paraben		
			β	95% CI		β	95% CI		β	95% CI	
Consumption frequency											
Fast food[a]											
Do not eat	578 (24.5)	1666968 (22.7)	ref.			ref.			ref.		
Less than once a week	1456 (61.8)	4591778 (62.6)	−0.199	−0.448	0.049	0.076	−0.272	0.424	−0.060	−0.357	0.237
Once a week or more	316 (13.5)	1067780 (14.6)	0.157	−0.285	0.600	***0.538***	***0.047***	***1.030***	0.008	−0.439	0.456
Once a day or more	5 (0.2)	10699 (0.1)	−0.687	−1.818	0.444	***2.385***	***1.349***	***3.421***	0.321	−1.097	1.739
Frozen food											
Do not eat	1000 (42.5)	3064147 (41.8)	ref.			ref.			ref.		
Less than once a week	1120 (47.6)	3478993 (47.4)	−0.068	−0.280	0.144	0.022	−0.278	0.321	−0.104	−0.323	0.115
Once a week or more	222 (9.4)	760698 (10.3)	−0.078	−0.555	0.399	−0.008	−0.527	0.511	−0.149	−0.740	0.441
Once a day or more	13 (0.5)	33386 (0.5)	1.369	−0.449	3.187	0.542	−2.584	3.668	1.568	−1.609	4.745
Beverages											
Do not eat	1114 (47.3)	3194723 (43.5)	ref.			ref.			ref.		
Less than once a week	536 (22.7)	1759684 (24.0)	−0.156	−0.519	0.208	−0.245	−0.654	0.163	−0.084	−0.461	0.292
Once a week or more	597 (25.4)	2073712 (28.3)	0.083	−0.270	0.435	***−0.398***	***−0.757***	***−0.038***	0.144	−0.270	0.558
Once a day or more	108 (4.6)	309107 (4.2)	−0.025	−0.520	0.471	0.095	−0.381	0.572	−0.056	−0.646	0.533
Canned food											
Do not eat	879 (37.3)	3028724 (41.3)	ref.			ref.			ref.		
Less than once a week	850 (36.1)	2408615 (32.8)	−0.206	−0.515	0.103	***0.464***	***0.043***	***0.884***	−0.124	−0.515	0.268
Once a week or more	571 (24.3)	1698995 (23.2)	0.006	−0.360	0.372	***0.609***	***0.192***	***1.026***	0.042	−0.401	0.485
Once a day or more	55 (2.3)	200891 (2.7)	−0.188	−1.196	0.820	0.596	−0.834	2.026	0.297	−0.752	1.345

NOTE: ***p < 0.05.***
[a] Pre-cooked meals, mass-produced ingredients.

Table 4
Multiple linear regression analysis and 95% confidence intervals for parabens in personal care products.

	N (%)	Weighted N (%)	Methyl paraben			Ethyl paraben			Propyl paraben		
			β	95% CI		β	95% CI		β	95% CI	
Product use frequency											
Liquid soap [a]											
No use	444 (18.9)	1410477 (19.2)	ref.			ref.			ref.		
Less than once a week	71 (3.0)	232412 (3.2)	***−0.595***	***−1.165***	***−0.025***	0.111	−0.614	0.836	−0.490	−1.078	0.097
Once a week and more	897 (38.1)	2709395 (36.9)	−0.277	−0.593	0.039	***0.311***	***0.013***	***0.609***	−0.198	−0.537	0.141
Within 2 days	943 (40.0)	2984941 (40.7)	0.032	−0.281	0.346	***0.386***	***0.052***	***0.720***	0.118	−0.236	0.473
Color cosmetics											
No use	1730 (73.5)	5384730 (73.4)	ref.			ref.			ref.		
Less than once a week	43 (1.8)	140440 (1.9)	0.135	−0.809	1.078	−0.168	−1.348	1.012	0.065	−0.937	1.066
Once a week and more	130 (5.5)	386090 (5.3)	0.148	−0.355	0.652	0.244	−0.327	0.815	0.370	−0.293	1.033
Within 2 days	452 (19.2)	1425965 (19.4)	−0.07	−0.422	0.283	0.297	−0.063	0.657	0.109	−0.423	0.642
Fragrance products											
No use	2142 (91.0)	6633288 (90.4)	ref.			ref.			ref.		
Less than once a week	45 (1.9)	148257 (2.1)	0.137	−0.998	1.271	−0.072	−1.427	1.283	−0.002	−1.375	1.372
Once a week and more	76 (3.2)	267292 (3.6)	1.451	−0.311	3.214	1.034	−1.335	3.403	1.685	−0.922	4.293
Within 2 days	92 (3.9)	288388 (3.9)	−0.447	−1.998	1.103	0.14	−0.651	0.93	***2.373***	***1.291***	***3.455***
Nail polish											
No use	2221 (94.3)	6949431 (94.7)	ref.			ref.			ref.		
Less than once a week	63 (2.7)	200905 (2.7)	0.202	−0.430	0.834	***0.976***	***0.284***	***1.668***	−0.085	−0.814	0.644
Once a week and more	13 (0.5)	33644 (0.5)	−1.008	−2.180	0.163	−0.771	−3.105	1.564	−0.746	−2.531	1.039
Within 2 days	58 (2.5)	153245 (2.1)	0.468	−0.191	1.127	0.285	−0.524	1.094	0.177	−0.641	0.995
Antiseptic products [b]											
No use	1724 (73.2)	5411351 (73.8)	ref.			ref.			ref.		
Less than once a week	262 (11.1)	801408 (10.9)	0.268	−0.043	0.580	0.045	−0.344	0.435	0.201	−0.180	0.582
Once a week and more	266 (11.3)	806904 (11.0)	0.254	−0.252	0.760	0.440	−0.158	1.038	0.244	−0.281	0.770
Within 2 days	103 (4.4)	317561 (4.3)	0.220	−0.229	0.669	0.325	−0.157	0.807	***0.414***	***−0.039***	***0.866***
Hair care products [c]											
No use	2189 (93.0)	6777098 (92.4)	ref.			ref.			ref.		
Less than once a week	27 (1.1)	99463 (1.4)	0.326	−0.941	1.594	−0.223	−1.714	1.267	0.686	−0.510	1.883
Once a week and more	84 (3.6)	282739 (3.9)	−0.265	−0.929	0.398	0.082	−0.756	0.920	−0.539	−1.216	0.138
Within 2 days	55 (2.3)	177925 (2.3)	0.065	−0.878	1.009	−0.548	−1.824	0.727	0.317	−0.634	1.268

NOTE: Adjusted for age, sex, regional area, monthly household income, exercise time and indoor activity time. ***p < 0.05***.
[a] Liquid soap includes shower gel and shampoo.

[b] Antibacterial products include those containing antibacterial (sterilizing) ingredients such as mouthwash, acne treatment, deodorant, and hand sanitizer.

[c] Hair products include hair gel, mousse, spray, wax, essence, and dye.

Table 5
Medication intake due to disease, and multiple linear regression and 95% confidence.

	N (%)	Weighted N (%)	Methyl paraben			Ethyl paraben			Propyl paraben		
			β	95% CI		β	95% CI		β	95% CI	
Take a medication											
Due to fever											
No	2185 (92.8)	6839186 (93.2)	ref.			ref.			ref.		
Yes	170 (7.2)	498039 (6.8)	***2.139***	***1.698***	***2.581***	***−0.542***	***−0.918***	***−0.167***	***2.019***	***1.495***	***2.544***
Due to dermatitis [a]											
No	2232 (94.8)	6951773 (94.7)	ref.			ref.			ref.		
Yes	123 (5.2)	385452 (5.3)	***0.887***	***0.409***	***1.365***	0.349	−0.167	0.864	***1.162***	***0.570***	***1.755***
Due to gastroenteritis											
No	2346 (99.6)	7316474 (99.7)	ref.			ref.			ref.		
Yes	9 (0.4)	20751 (0.3)	0.004	−2.261	2.269	***−0.695***	***−1.287***	***−0.104***	0.287	−2.452	3.027
Other reasons											
No	2323 (98.6)	7242916 (98.7)	ref.			ref.			ref.		
Yes	32 (1.4)	94308 (1.3)	0.395	−0.504	1.293	0.415	−0.160	0.990	0.387	−0.817	1.591

NOTE: Adjusted for age, sex, regional area, monthly household income, exercise time and indoor activity time. ***p < 0.05***.
[a] Both oral and dermal drugs included.

administered fever medication and are 7 years and younger, GM of methyl parabens was 502 μg/g creatinine; and for children who are older than 7 years of age who spent more than 770 min/day indoors, GM of methyl parabens was 129 μg/g creatinine. For children who did not administer fever medication, GM of methyl parabens was 106 μg/g creatinine in the group who are 8 years and younger and used dermatitis medication. For ethyl paraben, indoor activity time was an initial discriminator and fast-food consumption was next. For children who consumed fast food once a week or more showed 22.4 μg/g creatinine (GM). Lastly for propyl paraben, it showed a similar pattern as methyl paraben as the use of fever medication was the initial discriminator, followed by age. For children who administered fever medication and are 7 years and younger, GM of propyl parabens was 31.7 μg/g creatinine (Fig. 1).

4. Discussion

4.1. Concentration of parabens in Korean young population

The parabens were detected in more than 94% of the present children and adolescents, suggesting their widespread exposure among the young Korean population. The present observations showed that younger children generally exhibited greater levels of exposure to the parabens. This observation may be due to the fact that younger children tend to show greater intake amount and skin surface area per body weight (Miller et al., 2002), and is not different from those reported from other national biomonitoring programs (Table 2) (CDC, 2021; Health Canada, 2019; Murawski et al., 2020).

Urinary paraben concentrations are presented as unadjusted, i.e., μg paraben/L urine, and creatinine-adjusted urinary levels, i.e., μg paraben/g creatinine. Our results showed that urinary creatinine levels increased with age in children, similar to the observations of other reports (Aylward et al., 2011; Remer et al., 2002). Regardless of creatinine adjustment urinary methyl and propyl paraben concentrations were the highest in the 3–5 years old group. This observation suggests the presence of age specific sources of exposure among this group of young children. For ethyl paraben (unadjusted only), the highest levels were observed in the 12–18 years old group (Table S2).

Compared by sex, the concentrations of methyl and propyl parabens were higher in females. This finding is similar to those made on adults. Urinary levels of methyl, ethyl, and propyl parabens were reported to be higher in females (Engel et al., 2014; Genuis et al., 2013; Honda et al., 2018; Wang et al., 2013). In contrast, the concentrations of ethyl paraben were higher in male children and adolescents.

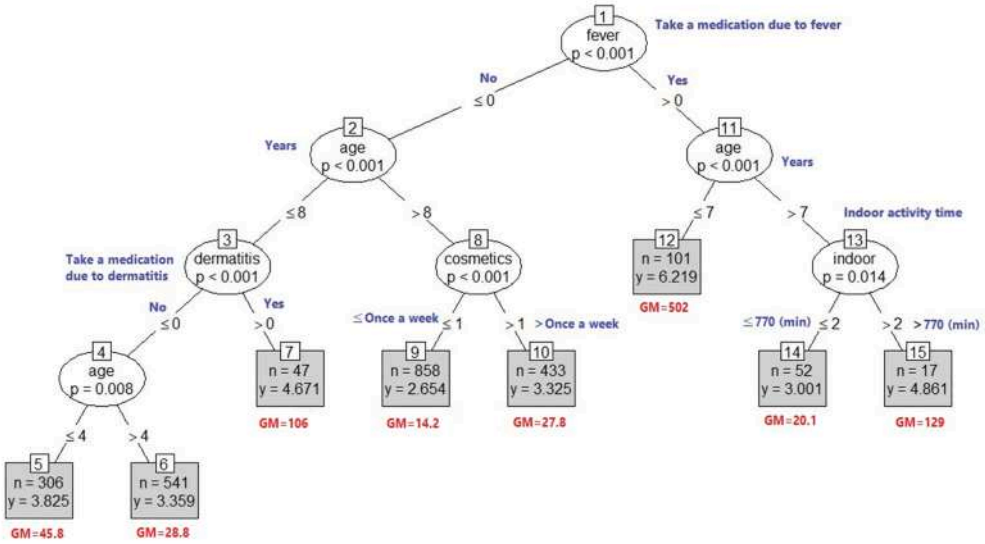
4.2. Comparison with other countries

The levels of methyl and propyl parabens among the Korean population were generally comparable to those of the US. Among the high exposure groups, e.g., 95th percentile, Korean children of 3–5 years of age showed more than two folds of methyl paraben levels than that of the US children of the matching age (5365 vs 2210 μg/g creatinine). For propyl parabens, a similar pattern, e.g., higher 95th percentile level than that of the US (824 vs 724 μg/g creatinine), was observed, while the GM was lower than that of the US (5.2 vs 7.5 μg/g creatinine). Compared to the Canadian and German children, Korean subjects showed up to 3 times higher levels of exposure on average for methyl parabens, and 2 times higher levels for propyl parabens. Another interesting finding was the frequent detection of ethyl parabens with consistently higher levels of exposure. Other biomonitoring programs generally showed very low or negligible levels of ethyl paraben in urine (CDC, 2021; Fillol et al., 2021; Health Canada, 2019; Murawski et al., 2020). Available literature indicates that the urinary ethyl paraben levels of the Korean population were always higher, i.e., up to 4 folds higher than those of the US children. In addition, ethyl paraben levels were more than 10-times higher than the levels reported from several Asian and Middle East countries (China, Japan, India, Saudi Arabia, Kuwait, and Vietnam: median concentration range: 0.19–2.74 ng/mL) (Honda et al., 2018). These observations suggest the presence of specific exposure sources and pathways of ethyl parabens among the Korean population.

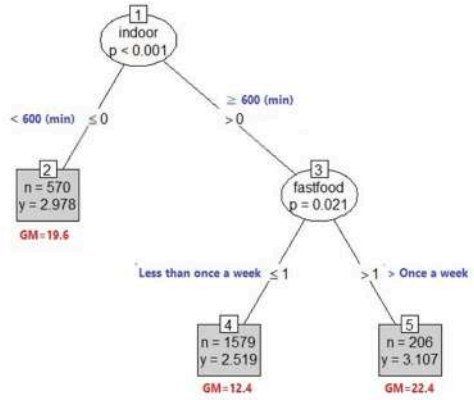
4.3. Exposure to ethyl parabens through food

Our findings suggest the presence of specific sources of exposure among Korean children and adolescents to the measured parabens. For ethyl parabens, fast foods and canned foods consumption were identified as major contributors of the exposure among the children and adolescents, in addition to the use of liquid soap and nail polish. While contribution of personal care products has frequently been reported for ethyl parabens (Fisher et al., 2017; Guo and Kannan, 2013; Li et al., 2020), dietary sources have seldom been investigated. In Korea, ethyl parabens are commonly used as a food preservative, and have been used in diverse condiments (Jeong et al., 2020). In particular, studies have reported detection of methyl and ethyl parabens in soy sauce and condiments (vinegar and sauces) (National Institute of Food and Drug Safety Evaluation, 2020), with the highest levels found in the fermented condiments (red pepper paste and soy paste) (Jo et al., 2020). A recent report on 'temple stay participants' of Korea found that urinary ethyl paraben levels increased after the 5-day temple stay and suggested that the increased consumption of traditional Korean condiments that are frequently used in a vegetarian style temple diet might explain this

(A) Methyl paraben



(B) Ethyl paraben



(C) Propyl paraben

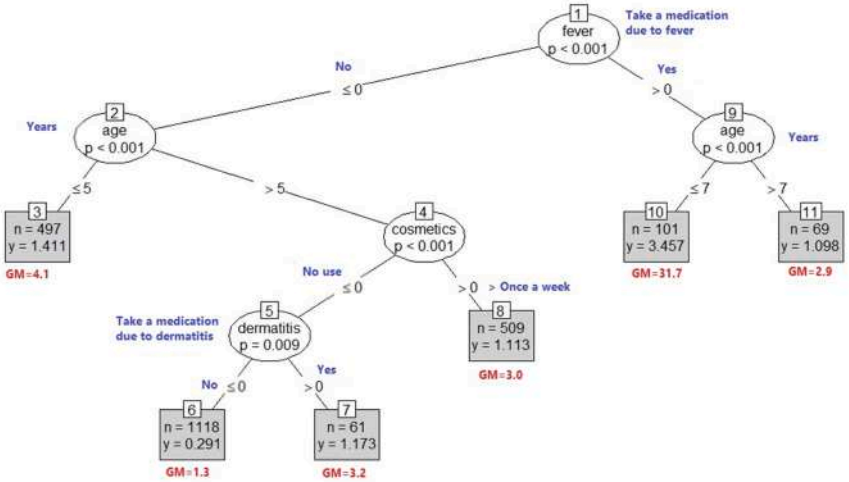


Fig. 1. Decision tree to identify sources of exposure to (A) methyl (B) ethyl and (C) propyl paraben. ***p<0.05.***

increase (Jo et al., 2020). Contribution of the dietary sources among Korean population to the ethyl parabens exposure should be subject to more refined exposure assessment in the future.

4.4. Exposure to parabens in personal care products

For methyl and propyl parabens, use of several personal care products such as nail polish, antiseptic products, hair care products, or color cosmetics were identified as potential sources. Both methyl and propyl parabens are predominantly used as preservatives in personal care products (Karthikraj and Kannan, 2018). Use of cosmetics and personal care products are among the well-known sources of paraben exposure in the general population (Wang et al., 2013). For example, urinary methyl paraben concentration is related to perfume and liquid soap among adults (Braun et al., 2014; Soni et al., 2002). For children and adolescents, the use of nail products and other cosmetics are also among the important sources of exposure to parabens, similar to other countries (Manová et al., 2013). The relationships of the use of color cosmetics, nail polish, and hair products with urinary paraben levels observed in the current study are in line with previous studies (Guo et al., 2014). Several studies have reported positive correlations between urinary biomarkers of parabens and personal care products(Guo et al., 2014; Kim et al., 2018; Kizhedath et al., 2019). A study conducted in Slovenia showed higher urinary methyl paraben levels in children who had used children cosmetic items such as lipstick and perfume (Tkalec et al., 2020). A positive association between methyl and propyl parabens and body lotion usage for a mother-children study has also been previously reported in Swedish literature (Larsson et al., 2014). This study results support previous international findings of association between parabens and personal care products.

4.5. Exposure to parabens in medications

Statistically significant associations were observed medications for between fever and atopic dermatitis medications, and the urinary levels of methyl and propyl parabens (Table 5), shed light on potential sources of these parabens among Korean children and deserve further discussions. Methyl parabens are widely used as a preservative in medicinal products, and it is frequently used in combination with propyl paraben (Brand et al., 2018). Paraben concentration was the highest in liquid medicines followed by soft gels, while medicines in solid form contained the least paraben content (Moreta et al., 2015). Among tablet, particle, oral liquid of pharmaceuticals products, the estimated daily intake (EDI) of parabens via liquid medications was the highest (Ma et al., 2016). While the levels of parabens used in the medicinal products of Korea are not readily available, the levels of parabens in medications varies by country (Moreta et al., 2015).

The importance of medication as a source of exposure to methyl and propyl parabens is also supported by the results of CTree analysis (Fig. 1). The results showed that a recent medication followed by the age of the medicated, were the most important discriminators that influence the urinary levels of both parabens. Paraben containing medications may be a source of high urinary methyl and propyl paraben concentrations reported previously (Dodge et al., 2015). In addition, a previous report showed that the neonatal who took liquid medications were found to be exposed to methyl and propyl parabens which might be used as their preservatives (Mulla et al., 2015). For Chinese children, the GM and median values of EDI of parabens via pharmaceutical ingestion were approximately three times higher than those of adults (Ma et al., 2016). Therefore, for young children, the use of medications containing parabens, e.g., antipyretic medicine in liquid formulation, warrants concern and further efforts to find safer alternatives. In line with this concern, more medications are sold in preservative-free or paraben-free formulations (Cutaia et al., 2018). In the present population who did not take the fever medicine, the use of ointment was again identified as the most important discriminator for urinary methyl paraben (Fig. 1A), outlining the importance of medication as sources of methyl paraben exposure among the children and adolescents of Korea. In addition, the CTree results suggested that the indoor activity time was another important discriminator among the Korean population. This discriminator implies that indoor pollution such as house dust and indoor air may contribute to the body burden of the parabens for the preschoolers and young children, which is comparable to several reports that were made elsewhere (Chen et al., 2018; Hartmann et al., 2016; Zhu et al., 2020).

5. Conclusions

National representative children and adolescents of Korea who participated in KoNEHS Cycle 3 showed that younger children tended to exhibit higher urinary paraben concentrations. Our observations demonstrate that urinary ethyl paraben levels are generally higher than those of other countries including the US and Canada. In addition, among the high exposure group, e.g., 95th percentile, levels of methyl and propyl parabens are higher in the young Korean population. For ethyl paraben, dietary sources such as fast food and canned food consumption were identified as major contributors. For methyl and propyl parabens, in addition to use of personal care and cosmetic products, use of fever medications and ointments was identified as major determinants of exposure, especially among the younger children. Further confirmation of the contribution of these exposure sources is needed. Considering that the young children are more likely to exhibit higher level of exposure and at the same time are expected to possess greater vulnerability to paraben exposure, identification of major exposure pathways for parabens and developing their mitigation measures are important. The present observations will help design further exposure assessments that would confirm major sources of paraben exposure among the children and adolescents of Korea and develop their management policies.

Disclaimer

The results and conclusions in this article are those of the authors and do not necessarily represent the views of the Ministry of Environment and the National Institute of Environmental Research of Korea.

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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.2021.113781.

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Web-based behavioral intervention to reduce exposure to phthalate metabolites, bisphenol A, triclosan, and parabens in mothers with young children: A randomized controlled trial

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ABSTRACT

In this study, a web-based behavioral intervention was designed, which aimed to reduce exposure to phthalate metabolites, bisphenol A, triclosan, and parabens in mothers with young children. A randomized controlled design with two groups was used to verify the effects of the intervention pre- and post-test. In total, 51 mothers participated in the study, categorizing 26 and 25 in the intervention and control groups, respectively. The web-based behavioral intervention focused on changes in diet, personal care products, and health behavior and reinforced behavior through encouragement. This program included an educational video, a game for locating endocrine disruptors at home, a method for locating facilities potentially emitting endocrine disruptors, resources, and a questions and answers mode. Data were collected from May 18 to June 30, 2020. Participants allocated to the intervention group were provided access to the behavioral intervention website via a computer or smartphone. Participants allocated to the control group were sent written information about endocrine disruptors via mail. For both the intervention and control groups, questionnaire results and maternal urine samples were assessed at baseline, during the intervention, and after one month. After the intervention, the urinary concentrations of mono (2-ethylhexyl) phthalate (MEHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), bisphenol A (BPA), methylparaben (MP), ethylparaben (EP), and propylparaben (PP) were found to be significantly decreased in the intervention group. Compared with the control group, the intervention group showed significantly decreased urinary geometric mean values of MEHP, MEOHP, BPA, MP, and PP after one month compared with those during the intervention (3.8%, 16.3%, 28.4%, 9.2%, and 24.4%, respectively). Hence, the web-based behavioral intervention was effective at reducing the exposure to endocrine disruptors in mothers with young children.

1. Introduction

Human health is regulated by the endocrine system, which releases and regulates certain hormones essential for metabolism, growth and development, sleep, and reproduction. Endocrine disrupting chemicals (EDCs), such as phthalates, bisphenol A (BPA), triclosan (TCS), and parabens, adversely affect health by altering or disrupting the functioning of hormonal systems (World Health Organization, 1996). Humans are exposed to EDCs through the ingestion of food and water and exposure to dust, inhalation of airborne gases and particles, and skin contact, which are closely related to the components of daily life, such as diet, food additives, personal care products (PCPs), and cosmetics (Kavlock et al., 2006). Several studies have associated phthalate metabolites, BPA, TCS, and parabens with disorders in the human reproductive system and nervous system development, and attention deficit and hyperactivity disorder, thyroid cancer, and breast cancer (Bai et al., 2015; CDC, 2019; Cho, 2012; Cullen et al., 2017; Fisher et al., 2017; Giulivo et al., 2016; Kim et al., 2018; Kim et al., 2020b; Larsson et al.,

Abbreviations: mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP); mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), methylparaben (MP); ethylparaben (EP), propylparaben (PP); bisphenol A (BPA), endocrine disrupting chemical (EDC); triclosan (TCS), personal care product (PCP); bisphenol S (BPS), di(2-ethylhexyl) phthalate (DEHP); short message service (SMS), limit of detection (LOD); Korean National Environmental Health Survey (KoNEHS), geometric mean (GM); monobutyl phthalate (MBP), monomethyl phthalate (MMP); 2-C-methyl-d-erythritol-2,4-cyclopyrophosphate (MECPP), monoethyl phthalate (MEP); mono-n-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP).

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2014; Ley et al., 2017; World Health Organization, 1996).

Interestingly, these EDCs have a half-life of 6–29 h and most are excreted through the urine (Haines et al., 2017; Koch et al., 2004; LaKind et al., 2019; Larsson et al., 2014; Moos et al., 2015; Völkel et al., 2002). Owing to the short half-life of EDCs, dietary and behavioral interventions, even for a short period, can reduce the exposure to these chemicals (Ackerman et al., 2014; Chen et al., 2015; Harley et al., 2016; Jo et al., 2020; Kim et al., 2020; Rudel et al., 2011; Sears et al., 2020). Chen et al. (2015) reported that handwashing and the reduced use of plastic cups are the most effective interventions among seven one-week strategies for reducing urinary phthalate levels in 4–13-year-old girls. Kim et al. (2020) performed a 3-day dietary intervention for mothers and infants from 37 families (93 people in total). Consequently, BPA levels decreased by 53.1% and bisphenol S (BPS) levels reduced by 63.9% in the maternal urine, and BPA levels decreased by 47.5% in the urine of infants. These findings suggest that dietary or behavioral strategies can effectively minimize exposure to EDCs on an individual level. From the perspective of the social cognition theory, behavioral change begins with the individual's perception of the effect of their behavior on health, and is reinforced by a sense of self-efficacy, such that they are able to perform a specific behavior that will produce desirable results (Middleton et al., 2013). Theory of Planned Behavior is widely used in the public health field as a conceptual framework for achieving human health-promoting behavior, which has been reported the most important variable in the process of intentional human behavioral change (Ajzen, 1985, 1991). These human intentions involve the attitude towards the behavior, subjective norm, and perceived behavioral control (Ajzen and Madden, 1986). Therefore, the intervention strategy should include not only educating subjects with information on exposure to EDCs but also recognizing the risk of exposure to EDCs and enhancing self-efficacy. In addition, mother–infant paired studies on urine concentrations of phthalate metabolites, BPA, TCS, and parabens have been conducted in many countries, and the results have indicated that the concentrations of toxic chemicals in mothers are highly correlated with those in their children (Ait Bamai et al., 2015; Covaci et al., 2015; Cullen et al., 2017; Hlisníková et al., 2019; Kim et al., 2020b; Tratnik et al., 2019). Particularly, infants and toddlers are highly dependent on their mothers, who are usually the primary caregivers (Dennedy and Dunne, 2010); therefore, the behavior of the mother greatly impacts the health of the child.

Previous studies on dietary and behavioral interventions have demonstrated that offline intervention for 3–7 days is effective in reducing EDC levels (Ackerman et al., 2014; Barrett et al., 2015; Chen et al., 2015; Harley et al., 2016; Jo et al., 2020; Kim et al., 2020; Rudel et al., 2011; Sathyanarayana et al., 2013. However, it is effective only for a short time. Behavioral change takes a long time, and long-term offline intervention is expensive and time-consuming. Thus, more effective alternate strategies are needed. A recent meta-analysis reported that web-based online intervention is more effective than offline intervention for changing lifestyle habits (Beleigoli et al., 2019). This web-based intervention has already been proven effective in many public health fields, such as prenatal and postnatal education, and education of cancer survivors and patients with chronic diseases (Jiao et al., 2019; Lee et al., 2014; Wantland et al., 2004). The advantages of web-based programs include their accessibility, user anonymity, and flexibility. In particular, as the participants in these previous studies were mothers who spent most of their time caring for their young children, web-based programs that can be remotely accessed have been shown to be suitable (Lee et al., 2014; Wantland et al., 2004). In addition, some questions are sensitive or personal, which may be difficult to answer in person, and hence, collection of honest and accurate answers is possible in web-based programs owing to user anonymity (Jiao et al., 2019; Wantland et al., 2004). Mothers can freely access the intervention program whenever possible and view or experience the content multiple times (Jiao et al., 2019). Moreover, owing to the restrictions associated with the COVID-19 pandemic, web-based programs are preferred by participants. In Korea, 99.8 and 96% of 20–40-year-old women are computer and smartphone users, respectively (Gallup Korea, 2020). This suggests that this technology is sufficient for a web-based behavioral intervention program that can be easily operated at home, and mothers being familiar with smartphone and internet usage further bolsters the application of these types of programs. Thus, we aimed to develop a web-based behavioral intervention to reduce the exposure of mothers with young infants to EDCs and confirm the effects of the intervention over a period of one month.

2. Materials and methods

2.1. Study design

This study involved the development of a web-based behavioral intervention to reduce exposure to phthalate metabolites, BPA, TCS, and parabens in mothers with young children. A randomized controlled design with two groups was used to evaluate the effects of the intervention pre- and post-test (Fig. 2).

2.2. Participants and randomization

Participants were recruited from 221 individuals who participated in the Endocrine Disruptors Project for Mothers, which prospectively studied the association between 15 toxic chemicals in the breast milk and urine, and the lifestyle of Korean women postpartum (Kim et al., 2020a, 2020b). The participants in this study were mothers with young children. The inclusion criteria were as follows: 1) mothers who stayed with the infant for the majority of the time during the day, and 2) mothers who understood the study purpose and provided informed consent. Mothers with metabolic disturbances or abnormal urine excretion were excluded. Among the 221 participants, 101 had a job and spent most of their time away from their children; six were receiving urinary system treatment, and 52 refused to participate. Subsequently, 62 mothers were randomized to either an experimental or control group by an independent statistician, using a random number function in Microsoft Excel. From five and six subjects in the experimental and control group, respectively, urine samples could not be collected and incomplete questionnaires were received. Finally, 51 mothers participated in this study (Fig. 1). This study was approved by the Institutional Review Board at Kyung Hee University, Seoul, Korea (KHSIRB-20-166).

2.3. Development of a web-based behavioral intervention

This program focused on the individuals' behavior (dietary habits, PCPs, and health) to reduce exposure to phthalate, BPA, TCS, and parabens, which was developed using previous evidence-based research. A literature review on endocrine disruptors (phthalate metabolites, BPA, TCS, and parabens) was conducted and the need for education was investigated to develop the intervention. Basic data and policies for these chemicals were retrieved using a search engine, including PubMed, as well as information provided by the World Health Organization (WHO), US Environmental Protection Agency (US EPA), US Food and Drug Administration (US FDA), and European Environment Agency (EEA). To develop an intervention program, we reviewed 12 studies published in the past decade. Among these, seven involved dietary interventions, two involved interventions against PCPs, two involved interventions against residential environments, and one involved both dietary and PCP interventions (Table 1). We encouraged the participants to eat fresh organic foods when possible, avoid foods containing high levels of fat and dairy products such as cheese and ice cream (Barrett et al., 2015; Dong et al., 2017; Harley et al., 2016; Hlisníková et al., 2019; Jo et al., 2016, 2020; Kim et al., 2020, 2020a; Larsson et al., 2014), and use stained glass or glassware instead of plastic products for cooking. Some chemicals such as phthalate are not chemically bound to the polymer; hence, they can be easily outgassed and inhaled into the human body (Koch and Calafat, 2009). Therefore, we requested mothers

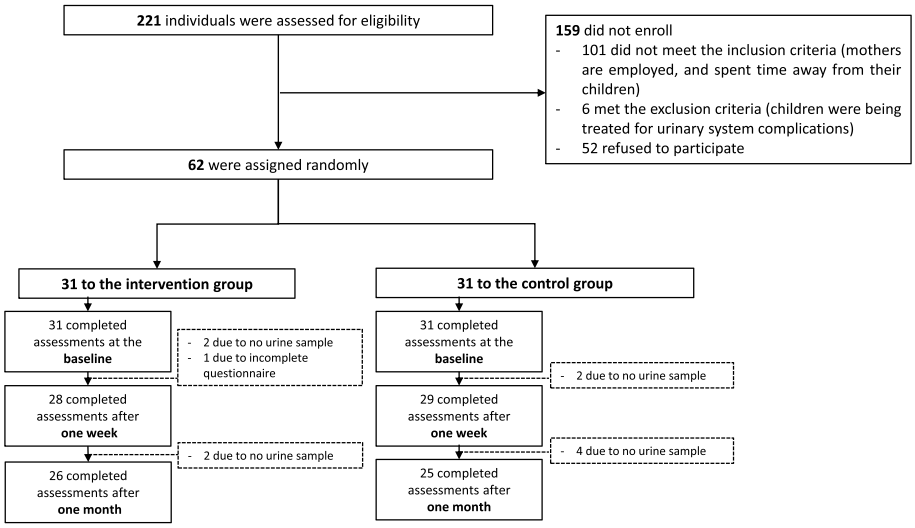

Fig. 1. Participant flow diagram.

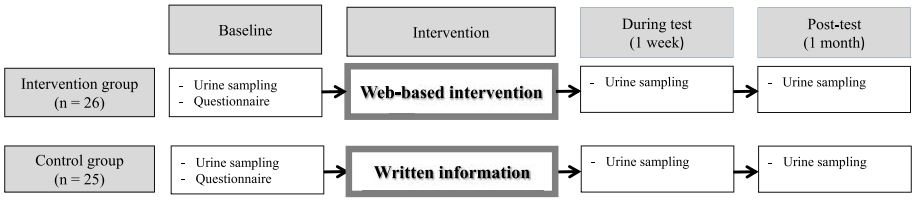

Fig. 2. Study design.

to avoid using new furniture and cars during the intervention period and refrain from using PCPs and cosmetics with strong fragrances and colors, as reported previously (Berger et al., 2019; Chen et al., 2015; Dodson et al., 2020; Kim et al., 2020a; Larsson et al., 2014; Nassan et al., 2017). In our intervention, we also encouraged participants to sweat for at least 30 min as a daily health activity, and frequently wash their hands and drink water to induce the release of endocrine disruptors, as reported previously (Kim et al., 2020a; Dong et al., Larsson et al., 2014). Based on previous research (Biedermann et al., 2010; Ferguson et al., 2017; Geens et al., 2012; Li et al., 2021; Liao and Kannan, 2014; Martínez et al., 2018; Mielke et al., 2011; Serrano et al., 2014), an intervention was developed that involved the following criteria: 1) intake of organic foods, 2) reduction in fish intake, 3) reduction in animal fat intake, 4) reduction in dairy products (cheese and ice cream) intake, 5) avoiding the use of new cars and new furniture, 6) using stainless steel and glassware for cooking, 7) avoiding the use of strong scented PCPs and cosmetics, 8) reduction in the use of color makeup, 9) exercising/sweating for more than 30 min daily, and 10) washing hands frequently.

To implement the behavioral intervention more effectively, we developed a web-based intervention program, which consisted of the following five components: an educational video explaining the health effects of endocrine disruptors as well as steps to reduce exposure to them; a game to find items containing endocrine disruptors at home; a search for facilities that release endocrine disruptors; resources; and a questions and answers mode (Q&A). The educational materials were validated by five experts. Moreover, the developed web-based program was shown to five mothers and their feedback on understanding the content, image speed, text, and effect processing was collected. The contents of the final web-based program are described in Supplementary Fig. S1.

2.4. Data collection

Data were collected from May 18 to June 30, 2020, to study the effects of the web-based behavioral intervention program on the participants' exposure to EDCs. Participants allocated to the intervention group were provided access to a behavioral intervention website via a computer or smartphone. Participants increased their EDC risk awareness by exploring facilities and materials, studying the videos, and asking questions via the Q&A mode. We encouraged the participants to access the web-based program at least three times a week. Furthermore, the researcher reinforced their behavior via weekly short message service (SMS) and phone calls for a month. We sent a message to the participants three times a week on the guidelines for preventing exposure to endocrine disruptors and encouraged them through phone calls once a week. In addition, we frequently communicated with the participants about precautions to be taken when collecting urine samples through SMS, allowing immediate feedback. Participants allocated to the control group were sent written information about endocrine disruptors by mail. The written information included the definition, forms, health effects, and methods of prevention of exposure to endocrine disruptors (Supplementary Fig. S2). They were provided access to a web-based intervention program upon completion of data collection for this study. Participants in both the intervention and control groups completed the questionnaire and provided urine samples for measurements at baseline (T1) and during the intervention at the end of week 1 (T2). Subsequently, urine samples were collected at the end of 1 month of the intervention and reinforcement (T3). The questionnaires included general characteristics, such as age, education level, household income, residential area, infant sex, and infant age. Samples (20 mL) were collected from the first morning urine in a container without exposure to endocrine disruptors and refrigerated immediately.

Table 1
Interventional studies on endocrine disruptors.

	Author (year)	Research design	Participants	Intervention period	Intervention	Analyte(s)
1	**This study (2021)**	Pre- and post-intervention, randomized controlled trial	51 mothers with infants (26 experimental/25 control)	4 weeks	**Web-based behavioral intervention** - dietary habits, personal care products, and health behavior	MEHP, MEOHP, MEHHP, BPA, TCS, parabens
2	Sears et al. (2020)	Pre- and post-intervention, randomized controlled trial	288 infants	NA	**Residential intervention** - removal of lead hazards in paint, dust, water, and soil in and around the home, extensive cleaning, and removal of dust - paint stabilization, involving repairing any deteriorating or water-damaged wall material, removing loose or peeling paint, reapplying paint, and thoroughly cleaning the area using wet methods	MEHHP, MEOHP, MEHP, MECPP, MEP, BzP, MCOP, MCNP, MiBP, MnBP
3	Kim et al. (2020)	Pre-, mid-, and post-intervention, one group	93 participants (37 mothers and their 56 infants)	3 days	**Dietary intervention** - refrain from canned foods and drinks - refrain from foods in plastic packaging, including takeaway and instant foods - refrain from bottled water -consume fresh home-cooked foods	BPA, BPS
4	Jo et al. (2020)	Pre- and post-intervention, one group	25 temple-stay participants	5 days	**Dietary intervention** - strict dietary replacement during temple stay, with Buddhist vegetarian diet excluding meat, eggs, dairy, and fish products.	Parabens
5	Rutkowska et al. (2020)	Pre- and post-intervention, one group	26 participants from 9 households	6 months	**Residential intervention** - introduction of recommended lifestyle changes to lower exposure to selected endocrine disruptors in the indoor home environment	BPA, BPS, 4-NP, DEP, DiBP, DEHP
6	Ley et al. (2017)	Cohort design, randomized trial	154 pregnant females (78/76)	NA	**Personal care products intervention** - participants provided commercially available wash products (liquid and bar soap, toothpaste, dishwashing liquid), which did or did not contain TCS	Triclosan, triclocarban
7	Galloway et al. (2018)	Pre- and post-intervention, one group	94 students	7 days	**Dietary intervention** - consuming a diet designed to reduce the consumption of BPA by avoiding processed foods and foods packaged in known sources of BPA - minimized intake of known sources of BPA according to a set of guidelines	BPA
8	Harley et al. (2016)	Pre- and post-intervention, one group	100 girls	3 days	**Personal care products intervention** - small polyethylene containers of shampoo, conditioner, body wash, and moisturizing lotion, a bar of hand soap, a container of liquid soap, and roll-on deodorant - four items from among liquid or powder foundation, mascara, eyeliner, lipstick/lip gloss/lip balm, and sunscreen	Phthalate, parabens, TCS, BP-3
9	Chen et al. (2015)	Pre- and post-intervention, one group	30 girls	7 days	**Intervention strategy** - nutrition supplements and medication, cosmetic and personal care products, plastic containers, microwaved food, food contained in a plastic bag or plastic wrapping, building material and handwashing, time-activity pattern	MMP, MEP, MBP, MBzP, MEHP, MEHHP, MEOHP, MECPP
10	Barrett et al. (2015)	Pre-, mid-, and post-intervention, one group	10 pregnant females	3 days	**Dietary intervention** - balanced diet intended to minimize dietary phthalate exposure	MEHHP, MEOHP, MEHP, MECCP, MBP, MEP, MiBP, MBzP, MCPP, MCOP, MCNP
11	Ackerman et al. (2014)	Pre- and post-intervention, one group	5 families, 20 participants	3 days	**Dietary intervention** - foods provided by a caterer, prepared from fresh ingredients (no canned or frozen foods), and packaged almost exclusively without contact with plastic	MEHHP, MEOHP, MEHP
12	Sathyanarayana et al. (2013)	Pre- and post-intervention,	10 families, 40 participants	5 days	**Dietary intervention**	Phthalate, BPA

(*continued on next page*)

Table 1 (*continued*)

	Author (year)	Research design	Participants	Intervention period	Intervention	Analyte(s)
		randomized, two arms			- complete dietary replacement with fresh and organic catered foods prepared without plastic	
13	Rudel et al. (2011)	Pre- and post-intervention, one group	20 participants	3 days	**Dietary intervention** - foods provided by a caterer, prepared from fresh ingredients (no canned or frozen foods), and packaged almost exclusively without contact with plastic	BPA, phthalate

2.5. Laboratory analysis of chemicals

To determine the extent of EDC exposure in this study, the concentrations of mono (2-ethylhexyl) phthalate (MEHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), BPA, TCS, MP, PP, and EP were quantitatively analyzed in the urine samples using previously reported procedures (Alves et al., 2016a, 2016b; Atkinson and Roy, 1995; Brotons et al., 1995; Colborn, 1995; Colerangle and Roy, 1997; Yamamoto and Yasuhara, 1999). All chemicals, reagents, and solvents were of analytical grade or higher quality. MEHP, MEHHP, and MEOHP were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA) and BPA, MP, EP, PP, and TCS were purchased from Sigma-Aldrich (St Louis, MO, USA). Individual isotope-labeled internal standards, $^{13}C_2$-MEHP, $^{13}C_4$-MEOHP, and $^{13}C_2$-MEHHP were purchased from Cambridge Isotope Laboratories, Inc., and $^{13}C_{12}$-BPA, $^{13}C_6$-MP, $^{13}C_6$-EP, and $^{13}C_6$-PP were purchased from Wellington Laboratories (Ontario, Canada). The limits of detection (LODs) were as follows: MEHP, 0.21 μg/g; MEOHP, 0.09 μg/g; MEHHP, 0.12 μg/g; BPA, 0.02 μg/g; MP, 0.09 μg/g; EP, 0.16 μg/g; PP, 0.10 μg/g; and TCS, 0.04 μg/g. The enzyme solution for hydrolysis was prepared by dissolving β-glucuronidase mixture from *Helix pomatia* (Sigma-Aldrich). β-Glucuronidase (*Escherichia coli* K12) was obtained from Roche Diagnostics GmbH (Mannheim, Germany). HPLC-grade water and acetonitrile were obtained from Merck. Hydrogen chloride, methyl tertiary butyl ether, ammonium acetate, and acetic acid were purchased from Sigma-Aldrich. To prepare samples, 1 mL of each sample (or blank, calibration curve level, and quality control) was added to 500 μL of ammonium acetate buffer (1 M, pH 6.5) and 5 μL of the internal standard working solution. Next, 30 μL of β-glucuronidase from *E. coli* K12 was added, and the solution was vortexed and incubated at 37 °C for 4 h. After incubation, 100 μL of 2 N HCl was added and the mixture was extracted using 3 mL of ethyl acetate. Further, 2 mL of supernatant was transferred to a new glass tube; the solution was evaporated; and the residue was dissolved in 300 μL of 60% acetonitrile. We used 5 μL of the resultant mixture for high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) analysis. All chemicals were analyzed using HPLC. For phthalate metabolites, the chromatographic separation was performed using an ACE Excel 2 C18-AR column (150 × 2.1 mm; particle size 2 μm; Advanced Chromatography Technologies, Ltd.). The CTC-PAL Leap cooling unit was set at 4 °C, and the sample injection volume was 10 μL. The mobile phase included 0.012% acetic acid in water (solvent A) and 0.012% acetic acid in acetonitrile (solvent B) passed through the column at a flow rate of 4 mL/min. The mobile phase gradient was as follows: 2% solvent B for 0.0 min; increased linearly to 80% solvent B from 0.1 min; maintained at 98% solvent B from 0.1 to 12.0 min; returned to 2% solvent B from 12.1 to 15.5 min; and finally, the gradient was maintained from 15.6 to 18.0 min. For phenols, chromatographic separation was performed on an ACE Excel 2 C18-AR column (150 × 2.1 mm; particle size 2 μm, Advanced Chromatography Technologies, Ltd.). The CTC-PAL Leap cooling unit was set at 4 °C and the sample injection volume was 3 μL. The mobile phase included 0.012% acetic acid in water (solvent A) and 0.012% acetic acid in acetonitrile (solvent B) passed through the column at a flow rate of 4 mL/min. The mobile phase gradient was as follows: 2% solvent B for 0.0 min; increased linearly to 80% solvent B from 0.1 min; maintained at 98% solvent B from 0.1 to 12.0 min; returned to 2% solvent B from 12.1 to 15.5 min; and lastly, the gradient was maintained from 15.6 to 18.0 min.

2.6. Statistical analysis

Concentrations below the LOD were assigned a proxy value as the LOD divided by the square root of two (Hornung and Reed, 1990). In accordance with the WHO criteria, spot urine samples with creatinine concentration as very dilute (<0.3 g/L) or concentrated (>3.0 g/L) were excluded from the analysis (World Health Organization, 1996). General characteristics of the subjects are presented using descriptive statistics, including frequency, percentage, average, geometric mean (GM), median, and standard deviation. The normality of distribution of variables (maternal age, education level, household income, resident area, infant sex, and infant age) was tested using the Shapiro–Wilk test. For verification of the homogeneity in baseline characteristics of the intervention and control groups, we performed a parametric test (Student's t-test, chi-square test) when variables followed a normal distribution through the normality test (maternal age, infant age, and household income), or a non-parametric test (Mann–Whitney *U* test) when variables did not follow a normal distribution (education level, residential area, and infant sex). We performed the Wilcoxon matched-pairs signed-rank test to assess if there was a difference in urinary concentration of chemicals before and after the intervention between the intervention and control groups. We used a mixed-effect regression model to determine if there was a difference in the urinary concentration of chemicals between the two groups with time after adjustment for age, education level, and household income. Statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Homogeneity of the baseline characteristics

We randomized 26 and 25 participants to the intervention and control groups, respectively, meeting the inclusion and exclusion criteria from among 221 subjects who participated in the Endocrine Disruptors Project for Mothers. We collected data on age, educational level, monthly income, residential area, infant sex, and infant age from both these groups. We found no significant differences in the baseline characteristics, including age, educational level, monthly income, residential area, infant sex, and infant age between the intervention and control groups (Table 2).

3.2. Chemical concentrations

We analyzed the concentrations of EDCs in the urine of participants in the intervention and control groups. Urinary creatinine-adjusted concentrations of chemicals measured pre-intervention are displayed in Table 3. MEHP, MEOHP, MEOHP, BPA, TCS, and parabens were detected in 80–100% of samples from the 51 subjects, with median concentrations of 0.14–41.86 μg/g and GM concentrations of

Table 2
Baseline characteristics of participants in the intervention and control groups.

Characteristic	Category	Intervention group (n = 26) n (%)/mean ± SD	Control group (n = 25) n (%)/mean ± SD	t/ x^2/ U	*p*-value
Age (years)		35.8 ± 3.9	35.1 ± 2.9	0.06	0.951
Education level	<College	4 (15.4)	5 (20.0)	0.97	0.156
	≥College	22 (84.6)	20 (80.0)		
Household income (USD/month)	<5000	13 (50.0)	14 (56.0)	2.43	0.407
	≥5000	13 (50.0)	11 (44.0)		
Residential area	Metropolitan	15 (57.7)	13 (52.0)	0.31	0.659
	Non-metropolitan	11 (42.3)	12 (48.0)		
Infant sex	Male	11 (42.3)	12 (48.0)	1.78	0.890
	Female	15 (57.7)	13 (52.0)		
Infant age (months)		22.3 ± 1.7	22.1 ± 1.8	0.38	0.705

SD, standard deviation; the Student's t-test, chi-square test, and Mann–Whitney *U* test were performed for testing homogeneity of baseline characteristics.

0.12–50.27 μg/g. EP concentration was detected at the highest level (GM: 50.27 μg/g), followed by MP (GM: 17.25 μg/g), MEHHP (8.44 μg/g), and MEOHP (GM: 4.24 μg/g). The concentrations of MEOHP, MEHHP, BPA, MP, EP, and PP were lower than those reported in the third Korean National Environmental Health Survey (KoNEHS) conducted in 2017 (12.1 μg/g, 16.1 μg/g, 1.18 μg/g, 41.7 μg/g, 39.3 μg/g, 3.9 μg/g, respectively, in median value) (Table 3).

3.3. Change in urinary concentrations of chemicals in the intervention and control groups

Table 4 shows the changes in the geometric mean (GM) of chemical concentrations before (T1) and after (T3) the intervention in the intervention and control groups. After the intervention, the urinary concentrations of MEHP, MEOHP, BPA, MP, EP, and PP decreased significantly in the intervention group ($p = 0.011$, $p = 0.036$, $p = 0.039$, $p = 0.013$, $p < 0.001$, and $p = 0.044$, respectively), whereas only the concentration of EP decreased significantly ($p = 0.044$) and that of MP increased significantly ($p = 0.036$) in the control group (Table 4).

Table 5 shows the changes in the GM of chemical concentrations before the intervention (T1) and during the intervention (T2) and the changes during and after the intervention (T3) in the intervention and control groups. The percentage change in the GM BPA concentration was only statistically significant in the intervention group between T1 and T2 (−35.5%, $p = 0.033$). Additionally, the urinary GM values for MEHP, MEOHP, BPA, MP, and PP were significantly lower (MEHP: −3.8%, MEOHP: −16.3%, BPA: −28.4%, MP: −9.2%, and PP: −24.4%) at T3 than at T2 in the intervention group compared with the control group. In the control group, urinary GM values of most chemicals, excluding BPA, MEOHP, and MEHHP, were increased from T2 to T3 (Table 5).

4. Discussion

In this study, we found that the urinary concentrations of six EDCs (MEHP, MEOHP, BPA, MP, EP, and PP) were significantly decreased in the intervention group after a month of intervention compared with those in the control group. Comparing the effects of the intervention between the two groups is difficult because most previous interventional studies have been performed using a single group. In this study, the urinary concentrations of phthalate metabolites (MEHP and MEOHP)

Table 3
Urinary creatinine-adjusted concentrations of chemicals measured pre-intervention (n = 51) in comparison with KoNEHS.

Analyte (μg/g creatinine)	LOD	% >LOD	GM	Percentile (μg/g)						KoNEHS*
				Min	25th	50th	75th	95th	Max	Median
MEHP	0.21	98	2.71	<LOD	1.28	2.42	3.82	6.67	14.51	–
MEOHP	0.09	93	4.24	<LOD	1.74	4.01	4.39	10.83	33.40	12.1
MEHHP	0.12	100	8.44	1.83	4.21	8.57	9.96	16.49	58.59	16.1
BPA	0.02	81	0.84	<LOD	0.3	0.71	1.37	2.78	8.92	1.18
TCS	0.04	80	0.12	<LOD	0.06	0.14	0.32	1.08	6.44	–
MP	0.09	84	17.25	<LOD	4.79	12.53	50.26	568.31	953.06	41.7
EP	0.16	100	50.27	1.17	12.59	41.86	154.24	868.29	2742.91	39.3
PP	0.1	92	0.63	<LOD	0.12	0.42	2.13	31.58	110.23	3.9

KoNEHS, third Korean National Environmental Health Survey (2017); LOD, limit of detection; GM, geometric mean; MEHP, mono (2-ethylhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl); BPA, bisphenol A; TCS, triclosan; MP, methylparaben; EP, ethylparaben; PP, propylparaben.

Table 4
Urinary concentration changes in chemicals pre- (T1) and post-intervention (T3) in the intervention (n = 26) and control groups (n = 25).

Analyte (μg/g creatinine)	Intervention group, GM (95% CI)			Control group, GM (95% CI)		
	Pre-intervention (T1)	Post-intervention (T3)	*p*-value	Pre-intervention (T1)	Post-intervention (T3)	*p*-value
MEHP	2.65 (1.07–13.09)	2.20 (1.02–7.31)	0.011	2.76 (1.26–14.51)	2.67 (1.87–13.84)	0.318
MEOHP	4.28 (1.70–30.92)	3.33 (1.07–13.96)	0.036	4.20 (1.67–33.40)	3.99 (1.01–33.61)	0.412
MEHHP	8.60 (2.87–43.10)	7.63 (3.14–51.02)	0.051	8.28 (1.83–58.59)	8.22 (1.13–68.88)	0.827
BPA	0.87 (0.53–8.92)	0.40 (0.23–4.11)	0.039	0.80 (0.07–5.90)	0.64 (0.07–5.12)	0.055
TCS	0.13 (0.01–6.39)	0.12 (0.06–10.33)	0.494	0.12 (0.04–6.44)	0.15 (0.03–10.20)	0.262
MP	16.76 (3.86–639.94)	11.0 (3.55–111.62)	0.013	17.76 (3.64–953.06)	20.09 (3.98–771.12)	0.044
EP	51.38 (1.17–2742.91)	33.0 (14.67–566.31)	<0.001	49.16 (2.07–2401.07)	42.00 (11.07–813.91)	0.036
PP	0.70 (0.02–87.19)	0.31 (0.17–43.15)	0.044	0.60 (0.08–110.23)	0.64 (0.04–113.62)	0.437

Pre-intervention, baseline measurement before the intervention (T1); post-intervention, measurement at the end of 1 month of the intervention (T3); CI, confidence interval; GM, geometric mean; MEHP, mono (2-ethylhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl); BPA, bisphenol A; TCS, triclosan; MP, methylparaben; EP, ethylparaben; PP, propylparaben.

Table 5
Change in geometric mean concentrations of phthalate metabolites, BPA, TCS, and parabens in the intervention and control groups over time.

	% change from T1 to T2 (T1 GM–T2 GM)			% change from T2 to T3 (T2 GM–T3 GM)		
Analyte (μg/g creatinine)	Intervention group	Control group	*p*-value	Intervention group	Control group	*p*-value
MEHP	−13.6 (2.65–2.29)	−5.4 (2.76–2.61)	0.491	−3.8 (2.29–2.20)	2.4 (2.61–2.67)	0.013
MEOHP	−7.0 (4.28–3.98)	−4.5 (4.20–4.01)	0.677	−16.3 (3.98–3.33)	−0.5 (4.01–3.99)	0.046
MEHHP	- 7.1 (8.60–7.99)	−0.4 (8.28–8.25)	0.092	−4.5 (7.99–7.63)	−0.4 (8.25–8.22)	0.299
BPA	−35.5 (0.87–0.56)	−11.3 (0.80–0.71)	0.033	−28.4 (0.56–0.40)	−9.9 (0.71–0.64)	0.038
TCS	2.4 (0.13–0.14)	17.2 (0.12–0.14)	0.721	−12.6 (0.14–0.12)	6.7 (0.14–0.15)	0.127
MP	−27.7 (16.76–12.11)	−15.3 (17.76–15.04)	0.091	−9.2 (12.11–11.00)	33.6 (15.04–20.09)	<0.001
EP	−38.5 (51.38–31.59)	−22.9 (49.16–37.88)	0.051	4.5 (31.59–33.00)	10.9 (37.88–42.00)	0.067
PP	−41.3 (0.70–0.41)	−34.1 (0.60–0.39)	0.088	−24.4 (0.41–0.31)	62.2 (0.39–0.64)	<0.001

Mixed-effect regression model examining the changes in geometric mean concentrations between two time points in the two groups after adjusting for age, education level, and monthly income. *P*-value for the interaction term group*time; T1, measurement at baseline before intervention; T2, measurement at 1 week during the intervention; T3, measurement at the end of 1 month of the intervention; MEHP, mono (2-ethylhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl); BPA, bisphenol A; TCS, triclosan; MP, methylparaben; EP, ethylparaben; PP, propylparaben.

were significantly decreased in the intervention group compared with the control group, which is comparable to the findings of previous studies (Ackerman et al., 2014; Chen et al., 2015; Harley et al., 2016; Rudel et al., 2011; Sears et al., 2020). Harley et al. (2016) found that the concentration of monoethyl phthalate (MEP) decreases significantly by 27.4% after a 3-day PCP intervention, while there are no significant changes in the concentrations of mono-n-butyl phthalate (MnBP) and mono-isobutyl phthalate (MiBP). Chen et al. (2015) proposed seven intervention strategies that combine diet and PCPs, and showed that hand washing, consuming fewer beverages in plastic cups, and using lower amounts of shampoo and shower gel reduces the urinary concentrations of monobutyl phthalate (MBP), monomethyl phthalate (MMP), MEHHP, 2-C-methyl-d-erythritol-2,4-cyclopyrophosphate (MECPP), and MEP. Two studies involving dietary interventions of fresh organic food and the avoidance of plastic packaging also reported a significant decrease of over 50% in the DEHP concentration in urine after the intervention (Ackerman et al., 2014; Rudel et al., 2011). However, several studies have reported conflicting results (Barrett et al., 2015; Sathyanarayana et al., 2013). Notably, Sathyanarayana et al. (2013) reported that the urinary DEHP concentration increased unexpectedly from a median of 283.7–7027.5 nmol/g after dietary intervention. The authors suspected that food contamination or ineffective self-guided intervention could account for these results. Therefore, a tailored and detailed system, such as a web-based program, is necessary to provide self-guided intervention and allow users to access and obtain information from anywhere at any time.

The urinary concentration of BPA was found to be significantly decreased in the intervention group compared with that in the control group, which was consistent with the results of previous studies (Kim et al., 2020; Rudel et al., 2011; Rutkowska et al., 2020). Kim et al. (2020) showed that the urinary BPA concentration decreases in both mothers (53.1%) and children (47.5%) after a 3-day dietary intervention. In addition, the frequent consumption of canned food, take-out drinks, and fast food has been associated with an increase in BPA concentration. In the studies of Rutkowska et al. (2020) and Rudel et al. (2011), the urinary concentration of BPA has been shown to be significantly decreased through dietary interventions and lifestyle changes, respectively. However, Sathyanarayana et al. (2013) reported that the urinary BPA concentration increases significantly after a 7-day dietary intervention, whereas Galloway et al. (2018) reported that dietary intervention has no effect on the BPA concentration. These discrepancies could be explained by either a non-stringent intervention or contamination of the food used.

The concentration of TCS in urine did not significantly change in the experimental and control groups before and after the intervention. This was inconsistent with the results of previous studies (Harley et al., 2016; Ley et al., 2017). Harley et al. (2016) confirmed that TCS concentration decreases significantly before and after intervention in a group using toothpaste containing TCS and reported that toothpaste is a major source of TCS exposure. Ley et al. (2017) also reported that the decrease in TCS concentration after intervention in the group using PCPs containing TCS is 7–10-fold higher than that in the group using PCPs without TCS. Although the US FDA banned the use of TCS in wash products in 2016 and in hospital products in 2018, owing to its toxicity, this compound is still used in some toothpastes, mouthwashes, and deodorants (Berger et al., 2019). This may explain the contradiction in the results obtained in the present and previous studies, as the daily use of toothpaste and mouthwash was not prohibited in the present study. However, TCS concentrations from T2 to T3 in the intervention group decreased by 12.6% and that in the control group increased in this study. TCS is used as a preservative in many PCPs, such as makeup products, soaps, and deodorants (Bever et al., 2018; Toms et al., 2011). Our intervention included avoiding the use of PCPs with strong scents and colors, but it would not be easy for subjects to replace their products with alternatives in a short period of time because most PCPs, including TCS-containing products, are a daily necessity. We believe this is the reason behind the urinary TCS concentration after the intervention not decreasing significantly. As the use of these products for personal hygiene is steadily rising, further research is needed to determine whether they are the main sources of TCS exposure.

In this study, the urine concentrations of MP, EP, and PP decreased significantly in the intervention group, which was consistent with the findings of Harley et al. (2016), who conducted an interventional study on the use of PCPs for 3 days in 100 girls. Interestingly, the concentrations of MP and PP decreased continuously from T2 to T3, whereas the concentration of EP increased from T2 to T3. This may be because MP and PP are the most frequently used materials in PCPs such as sunscreens, hand/body lotions, and shampoos, whereas EP is mainly used as a food preservative (Harley et al., 2016; Nassan et al., 2017). Koreans have unique cultural eating habits, including the use of traditional fermented cabbage with seasoning (*Kimchi*) and traditional pepper/-bean/soy seasoning (*Gochujang*, *Doenjang*, and *Ganjang*) (Jo et al., 2020). The *Gochujang* and soy sauce sold in Korea are reported to contain up to 29.7 mg/kg of EP (Choi et al., 2008; Jo et al., 2020). For this reason, many studies have reported that the urinary concentration of EP is higher in Koreans than in individuals from other countries (Honda et al., 2018; Kang et al., 2016; Kim et al., 2018, 2020b). Therefore, studies should be undertaken to investigate additional causes resulting in high levels of EP in traditional Korean foods.

Our study has a few limitations. Firstly, although we demonstrated that web-based behavioral programs were effective at reducing exposure to EDCs, the sample size used in this study was relatively small to allow for generalizations. However, when assuming a significance level of 0.05, a power of 0.85, a medium effect size of 0.2, two groups, three measurement time points (baseline, 1 week, 1 month), and a 0.5 correlation between points using the G-power 3.1.9.4 program, the target sample size for the experiment was 48 subjects. Hence, in this study, the sample size was sufficient to determine the effects of the intervention on reducing the exposure of mothers with young infants to phthalate

metabolites, BPA, TCS, and parabens. Secondly, we compared the concentration of EDCs in morning spot urine samples before and after the intervention based on a previous study, which showed the first morning urine to have a higher reproducibility than the lunch-time and bed-time urine (Kim et al., 2020b). However, the levels of chemicals with a short half-life may also show inter- and intra-day variations (Kim et al., 2020b; Koch et al., 2004; LaKind et al., 2019). Hence, additional studies are needed to measure the effectiveness of web-based interventions, not only using morning urine, but also using urine samples obtained at lunch or before bedtime. Lastly, we confirmed the effect of the web-based behavioral intervention program only by noting changes in the concentration of EDCs in the urine. In the future, it is necessary to investigate the changes in subjects' health behavior during the intervention period and the barriers and facilitators for the behavior change.

5. Conclusions

This study was conducted to develop a web-based behavioral intervention and confirm the effectiveness in reducing EDC exposure by evaluating the urinary concentration of MEHP, MEOHP, MEHHP, BPA, TCS, and parabens after a month. The web-based intervention was developed based on evidence from previous studies (Berger et al., 2019; Chen et al., 2015; Dodson et al., 2020; Kim et al., 2020a; Larsson et al., 2014; Nassan et al., 2017), including dietary, PCP, and health-promoting interventions, and reinforced through SMS and phone calls. After the intervention, the urinary concentrations of MEHP, MEOHP, BPA, MP, EP, and PP decreased significantly in the intervention group. These findings suggested that web-based behavioral intervention was effective for reducing the exposure to EDCs among mothers with young children. In the future, the web-based intervention program would be broadened and customized to each life cycle. Additionally, it will be necessary to develop an integrated program to reduce the exposure to environmentally harmful factors through the development of programs for EDCs as well as other environmental hazards, including air pollution.

Author contributions

Conceptualization and methodology, JHK; investigation, JHK, JMK, and HK; formal analysis, JHK and JMK; visualization, resources, and data curation, JMK and HK; validation, JHK and JMK; writing—original draft, JHK; writing—review and editing, JHK, JMK, and HK; supervision, JHK; project administration, JHK; funding acquisition, JHK. All authors have read and agreed to the published version of the manuscript.

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Declaration of competing interest

The authors declare no conflict of interest.

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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.2021.113798.

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Maternal $PM_{2.5}$ exposure associated with stillbirth: A large birth cohort study in seven Chinese cities

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ABSTRACT

Background: Maternal exposure to fine particulate matter ($PM_{2.5}$) has been associated with a few adverse birth outcomes. However, its effect on stillbirth remains unknown in China, especially the susceptible windows and potential modifiers.

Objective: This study aimed to evaluate the associations between maternal $PM_{2.5}$ exposure and stillbirth in seven Chinese cities.

Methods: We used birth cohort data of 1,273,924 mother-and-birth pairs in seven cities in southern China between 2014 and 2017 to examine these associations. Pregnant women were recruited in the cohort at their first visit to a doctor for pregnancy, and stillbirths were recorded at the time of birth. Air pollution exposures were assessed through linking daily air pollutant concentrations from nearby monitoring stations to the mother's residential community. Cox regression models were applied to determine the associations between $PM_{2.5}$ and stillbirth for different gestational periods.

Results: Among the participants, 3150 (2.47‰) were identified as stillbirth cases. The hazard ratio (HR) of stillbirths was 1.52 (95% CI: 1.42, 1.62) for each 10 $\mu g/m^3$ increase in $PM_{2.5}$ during the entire pregnancy after controlling for some important covariates. Relatively stronger associations were observed during the second trimester [adjusted HR = 1.67 (95% CI: 1.57, 1.77)] than trimesters 1 [HR = 1.44 (95% CI: 1.37, 1.52)] and trimester 3 [HR = 1.23 (95% CI: 1.16, 1.30)]. Stratified analyses also showed a stronger association among pregnant women without previous pregnancy and previous delivery experiences.

Conclusion: The study indicates that maternal exposure to $PM_{2.5}$, especially during the midpoint period of pregnancy, might increase the risk of stillbirths. Maternal previous pregnancy and delivery may modify this association.

1. Introduction

Stillbirth is one of the important adverse birth outcomes (Blencowe et al., 2016; Yakoob et al., 2010). It is estimated that about 2.6 million stillbirths occurred internationally in 2015, with the majority occurring in developing countries (Lawn et al., 2016). A stillbirth can lead to tremendous societal and familial burden, such as the mental health problems and an increased risk of recurrent stillbirths (Heazell et al., 2016; Lamont et al., 2015).

Previous studies have identified a few important risk factors for stillbirth, such as genetics, childbirth or pregnancy complications, fetal growth restriction, and congenital abnormalities (Flenady et al., 2011; Jason et al., 2013). However, most of these factors are not easily preventable or modifiable, and thus are difficult for specific intervention

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measures (Mendola et al., 2017). Ambient air pollution exposure is another potential risk factor, which has been linked to a few adverse birth outcomes, such as preterm birth (Guo et al., 2018; Xiao et al., 2018) and low birth weight (Basu et al., 2014; Harris et al., 2014). However, only a few studies have assessed the association between stillbirths and $PM_{2.5}$ (Green et al., 2015; Siddika et al., 2016). One study conducted in California suggested that $PM_{2.5}$ exposure throughout pregnancy was associated with an increased risk of stillbirth (Green et al., 2015). Another study in Ohio showed $PM_{2.5}$ exposure during the third trimester of pregnancy was associated with stillbirth (DeFranco et al., 2015). At the same time, a few studies reported a non-significant association between $PM_{2.5}$ and stillbirths (Faiz et al., 2012, 2013). In China, only one study has found an increased risk of stillbirth associated with high $PM_{2.5}$ levels during pregnancy (Yang et al., 2018).

We investigated the associations between maternal $PM_{2.5}$ exposures and stillbirths in seven Chinese cities using a birth cohort study design. We also performed stratified analyses to explore potential modifiers, such as maternal age, previous pregnancy, and previous delivery condition.

2. Methods and materials

2.1. Study settings

This study was conducted in seven cities in the Pearl River Delta (PRD) region, Dongguan, Foshan, Huizhou, Guangzhou, Jiangmen, Zhaoqing, and Zhongshan, between January 1, 2014 and December 31, 2017 (Fig. 1). Participants were recruited in this cohort from their first hospital visit after becoming pregnant until delivery. Our birth cohort was linked to the birth registry datasets in order to track the occurrence of stillbirths. In this registry system, birth information is acquired from all midwifery clinics and hospitals in Guangdong province. In accordance with previous studies (Darrow et al., 2009; He et al., 2016), all singleton births that occurred at 20–42 weeks of gestation were included in the data. We also collected maternal and birth information, including date of birth, maternal age, gestational age at birth, the infant's sex, birth weight, birth outcome, mode of delivery, and parity. Gestational age was determined by ultrasound examinations during pregnancy (Fu and Yu, 2011). In the absence of ultrasound information, the gestational age was determined according to the last menstrual period (He et al., 2016).

Approval to perform the study was obtained from the institutional ethical committee of Guangdong Women and Children Hospital (Approval no 202101138). There was no identification information at an individual level in the dataset and all information was anonymous.

2.2. Stillbirths

Stillbirths were collected through the Guangdong Maternal and Child Health Management Information System. The definition of stillbirth used for this study is a fetus born between 20 weeks' and 42 weeks' gestation with no evidence of life such as breathing, heartbeat, or movement, as originally defined by the World Health Organization (WHO) (Yang et al., 2018).

2.3. Air pollution exposure assessment

Ambient air pollution data were collected at each city's air monitoring stations from February 1, 2013 to December 31, 2017. There are ten stations in Guangzhou, five in Dongguan, eight in Foshan, four in Zhongshan, five in Huizhou, three in Zhaoqing, and four in Jiangmen (Fig. 1).

All of the monitoring stations are located in areas with both commercial and residential activities. The air pollutant samples were collected from about 10 to 20 m above ground level. Daily 24 h mean concentrations of $PM_{2.5}$, nitrogen dioxide (NO_2), and sulfur dioxide (SO_2) as well as 8 h mean ozone (O_3) were obtained from each station. The quality control and quality assurance were processed according to

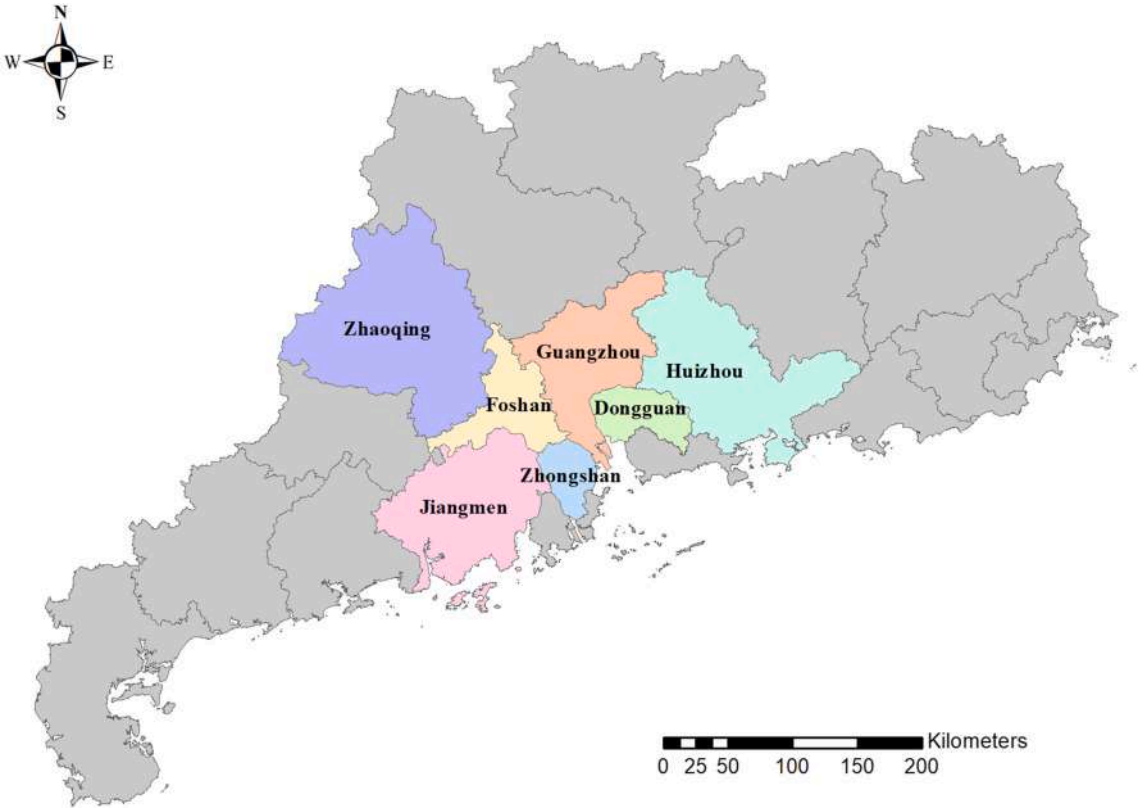

Fig. 1. The geographical distribution of the seven study cities in China.

the National Environmental Protection Administration of China (Lin et al., 2016). The missing data (fewer than 5%) were imputed based on the "na.approx" function in R (Vivian Chit et al., 2014). Air pollution exposure for each participant was estimated by matching the data from air monitoring stations to the mothers. Specifically, exposures were calculated based on the mother's residential district during pregnancy, and those districts without a monitoring station within a radius of about 5 km were excluded from the study, for a final total of 25 districts. To examine the effects at each of different trimesters of pregnancy (the first trimester from 0 to 12 weeks, the second trimester from 13 to 28 weeks, and the third trimester from after 28 weeks), the average concentrations during each trimester as well as over the entire gestation period were calculated (Chen et al., 2018). Participants who gave birth before or at 28 gestational weeks were excluded in the analyses for trimester 3.

We collected daily meteorological data from the National Weather Data Sharing System (http://cdc.cma.gov.cn/home.do). Daily mean temperature (°C) and relative humidity (%) were collected from one automatic weather observation station in each city. Meteorological data were matched to each participant through the same method used with air pollution and as described above.

Other covariates used in this study were continuous variables including gestational age (weeks), maternal age (years), and birth weight (grams) as well as categorical variables including baby gender, previous pregnancy and delivery condition.

3. Statistical analysis

A Cox proportional hazards model was applied to estimate the associations between $PM_{2.5}$ exposures during different trimesters and stillbirth, with gestational age as the time axis and stillbirth as the outcome (Wang et al., 2013). Before the formal analysis, we performed Schoenfeld residuals to assess the proportional hazards assumption and there were no violations.

To account for non-linear effects, both mean temperature and relative humidity were adjusted in model 1 using natural cubic splines with degrees of freedom of 6 and 3, respectively (Liang et al., 2019). Other covariates were also controlled in the model 2 analyses, including the maternal age, newborn gender, previous pregnancy, and season of conception (spring: March–May; summer: June–August; fall: September–November; winter: December–February) (Qian et al., 2016). We estimated the effects for different trimesters of pregnancy using an individual model of each trimester.

We examined these associations by both single-pollutant and two-pollutants in model 2. $PM_{2.5}$ was included alone in the single-pollutant model; while $PM_{2.5}$ and SO_2 (or NO_2, O_3) were included in the two-pollutant models (Lin et al., 2016). The associations were shown in hazard ratios (HRs) and 95% confidence intervals (95% CI) for each 10 $\mu g/m^3$ increase in $PM_{2.5}$ concentrations.

In our stratified analyses, the potential effect modifications were analyzed by variables such as baby's gender, maternal age group (<35 years or ≥35 years), previous pregnancy (first pregnancy or not), and previous delivery condition (first delivery or not). The statistical differences of associations between different subgroups were calculated by using the following strata:

$$(b_1 - b_2) \pm 1.96\sqrt{(se_1)^2 + (se_2)^2}$$

In this strata, b_1 and b_2 are presented for the effect estimates for each stratum, and the se_1 and se_2 represent their corresponding standard errors (Lin et al., 2016).

The robustness of these effects was examined by several sensitivity analyses. For the model 2, we conducted these analyses by changing the degrees of freedom for mean temperature (5–7 degrees of freedom) and relative humidity (2–4 degrees of freedom). We also performed one analysis by excluding the season of conception covariate in the model 2 to check the robustness of the findings. All data analyses were performed using R software (version 3.4.4). The "smoothHR" package was used to fix the Cox models.

4. Results

Our cohort included a total of 1,273,924 singleton live births at between gestational age of 20–42 weeks. Among those, 3150 (2.47‰) were stillbirths. Table 1 summarizes the characteristics. Among all the pregnant women, 54.29% had been previously pregnant and 43.68% had previously delivered a baby. A higher risk of stillbirth was found among women younger than 35 years and those with pregnancy experience. Mothers delivered male babies had higher risk of stillbirths.

Table 2 displays the meteorological factors and air pollution during pregnancy for all participants. The mean concentration of $PM_{2.5}$ during the entire gestation period was 36.78 $\mu g/m^3$, and the mean of daily average temperature and relative humidity during the entire pregnancy was 22.72 °C and 78.92% respectively. The results of the relation between air pollutants and meteorological data are showed in Supplementary Table S1. Both pollutants were positively correlated with $PM_{2.5}$ and SO_2 (Pearson's correlation coefficient: r ranged from 0.04 to 0.65), and a negative correlation between O_3 and NO_2 ($r = -0.16$). The daily average temperature and relative humidity were both negatively correlated with air pollutants except for O_3 and NO_2 with $r = 0.46$ and $r = 0.01$, respectively.

We observed associations between $PM_{2.5}$ exposure and stillbirths during all of the study periods in both single-pollutant (Table 3) and two-pollutant models (Table 4). For example, the associations between $PM_{2.5}$ and stillbirth in single-pollutant models were relatively higher in the second trimester for each 10 $\mu g/m^3$ increase in $PM_{2.5}$ concentration, with a HR of 1.61 (95% CI: 1.52, 1.71) in model 1 and an HR of 1.67 (95% CI: 1.57, 1.77) in model 2, while the HR was 1.44 (95% CI: 1.37, 1.52) for trimester 1, 1.23 (95% CI: 1.16, 1.30) for trimester 3, and 1.52 (95% CI: 1.42, 1.62) for the entire gestation period in model 2. Similar results were obtained in the two-pollutant models, with an HR of 1.59 (95% CI: 1.49, 1.70) after adjusting for O_3, an HR of 1.44 (95% CI: 1.33, 1.56) after adjusting for NO_2 and an HR of 1.89 (95% CI: 1.72, 2.08) after adjusting for SO_2 during the entire pregnancy.

Table 5 displays the effects of stratified analyses by sex, maternal age, previous pregnancy, and previous delivery condition. The associations varied by sex, maternal age, previous pregnancy and previous delivery experience, but only previous pregnancy and delivery were different at a statistically significant level during the entire pregnancy.

Table 1
Characteristics of all participants in seven cities of PRD region (2014–2017).

Variables	Live births (n = 1,270,774)	Stillbirths (n = 3,150)	Total births (n = 1,273,924)
Baby gender			
Male	681281 (53.61%)	1618 (51.37%)	682899 (53.61%)
Female	589377 (46.37%)	1498 (47.55%)	590875 (46.38%)
Uncertain	116 (0.01%)	34 (1.08%)	150 (0.01%)
Maternal age			
<35 years	1083764 (85.28%)	2593 (82.32%)	1086357 (85.28%)
≥35 years	186568 (14.68%)	551 (17.49%)	187119 (14.69%)
Missing	442 (0.03%)	6 (0.19%)	448 (0.03%)
Previous pregnancy			
Yes	689492 (54.26%)	2124 (67.43%)	691616 (54.29%)
No	333981 (26.28%)	1026 (32.57%)	335007 (26.30%)
Unrecorded	247301 (19.46%)	0	247301 (19.41%)
Previous delivery			
Yes	554838 (43.66%)	1567 (49.75%)	556405 (43.68%)
No	468741 (36.89%)	1583 (50.25%)	470324 (36.92%)
Unrecorded	247195 (19.45%)	0	247195 (19.40%)
Season of conception			
Spring	280823 (22.10%)	717 (22.76%)	281540 (22.10%)
Summer	285603 (22.47%)	708 (22.48%)	286311 (22.47%)
Fall	325760 (25.64%)	836 (26.54%)	326596 (25.64%)
Winter	378588 (29.79%)	889 (28.22%)	379477 (29.79%)

Table 2
Summary of air pollution and meteorological variables during pregnancy of all participants.

Variables	Mean	Min	Max	Percentiles		
				25th	50th	75th
$PM_{2.5}$ (μg/m^3)	36.78	19.17	73.46	32.73	36.24	40.99
O_3 (μg/m^3)	80.70	37.81	125.35	73.38	81.67	87.92
NO_2 (μg/m^3)	41.20	12.91	75.55	33.37	41.64	48.95
SO_2 (μg/m^3)	13.42	6.75	45.23	10.91	12.50	14.95
Temperature (°C)	22.72	14.17	28.36	21.20	22.54	24.35
Relative humidity (%)	78.92	64.09	88.32	76.79	79.03	81.13

Table 3
The HRs (95% CIs) for stillbirths with each 10 μg/m^3 increase in $PM_{2.5}$ concentration during study periods in single-pollutant models.

Trimesters	Model 1[a]	Model 2[b]
Trimester 1	1.45 (1.38, 1.53)	1.44 (1.37, 1.52)
Trimester 2	1.61 (1.52, 1.71)	1.67 (1.57, 1.77)
Trimester 3	1.19 (1.13, 1.26)	1.23 (1.16, 1.30)
Entire pregnancy	1.23 (1.16, 1.31)	1.52 (1.42, 1.62)

Note: HR, hazard ratio; CI, confidence interval.
[a] All models adjusted for mean ambient temperature and relative humidity with degrees of freedom of 6 and 3, respectively.
[b] All models adjusted for maternal age, newborn gender, season of conception, previous pregnancy and previous delivery condition, in addition to Model 1.

Table 4
The HRs (95% CIs) for stillbirths with each 10 μg/m^3 increase in $PM_{2.5}$ concentration during study periods in two-pollutant models.

Pollutants	Model 2[a]
Trimester 1	
$PM_{2.5}$	1.44 (1.37, 1.52)
$+O_3$	1.51 (1.43, 1.60)
$+NO_2$	1.47 (1.38, 1.56)
$+SO_2$	1.41 (1.32, 1.50)
Trimester 2	
$PM_{2.5}$	1.67 (1.57, 1.77)
$+O_3$	1.77 (1.66, 1.89)
$+NO_2$	1.63 (1.52, 1.75)
$+SO_2$	1.60 (1.48, 1.72)
Trimester 3	
$PM_{2.5}$	1.23 (1.16, 1.30)
$+O_3$	1.23 (1.17, 1.31)
$+NO_2$	1.09 (1.01, 1.16)
$+SO_2$	1.35 (1.26, 1.45)
Entire pregnancy	
$PM_{2.5}$	1.52 (1.42, 1.62)
$+O_3$	1.59 (1.49, 1.70)
$+NO_2$	1.44 (1.33, 1.56)
$+SO_2$	1.89 (1.72, 2.08)

Note: HR, hazard ratio; CI, confidence interval.
[a] All models adjusted for maternal age, newborn gender, season of conception, previous pregnancy and previous delivery condition, mean ambient temperature and relative humidity with degrees of freedom of 6 and 3.

Over an average during the entire pregnancy, the estimated effect of $PM_{2.5}$ was higher among pregnant women who had not been pregnant previously than among women who had been previously pregnant with an adjusted HR of 2.02 (95% CI: 1.78, 2.28) vs 1.33 (95% CI: 1.23, 1.45), and among pregnant women without previous delivery than those with previous delivery with an adjusted HR of 1.84 (95% CI: 1.66, 2.03) vs 1.28 (95% CI: 1.17, 1.41).

Altering the degrees of freedom for the adjustment of temperature and relative humidity did not substantially change effect estimates for the association of $PM_{2.5}$ to stillbirth. HR estimates for stillbirth of entire pregnancy ranged from 1.52 to 1.53 for the adjustment of temperature and from 1.52 to 1.56 for the adjustment of relative humidity (See Supplementary Table S2). Further excluding the season of conception covariate did not alter the effect estimates (Supplementary Table S3), for example, the strongest HR of including the season of conception was 1.67 (95% CI: 1.57, 1.77) for each 10 μg/m^3 increase in $PM_{2.5}$ at trimester 2, which similarly to the HR of excluding the season of conception covariate 1.63 (95% CI: 1.54, 1.73). The HRs (95% CIs) of other adjusting covariates for stillbirths during the entire pregnancy are showed in Supplementary Table S4.

5. Discussion

To our knowledge, this study is among the largest multi-city birth cohort study to investigate the association between $PM_{2.5}$ and stillbirths among a Chinese population. Based on our cohort of 1.27 million births, this study illustrated that gestational exposure to $PM_{2.5}$ was associated with increased risk of stillbirths. Our study also demonstrated that the second trimester might be more vulnerable exposure window than the first trimester and the third trimester.

The above findings are generally consistent with the results of previous literature which also showed the significant associations between $PM_{2.5}$ exposure and increased stillbirth (Arroyo et al., 2016; DeFranco et al., 2015; Green et al., 2015). For example, Arroyo's study in Spain and DeFranco's study in the United States both revealed that the risk of stillbirths increased with high concentrations of $PM_{2.5}$ exposure (Arroyo et al., 2016; DeFranco et al., 2015). Green's study also indicated an association between exposure to high level $PM_{2.5}$ and an increased stillbirth throughout pregnancy in California (Green et al., 2015). However, other two studies in USA and one study in China found no positive association between $PM_{2.5}$ and stillbirths (Faiz et al., 2012, 2013; Hou et al., 2014). Inconsistent findings across these studies might be due to the complexity of $PM_{2.5}$ composition, the difference of air pollutant concentrations, and the different susceptibilities and confounders of the underlying populations. Thus, large multicity birth cohort studies focused on identifying the association of different air pollutant and the risk of stillbirths are necessary to prevent the adverse effect of air pollutants and make effective measurements.

The present study suggested that $PM_{2.5}$ was a potential risk factor of stillbirths; however, the mechanism by which $PM_{2.5}$ causes stillbirths remains unsolved. One study found that exposure to $PM_{2.5}$ might cause hypercoagulability with vascular thrombosis and placental inflammation, which would affect placental development (Liu et al., 2016). Another study found that prenatal traffic exposure might increase the risk of preeclampsia and lead to placental abruption (Yorifuji et al., 2015). This placental abnormality is a main cause of stillbirths (Tikkanen, 2011). Experiments conducted on animals show that $PM_{2.5}$ exposure could exacerbate the deviation of Th1 (type 1 helper T cell) or Th2 (type 2 helper T cell) and affect the immune system (Hong et al., 2013). Immune or endocrine system dysfunction could affect fetal growth, leading to a stillbirth (Ebisu et al., 2018). Toxic pollutants may also affect blood flow and, thereby, lessen nutrition transfer from the mother to the fetus (Maisonet et al., 2004), which heightens the risk of stillbirths.

Trimester-specific susceptibility to air pollution exposure is another factor that remains unanswered. The sensitive window to air pollutant exposure is inconsistent across studies (Arroyo et al., 2016; DeFranco et al., 2015; Hwang et al., 2011). Yang's study in China reported that air pollutant exposure in the third trimester was significantly associated with stillbirth (Yang et al., 2018). In the present study, the second trimester was shown to be the sensitive window of stillbirth to $PM_{2.5}$ exposure. This variation in susceptibilities may be caused by different air pollutant exposures at different period of pregnancy, and associations may also fluctuate with seasonal changes. Different sample sizes, study design, air pollution levels, and methodologies might also contribute to

Table 5
Baby's sex, maternal age group, previous pregnancy, and previous delivery condition specific HRs (95%CIs) for stillbirths associated with each 10 μg/m^3 increase in $PM_{2.5}$ in the study population.

Variables	Model 1[a]				Model 2[b]			
	Trimester 1	Trimester 2	Trimester 3	Entire pregnancy	Trimester 1	Trimester 2	Trimester 3	Entire pregnancy
Baby gender								
Male	1.49 (1.38, 1.61)	**1.69 (1.55, 1.83)**	**1.26 (1.17, 1.36)**	1.26 (1.16, 1.37)	1.47 (1.37, 1.58)	**1.74 (1.60, 1.89)**	**1.32 (1.22, 1.43)**	1.55 (1.42, 1.71)
Female	1.36 (1.26, 1.47)	**1.48 (1.35, 1.61)**	**1.11 (1.03, 1.21)**	1.20 (1.09, 1.31)	1.36 (1.26, 1.47)	**1.53 (1.40, 1.67)**	**1.13 (1.04, 1.23)**	1.47 (1.33, 1.62)
Maternal age								
<35 years	1.48 (1.40, 1.57)	**1.66 (1.56, 1.78)**	**1.23(1.16, 1.30)**	1.25 (1.17, 1.34)	1.46 (1.38, 1.55)	**1.72 (1.61, 1.84)**	1.26 (1.19, 1.34)	1.53 (1.42, 1.65)
≥35 years	1.31 (1.15, 1.49)	**1.34 (1.16, 1.56)**	**1.06 (0.93, 1.20)**	1.17 (1.01, 1.36)	1.33 (1.17, 1.52)	**1.45 (1.24, 1.69)**	1.11 (0.97, 1.28)	1.50 (1.27, 1.76)
Previous pregnancy								
Yes	**1.30 (1.23 1.39)**	**1.46 (1.36, 1.57)**	1.19 (1.11, 1.26)	**1.25 (1.16, 1.35)**	**1.28 (1.20, 1.37)**	**1.47 (1.36, 1.58)**	1.18 (1.11, 1.27)	**1.33 (1.23, 1.45)**
No	**1.85 (1.69, 2.02)**	**2.15 (1.95, 2.38)**	1.31 (1.18, 1.44)	**1.75 (1.57, 1.96)**	**1.83 (1.67, 2.00)**	**2.24 (2.01, 2.49)**	1.32 (1.19, 1.46)	**2.02 (1.78, 2.28)**
Previous delivery								
Yes	**1.30 (1.20, 1.39)**	**1.46 (1.35, 1.58)**	1.17 (1.09, 1.26)	**1.22 (1.12, 1.33)**	**1.27 (1.18, 1.37)**	**1.47 (1.35, 1.60)**	1.18 (1.09, 1.27)	**1.28 (1.17, 1.41)**
No	**1.64 (1.52, 1.76)**	**1.88 (1.73, 2.04)**	1.30 (1.20, 1.41)	**1.60 (1.46, 1.76)**	**1.62 (1.51, 1.75)**	**1.92 (1.76, 2.09)**	1.30 (1.19, 1.41)	**1.84 (1.66, 2.03)**

Note: Bolded text indicates statistically significant values ($p < 0.05$), HR, hazard ratio; CI, confidence interval.
[a] All models adjusted for mean ambient temperature and relative humidity with degrees of freedom of 6 and 3, respectively.
[b] All models adjusted for maternal age, newborn gender, season of conception, previous pregnancy and previous delivery condition, in addition to Model 1.

differing results across ours and previous studies. For example, our study included a large sample size of participants who had been exposed to high $PM_{2.5}$ pollution levels in seven cities in the PRD region. Another example is that the mean concentrations of air pollutants at each trimester of pregnancy were considered in our analysis by using Cox proportional hazards models.

The observed associations between $PM_{2.5}$ and stillbirth seemed not to be confounded by other air pollutants, expect for SO_2. It might be due to the relatively high correlations between $PM_{2.5}$ and SO_2 ($r = 0.65$, shown in Table S1), which change the HR of $PM_{2.5}$ most after controlling for SO_2 in the model.

In this study, we examined potential effect modification and found higher estimated risk of $PM_{2.5}$ exposure on stillbirth among pregnant women without previous experiences of pregnancy and delivery. This finding had some important public health implications, particularly, special air pollution protection should be considered for these women. The underlying reasons for these findings remain unknown. It is possible that women who had not previously been pregnant and or delivered a child were less experienced on how to protect themselves and were exposed to more outdoor air pollution.

Our study was a population based birth cohort study which included 1,273,924 newborns across in seven cities in the PRD region. The air pollution levels in the studied region are relatively higher than that in the previously studied regions. A study in U.S. found the $PM_{2.5}$ concentration during the entire pregnancy ranged from 9.9 μg/m^3 in Florida to 18.7 μg/m^3 in California (Sun et al., 2016). In this study, the mean concentration of $PM_{2.5}$ during the entire pregnancy was 36.78 μg/m^3. Our study also added new evidence for the adverse effects of maternal $PM_{2.5}$ exposures on neonatal health. Pregnant women seemed to be more sensitive to the adverse effect of air pollutants, and thus effective air quality control policies and stricter enforcement are necessary to improve maternal and neonatal health. More studies focused on air pollutants and neonatal health are important for building a comprehensive understanding of their associations and interactions.

Our study was subject to a few limitations. First, exposure misclassification was possible, since the air pollutant data from nearby air monitoring stations was used, future studies are suggested to have a better exposure assessment at the individual level (Copeland et al., 1977). Secondly, we did not consider residence mobility of some participants during their pregnancy because of the unavailability of this information. We also lacked information on other important factors, such as genetics, childbirth or pregnancy complications, fetal growth restrictions, cogential abnormalties, maternal nutritional status and socio-economic status (Goldenberg et al., 2008). Furthermore, some previous studies on stillbirth treated exposure to indoor cigarette smoking and alcohol consumption as potential confounders, but this study did not have categorized parental occupations and behaviors available in our data (Hao et al., 2016; Woodruff et al., 2009).

6. Conclusions

Our study demonstrates that maternal $PM_{2.5}$ exposure might be an important risk factor for stillbirths, especially at the middle stage of pregnancy. Our analysis also suggests that whether the mother has been pregnant and/or delivered a child before might modify the associations between $PM_{2.5}$ and stillbirths.

Contributors

ZJ.L, HL. L and GC.L designed this study and wrote the manuscript. ZJ.L and J. Y collected the health data and analyzed the data. Y.Y and HL. L collected the air pollution and weather data. ZM. Q, ZL. Z, SE. M and E.L revised the manuscript and were involved in the discussion of the results.

Declaration of competing interest

The authors declare they have no actual or potential competing financial 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.2021.113795.

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Review

Substitutes mimic the exposure behaviour of REACH regulated phthalates – A review of the German HBM system on the example of plasticizers

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ABSTRACT

The population is constantly exposed to potentially harmful substances present in the environment, including inter alia food and drinking water, consumer products, and indoor air. Human biomonitoring (HBM) is a valuable tool to determine the integral, internal exposure of the general population, including vulnerable subgroups, to provide the basis for risk assessment and policy advice. The German HBM system comprises of five pillars: (1) the development of suitable analytical methods for new substances of concern, (2) cross-sectional population-representative German Environmental Surveys (GerES), (3) time trend analyses using archived samples from the Environmental Specimen Bank (ESB), (4) the derivation of health-based guidance values as a risk assessment tool, and (5) transfer of data into the European cooperation network HBM4EU. The goal of this paper is to present the complementary elements of the German HBM system and to show its strengths and limitations on the example of plasticizers. Plasticizers have been identified by EU services and HBM4EU partners as priority substances for chemical policy at EU level. Using the complementary elements of the German HBM system, the internal exposure to classical phthalates and novel alternative plasticizers can be reliably monitored. It is shown that market changes, due to regulation of certain phthalates and the rise of substitutes, are rapidly reflected in the internal exposure of the population. It was shown that exposure to DEHP, DiBP, DnBP, and BBzP decreased considerably, whereas exposure to the novel substitutes such as DPHP, DEHTP, and Hexamoll®DINCH has increased significantly. While health-based guidance values for several phthalates (esp. DnBP, DiBP, DEHP) were exceeded quite often at the turn of the millennium, exceedances today have become rarer. Still, also the latest GerES reveals the ubiquitous and concurrent exposures to many plasticizers. Of concern is that the youngest children showed the highest exposures to most of the investigated plasticizers and in some cases their levels of DiBP and DnBP still exceeded health-based guidance values. Over the last years, mixture exposures are increasingly recognized as relevant, especially if the toxicological modes of action are similar. This is supported by a cumulative risk assessment for four endocrine active phthalates which confirms the still concerning cumulative exposure in many young children. Given the adverse health effects of some phthalates and the limited toxicological knowledge of substitutes, exposure reduction and surveillance are needed on German and EU-level. Substitutes need to be monitored, to intervene if exposures are threatening to exceed acceptable levels, or if new toxicological data question their appropriateness. It is strongly recommended to reconsider the use of plastics and plasticizers.

1. Introduction

Although chemical safety has been continuously improved within the last century, public concern about the exposure to environmental chemicals, the number of insufficiently investigated substances as well as the aggregated exposure remains high. Challenging the task to inform the public and support a science-based chemical policy, the German Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) has established a comprehensive human biomonitoring (HBM) system at the German Environment Agency (UBA). The aims of the HBM system are the monitoring of internal and aggregated exposure to

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Abbreviations

BMU	German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety
BBzP	butylbenzyl phthalate
5cx-MEPP	mono(2-ethyl-5-carboxy-pentyl) phthalate
5cx-MEPTP	mono(2-ethyl-5-carboxyl-pentyl) benzene-1,4-dicarboxylate
cx-MiDP	mono(2,7-methyl-7-carboxy-heptyl) phthalate
cx-MINCH	cyclohexane-1,2-dicarboxylic mono carboxyisooctyl ester
DEP	diethyl phthalate
DEHA	di(2-ethylhexyl) adipate
DEHP	di(2-ethylhexyl) phthalate
DEHTP	diethylhexyl terephthalate
DI	daily intake
DiBP	di-iso-butyl phthalate
DiDP	di-iso-decyl phthalate
DINCH	di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate
DINA	diisononyl adipate
DiNP	di-iso-nonyl phthalate
DnBA	di-n-butyl adipate
DnBP	di-n-butyl phthalate
DMP	dimethyl phthalate
DPHP	di(2-propylheptyl) phthalate
ECHA	European Chemicals Agency
ESB	German Environmental Specimen Bank
GerES	German Environmental Survey
HBM	human biomonitoring
HBM4EU	European Human Biomonitoring Initiative
HBM-I value	human biomonitoring value I
HBM-II value	human biomonitoring value II
HBM-GV	human biomonitoring guidance value
HMW	high molecular weight
REACH	registration, evaluation, authorisation and restriction of chemicals
LMW	low molecular weight
MBzP	monobenzyl phthalate
MEP	monoethyl phthalate
MEHP	mono(2-ethylhexyl) phthalate
MiBP	mono-iso-butyl phthalate
MMP	monomethyl phthalate
MnBP	mono-n-butyl phthalate
5OH-MEHP	mono(2-ethyl-5-hydroxy-hexyl) phthalate
OH-MiDP	6-OH-mono-propyl-heptyl phthalate
OH-MiNP	7-OH-(mono-methyl-octyl) phthalate
OH-MINCH	cyclohexane-1,2-dicarboxylic mono hydroxyisononyl ester
OH-MPHP	mono(2-propyl-6-hydroxy-heptyl) phthalate
oxo-MPHP	mono(2-propyl-6-oxo-heptyl) phthalate
5oxo-MEHP	mono(2-ethyl-5-oxo-hexyl) phthalate
oxo-MiDP	6-oxo-mono-propyl-heptyl phthalate
oxo-MiNP	7-oxo-(mono-methyl-octyl) phthalate
PVC	polyvinyl chloride
TDI	tolerable daily intake
TOTM	tris(2-ethylhexyl) tri-mellitate
UBA	German Environment Agency
VCI	German Chemical Industry Association (Verband der Chemischen Industrie)

chemicals, the identification of potential health risks, and building of scientific bases for political actions.

The most prominent instrument of the German HBM system is the regular analysis of environmental chemicals and/or their metabolites in human matrices, mostly blood and urine. Thus, the internal exposure to certain chemicals and cumulative exposure to multiple chemicals simultaneously can be assessed accurately. The origin of the German HBM activities dates back to the early 1970s, where, after a massive lead intoxication of livestock in Nordenham (Germany), an HBM research project was initiated with the aim to assess the lead levels of the population (Kolossa-Gehring et al., 2012a). Successively, new instruments have been added to the German HBM toolbox and have continuously been developed further, resulting in a highly valuable instrument for exposure and risk assessment of the population.

Today, the German HBM system comprises five complementing components, providing strong scientific evidence for policy advice and information of the public (cf. Fig. 1): (1) the development of new analytical methods for the monitoring of substances of health relevance - to which the population might be exposed to a considerable extent and for which no sufficiently sensitive and specific HBM methods yet exist - in a co-operation between the BMU and the German Chemical Industry Association (VCI); (2) population-representative cross-sectional German Environmental Surveys (GerES) to assess the exposure of the general population, to identify exposure sources, exposure-relevant habits, higher exposed subgroups, and thus, to develop exposure reduction measures; (3) time-trend analyses using samples from the Environmental Specimen Bank (ESB) to identify trends in exposure and to monitor the effectiveness of exposure reduction measures and policy actions; (4) the toxicological risk assessment by independent experts from various disciplines in the German Human Biomonitoring Commission allowing for the evaluation and interpretation of HBM results in the context of health risks; (5) the sharing of experience, methodologies, and results for harmonisation at the EU level in the HBM network of the European Joint Programme HBM4EU.

This review aims at presenting the progress and achievements of the German HBM system, using the monitoring of exposure to phthalates and alternative plasticizers as an example. Plasticizers were identified by EU services and HBM4EU partners as priority substances for which open policy-relevant research questions still have to be answered to support chemical policy at the EU level (Ganzleben et al., 2017).

The strengths and limitations of the single components of the German HBM system are discussed in detail and conclusions for plasticizers risk assessment and exposure mitigation are drawn. The example is also used to show the progress of the German HBM system in the last decade, to emphasize the importance of its components, and to illustrate its contribution to European chemical policy. By using multiple tools complementing each other, conclusions can be drawn which allow for deriving recommendations for policy makers.

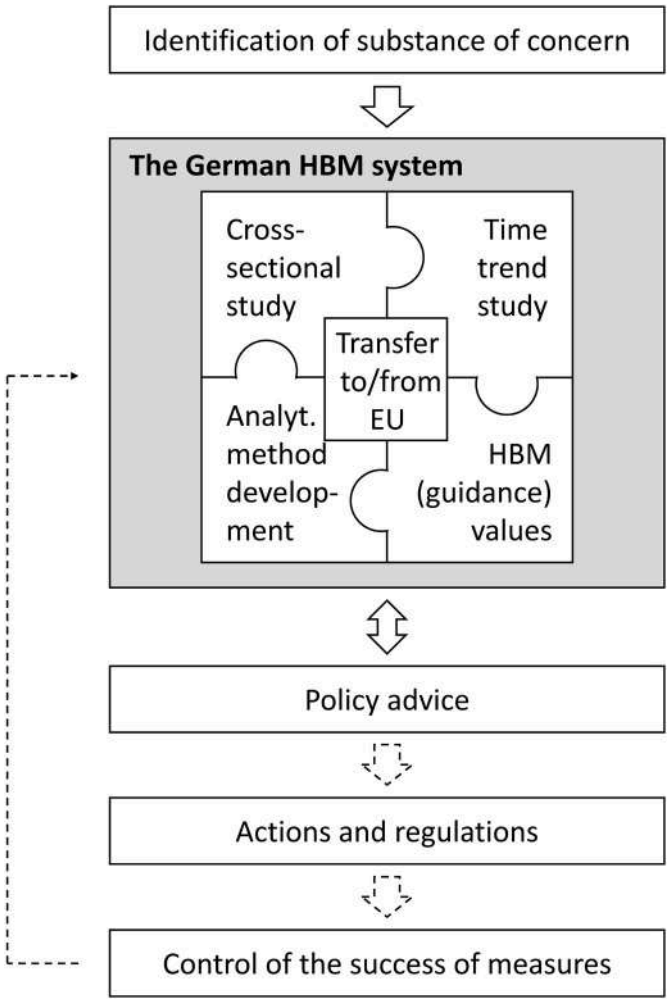

Fig. 1. The German HBM system and its embedding in the process from identification of substances of concern to policy advice and action.

2. The German HBM system

From the multitude of chemicals used in Europe only a selection can be monitored in HBM studies because of logistical and financial limitations. The criteria for the prioritisation of compounds for inclusion in HBM are their production volume, toxicological properties and potential impact on human health, accumulation potential and persistency, occurrence in the environment, and the availability of sensitive and specific biomarkers preferably in blood or urine. High tonnage compounds with critical toxicological characteristics such as carcinogenicity, mutagenicity, reproductive toxicity (CMR) or endocrine disrupting (ED) properties, as well as long term and organ specific effects are of high priority for investigation in HBM studies.

One major bottleneck of HBM is the availability of suitable analytical methods for emerging substances of concern and newly introduced substitutes for regulated chemicals like the reprotoxic phthalates. To close this gap, a cooperation between BMU and VCI with clearly separated responsibilities started in 2010 (Kolossa-Gehring et al., 2017; Leng and Gries, 2017). Within this BMU/VCI cooperation, up to 50 new HBM methods were to be developed for substances (1) for which substantial exposure of the general population is to be expected, (2) that might have health-relevant effects on the human organism, and (3) for which no specific and sensitive HBM methods exist. More than 20 HBM methods have already been developed and validated, inter alia for phthalates and alternative plasticizers (Gries et al., 2012a; Höllerer et al., 2018b; Kuhlmann et al., 2021; Leng and Gries, 2017; Lessmann et al., 2016a; Schütze et al., 2012). After analytical method validation and peer-reviewed publication, the respective methods are applied to samples of the two German HBM studies at federal level: the German Environmental Survey (GerES) and the German Environmental Specimen Bank (ESB).

GerES is a population-representative cross-sectional study investigating the exposure of the population in Germany to chemicals. It has repeatedly been carried out since 1985 (Kolossa-Gehring et al., 2012b; Schulz et al., 2007). GerES has always been conducted in close cooperation with the German health interview and examination surveys of the Robert Koch Institute (RKI) (Kamtsiuris et al., 2007; Kurth et al., 2008; Mauz et al., 2017). The latest cycle, GerES V (2014–2017), examined children and adolescents aged 3–17 years (Schulz et al., 2017). Some of the former GerES cycles focussed on other age groups. The HBM of GerES is complemented, inter alia, by the monitoring of indoor air, house dust, and drinking water. It is also supplemented by questionnaire data on dietary behaviour, lifestyle, and living environment and can be supplemented by the data derived by the health surveys of RKI. Thus, allowing for the identification of potential exposure pathways.

The ESB is an archive for human samples collected annually (Kolossa-Gehring et al., 2012a; Wiesmüller et al., 2007) and specimen of various environmental compartments (ESB, 2021a). Collection of human samples started in 1981. While a base set of substances (mainly heavy metals) is routinely analysed in all samples, cryo-archived samples are used for retrospective analyses of relevant substances if there is a need and an analytical method exists, becomes newly available or is sufficiently refined to detect exposure levels originating from environmental exposure. Thus, time trends of pollutant concentrations, e.g. in human blood, plasma, or urine can be analysed and the effects of the marketing of chemicals and their potential regulation on human internal exposure can be investigated. The ESB investigates a non-representative group of 20–29-year-old students at four sampling locations. The strength of the ESB is to supply valuable information on the development of exposure over time in continuously collected, comparable samples investigated by standardized procedures and methods (ESB, 2021a, b) – while ESB samples (not population-representative, from not specifically exposed volunteers, limited to 20–29 year old adults) are not suited to draw conclusions for other subgroups and living conditions of the whole population in Germany (as does GerES). A unique selling point of the ESB is the option for thorough time trend analyses dating back even to times before a substance was of concern. The time trends can help to interpret GerES results when exposure levels measured in GerES at different time points shall be compared. ESB and GerES together allow a complementary cross-sectional and time trend assessment of the chemical body burden of the population in Germany.

The results of the HBM studies are evaluated by using the health-based HBM values derived by the German HBM Commission (German HBM Commission, 1996) whenever available. The Commission derives so-called HBM-I and HBM-II values. The HBM-I value is defined as the concentration of a substance or its biomarker(s) at or below which there is no risk for human health, according to current knowledge. The HBM-II value represents the concentration of a substance or its biomarker(s) in human biological material at or above which there is an increased risk for adverse health effects, according to current knowledge (Apel et al., 2017). If the exposure levels are at or below HBM-I value, there is no need for action. If there is an exceedance of the HBM-II value, a health risk is anticipated. Thus, the HBM-I value can be regarded as a control value and the HBM-II value as an intervention value (Angerer et al., 2011; Apel et al., 2017).

In 2017, the European Human Biomonitoring Initiative (HBM4EU) has been set up. HBM4EU is a joint effort of 30 countries and the European Environment Agency, co-funded under the European Commission's Horizon 2020 program. HBM4EU, coordinated by UBA, creates a European network that improves the knowledge and factual basis for the European Union's environmental and chemical policy by harmonizing the planning and implementation of HBM studies, sample analysis and

data analysis across national borders (Ganzleben et al., 2017). One key aim is the knowledge transfer from countries with a long tradition in HBM to countries without own national programs to advance and implement HBM on a European scale and to provide scientific evidence to improve chemical policy making. Another goal of the project is to bring together already existing data, including the results of the GerES and ESB studies, and evaluate them according to uniform criteria. Moreover, new joint studies are conducted. For this purpose, questions, procedures, analytical methods, and evaluations are standardized and quality assured. HBM4EU also aims at harmonizing health risk assessments by deriving human biomonitoring guidance values (HBM-GVs) in a coordinated manner (Apel et al., 2020b). HBM-GVs derived for the general population correspond to the HBM-I values of the German HBM Commission and provide a valuable addition and partial update of already existing HBM-I values.

Plasticizers were chosen as an example to show the complementary elements of the German HBM system. The full scope of activities on plasticizers within the German HBM system is given in Table 1.

3. Human biomonitoring of phthalates and substitute plasticizers

Phthalates are esters of phthalic acid (benzene-1,2-dicarboxylic acid) and are widely used in industrial applications, e.g. as solvents, formulating agents, and plasticizers for soft polyvinyl chloride (PVC) (Koch et al., 2017). The first phthalates were introduced into the market in the 1920s and production skyrocketed after di-(2-ethylhexyl) phthalate (DEHP) was synthesized in 1933 and the PVC industry started growing (Graham, 1973). As of 2011, 8 mio tons of phthalates per year were consumed worldwide and about 840 ktons in Europe (ECHA, 2013), decreasing to 810 ktons in 2017 (European Plasticisers initiative CEFIC sector group, 2018).

High molecular weight (HMW) phthalates such as DEHP, di-iso-nonyl phthalate (DiNP), di-iso-decyl phthalate (DiDP) and di-(2-propylheptyl) phthalate (DPHP) were mostly used as plasticizers for the production of PVC (Fréry et al., 2020). Low molecular weight (LMW) phthalates like di-*n*-butyl phthalate (DnBP), di-*iso*-butyl phthalate (DiBP), butylbenzyl phthalate (BBzP), diethyl phthalate (DEP) and dimethyl phthalate (DMP) used to be contained in consumer products like textiles, pesticides, paints, adhesives and cosmetics (Fréry et al., 2020).

Until 2000, DEHP was the most commonly used phthalate in Europe with over 400 ktons of annual consumption (Bizzari et al., 2013). However, from the late 1990s, the use of DiNP, DiDP and DPHP started increasing and partially replaced DEHP on the plasticizer market (ECHA, 2013).

Health impacts of phthalates raised increasing concern after their reprotoxic properties were identified. Even more so as ubiquitous exposure of humans was observed due to the high production volumes and the use in products in close contact to consumers (Koch et al., 2017; Wittassek et al., 2011). Especially phthalates with a backbone of three to six carbon atoms such as DiBP, DnBP, and BBzP, and the HMW phthalate DEHP show endocrine disrupting properties related to the so-called phthalate syndrome (Koch et al., 2017). The phthalate syndrome includes developmental effects and structural as well as functional damage of the male reproductive system when the foetus is exposed during a critical window of sexual development (Apel et al., 2020a). Among others, the observed effects include malformations of the testes, epididymis and gubernaculum testis, cryptorchidism, hypospadias, reduced anogenital distance and reduced semen count (summarised in e.g. NRC, 2008; US CPSC, 2014). As phthalates are not chemically bound to the carrier material, they are easily released into the environment,

Table 1
Overview of German HBM activities on phthalates and substitute plasticizers – monitoring, assessment values, method development.

	Population-representative monitoring		Time trends	HBM (guidance) values	Analytical methods
	GerES IV	GerES V	ESB		BMU/VCI cooperation
Phthalates					
DMP, DEP, DiDP, DnOP, DCHP, DnPeP		Schwedler et al. (2020c)	2007–2015: Koch et al. (2017)		
BBzP, DnBP, DiBP	Becker et al. (2009)	Schwedler et al. (2020c)	1988–2015: Apel et al. (2020a); Göen et al. (2011); Koch et al. (2017); Wittassek et al. (2007b)	HBM-GV: Lange et al. (2021)	
DEHP	Becker et al. (2009)	Schwedler et al. (2020c)	1988–2015: Apel et al. (2020a); Göen et al. (2011); Koch et al. (2017); Wittassek et al. (2007b)	HBM-I: Apel et al. (2017), HBM-GV: Apel and Ougier (2017)	
DiNP	Becker et al. (2009)	Schwedler et al. (2020c)	1988–2015: Apel et al. (2020a); Göen et al. (2011); Koch et al. (2017); Wittassek et al. (2007b)		
DPHP		Schwedler et al. (2020a)	1999–2017: Schmidtkunz et al. (2019); Schütze et al. (2015)	HBM-I: Apel et al. (2017), HBM-GV: Lange et al. (2021)	Gries et al. (2012b); Leng and Gries (2017)
Substitute plasticizers					
DEHTP		Schwedler et al. (2020b)	1999–2017: Lessmann et al. (2019)	HBM-I: Apel et al. (2017)	Lessmann et al. (2016a)
DINCH		Schwedler et al. (2020a)	1999–2017: Kasper-Sonnenberg et al. (2019); Schütze et al. (2014)	HBM-I: Apel et al. (2017), HBM-GV: Apel and Ougier (2017)	Schütze et al. (2012)
TOTM		Murawski et al. (2021)			Höllerer et al. (2018b); Kuhlmann et al. (2021)
DEHA					Nehring et al. (2019)
DnBA					Ringbeck et al. (2020)
DINA					Gotthardt et al. (2021)

GerES IV: 2003–06, children (3–14 years), N = 599; GerES V: 2015–17, children and adolescents (3–17 years), N = 439–2256; ESB: young adults (20–29 years), N = 60/year.

DMP: Dimethyl phthalate, DEP: Diethyl phthalate, DiDP: Di-iso-decyl phthalate, DnOP: Di-n-octyl phthalate, DCHP: Dicyclohexyl phthalate, DnPeP: Di-n-pentyl phthalate, BBzP: Butylbenzyl phthalate, DnBP: Di-n-butyl phthalate, DiBP: Di-iso-butyl phthalate, DEHP: Di(2-ethylhexyl) phthalate, DiNP: Di-iso-nonyl phthalate, DPHP: Di(2-propylheptyl) phthalate, DEHTP: Diethylhexyl terephthalate, DINCH: Di-iso-nonyl-cyclohexane-1,2-dicarboxylat, TOTM: Tri-(2-ethylhexyl) trimellitate, DEHA: Di(2-ethylhexyl) adipate, DnBA: Di-n-butyl adipate, DINA: Diisononyl adipate.

resulting in ubiquitous exposure of the population, e.g. by uptake via food, indoor air, and dust (Bui et al., 2016; Fréry et al., 2020; Schwedler et al., 2020c). After implementation of different steps of regulation, HBM studies were conducted to evaluate the success of the reduction measures. First mitigation measures were already enacted in 1999 (ECHA, 2013). The classification of DnBP, DEHP, BBzP and later DiBP as reproductive toxicants (Regulation (EC) No 1272/2008, EU, 2008) was followed by further restrictions and bans, e.g. use in toys and childcare articles (Directive, 2009/48/EC, EU, 2009a), cosmetics (Directive, 2009/48/EC, EU, 2009b) and food contact materials (Regulation (EC) 10/2011, EU, 2011). These four phthalates are regulated under REACH (registration, evaluation, authorisation and restriction of chemicals, Regulation (EC) No 1907/2006, EU, 2006) after classification as substances of very high concern (SVHC) (Schwedler et al., 2020c) and since 2015 authorisation is required for DiBP, DnBP, BBzP, and DEHP (REACH Annex XIV) (ECHA, 2013; Koch et al., 2017; Schwedler et al., 2020c). The latest amendment of REACH Annex XVII took effect in July 2020, restricting the general use of DiBP, DnBP, BBzP, and DEHP in consumer articles (ECHA, 2020).

One effect of the increasing regulation of certain phthalates is the decrease in use of the regulated phthalates and a shift to the use of non-regulated phthalates or substitute substances (Apel et al., 2020a; Frederiksen et al., 2020; Koch et al., 2017). DEHP is the most prominent regulated phthalate. The regulation resulted in a shift to the less regulated phthalates DiNP and DiDP, which in turn were substituted by DPHP, diethylhexyl terephthalate (DEHTP), di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate (Hexamoll®DINCH, abbr. DINCH), tris (2-ethylhexyl) tri-mellitate (TOTM) and di (2-ethylhexyl) adipate (DEHA). This effect is apparent in the increasing consumption of DINCH and DEHTP in Europe, rocketing from 9 to 2 ktons in 2002 to 55 and 100 ktons in 2014, respectively (Kasper-Sonnenberg et al., 2019; Lessmann et al., 2019). In contrast to the substituted phthalates, the substitutes thus far are not classified as toxic to reproduction or as endocrine disruptors. DINCH, introduced into the marked in 2002, did not show reproductive toxicity or developmental toxicity in animal studies (German HBM Commission, 2014). Effects on the kidneys were considered most relevant (EFSA, 2006). For DPHP, EFSA and also the German HBM Commission based their guidance values on the combined chronic and carcinogenicity dietary study in rats by Deyo (2008), in which effects on the retina were considered critical (Apel et al., 2017; Deyo, 2008; EFSA, 2008). Adverse systemic effects for TOTM following short-term and sub-chronic exposure refer to haematology, clinical chemistry, liver and spleen. Developmental toxicity considered to be biologically significant or substance-induced was not observed. However, toxicity to reproduction of TOTM remains to be elucidated as studies show contradictory results (CPSC, 2018).

DINCH and DEHTP are not only used as substitute plasticizers for reprotoxic HMW phthalates like DEHP, but also for DnBP, DiBP, BBzP, especially in regulated products and products resulting in potentially high exposure, e.g. toys, food contact materials, and medical applications (Kasper-Sonnenberg et al., 2019; Lessmann et al., 2019). DINCH and DEHTP, like other plasticizers, are not bound to the polymer and can easily migrate. A considerable exposure of the population is the result (Lessmann et al., 2016a; Schütze et al., 2012). The shift in the market and its consequences for human exposure and health require the inclusion of substitute compounds into the monitoring of human internal exposure.

4. Analytical methods for phthalate determination

Early methods for phthalate determination in urine could not distinguish between different specific phthalates and were replaced by liquid chromatography coupled to mass spectrometry (Blount et al., 2000; Kato et al., 2005; Koch et al., 2003b), which also allowed the simultaneous determination of various specific phthalate metabolites (Barr et al., 2003). Additionally, this method prevented interference with contamination by the ubiquitous parent compounds. Multi-methods enabling the quantification of many different phthalates are frequently expanded to include an even higher number of urinary metabolites. Nowadays, routine measurements of more than 10 different phthalates are available (Koch et al., 2017). Due to the short half-lives, phthalates and their substitutes, DINCH, DEHTP, DEHA, di-n-butyl adipate (DnBA) and diisononyl adipate (DINA), are rapidly eliminated from the body (Anderson et al., 2001a, 2011; Gotthardt et al., 2021; Koch et al., 2012, 2013; Lessmann et al., 2016b; Nehring et al., 2019; Ringbeck et al., 2020), whereas TOTM has a longer elimination half-life due to the molecular structure (most of the 2-ethylhexanol group is eliminated within 6 days; trimellitic acid core is excreted slowly) (CPSC, 2018). Thus, HBM measurements for the phthalates and their substitutes reflect only very recent exposure, except for TOTM which has a slower urinary excretion rate than e.g. DEHP with metabolites still detectable after 48 h and 72 h post-exposure (Höllerer et al., 2018a).

To keep up with the market development and the introduction of non-regulated substitute plasticizers, new analytical methods for the biomonitoring of these emerging substances were needed. Within the German BMU/VCI cooperation, new methods for the determination of DEHTP (Lessmann et al., 2016a), DINCH (Schütze et al., 2012), TOTM (Höllerer et al., 2018b; Kuhlmann et al., 2021), DEHA (Nehring et al., 2019), DnBA (Ringbeck et al., 2020) and DINA (Gotthardt et al., 2021) have been developed, thereby greatly expanding the toolbox of plasticizers' and phthalate substitutes' monitoring in the general population. Moreover, existing methods were improved to allow for the distinction of the structurally similar phthalates DiDP and DPHP (Gries et al., 2012b; Leng and Gries, 2017).

5. Internal exposure levels reflect regulation of phthalates and the emergence of substitute plasticizers

ESB and GerES provide complementary information on the population's exposure to pollutants: GerES supplies information on the exposure distribution in sub-groups and the whole society, on potential risk groups, risk factors, and on sources of exposure, while the ESB answers the question if either a new regulation has to be considered because of increasing exposure time trends or if implemented measures where sufficiently successful. Additionally, this archive allows for the retrospective analyses of newly emerging substances.

HBM data on 15 phthalates and plasticizer substitutes are to date available from ESB and GerES. An overview is given in Table 1. Daily intakes (DI) derived from ESB and GerES data are presented in Fig. 2. DI from 24-h-urine samples of the ESB were derived using equation (1) as also described by Apel et al. (2020a). For the first-morning void urine samples of GerES, a creatinine excretion-based approach described by Wittassek et al. (2007a) was applied as shown in equation (2).

$$\mathrm{DI}(\mu g / kg_{bw} / day) = \frac{UE_{vol,sum} \cdot uv}{F_{UE} \cdot bw} \cdot MW \quad (1)$$

$$\mathrm{DI}(\mu g / kg_{bw} / day) = \frac{UE_{crea,sum} \cdot CE}{F_{UE} \cdot bw} \cdot MW \quad (2)$$

$UE_{vol,sum}$ - molar urinary excretion sums of specific metabolites in µmol/L; $UE_{crea,sum}$ – molar urinary excretion sums of specific metabolites in µmol/g_{crea}; uv – 24-h urine volume; CE – sex- and body height-specific daily creatinine excretion given by Remer et al. (2002); F_{UE} – summarised urinary excretion fraction for all considered metabolites given in Table 2; bw – bodyweight; MW – molecular weight of respective substance.

Exposure to BBzP, DnBP, DEHP and, much later, DiBP decreased after regulatory measures were taken (Koch et al., 2017), reflecting the decrease of consumption (German HBM Commission, 2011). A very strong correlation between production and daily intake of DEHP was also already revealed by Helm (2007), indicating that human internal

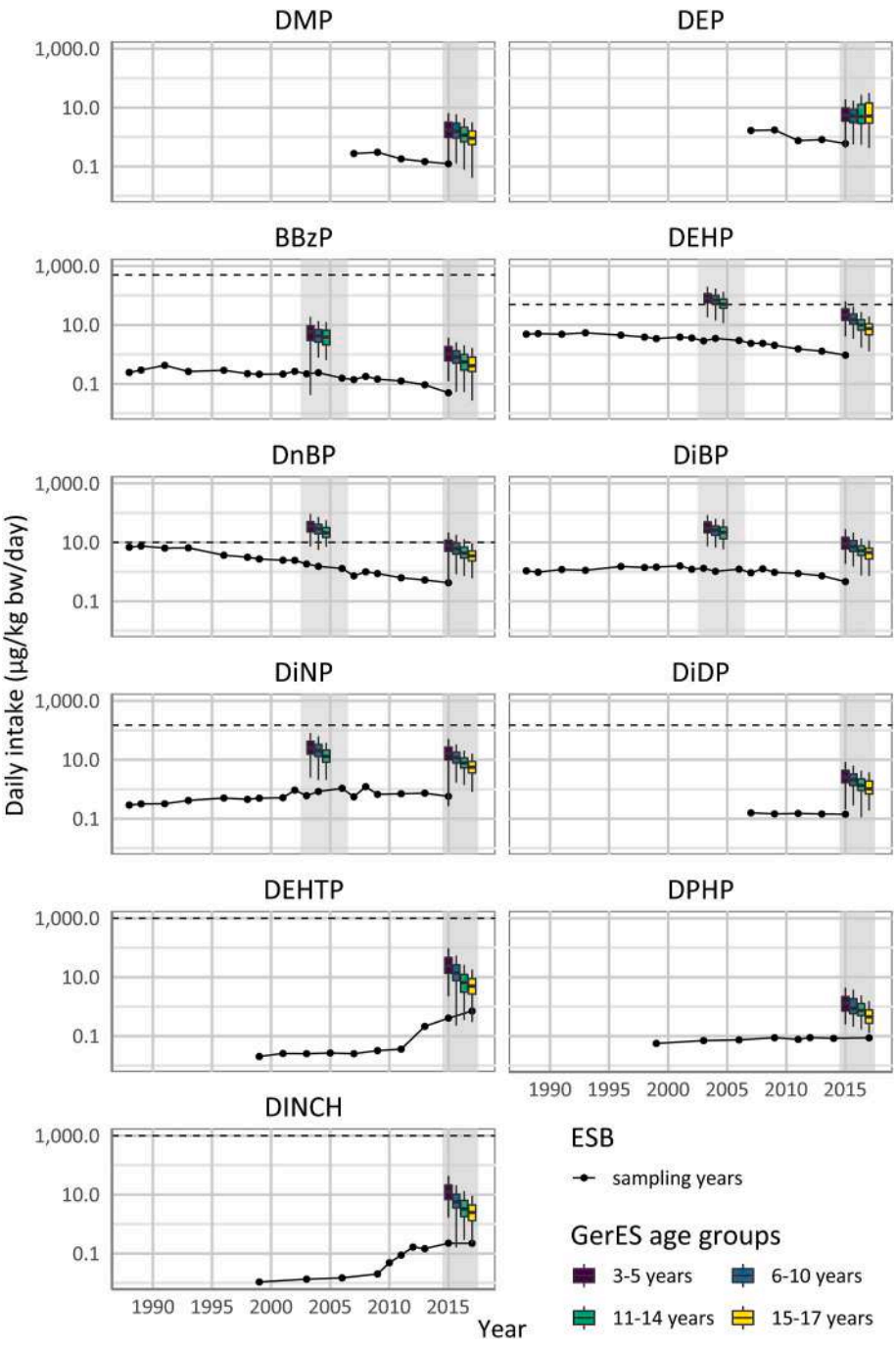

Fig. 2. Daily intake (µg/kg_{bw}/day) of plasticizers measured in samples of the ESB, GerES IV, and GerES V. Annual median values for ESB are presented by line-connected dots. GerES data are presented by colour-coded boxplots for each age group (whiskers spanning the 1.5-fold interquartile range at max, outliers are not shown). Grey shaded areas denote the sampling period of the respective GerES cycle (GerES IV – 2003–2006, GerES V – 2015–2017). The position of boxplots within the grey shaded area is for presentation purposes only; each boxplot represents the full sampling period of the respective GerES. Horizontal dashed lines denote the TDI of the substance (not available for DMP, DEP, DiBP, and DPHP). DCHP, DnPeP, DnOP, and TOTM are not shown as too few samples contained quantifiable amounts for statistical summaries. Note the logarithmic scale!

exposure follows production of plasticizers closely and immediately. Simultaneously with the decrease of regulated plasticizers, new phthalates, and substitute plasticizers such as DPHP, DEHTP, DINCH, DEHA and TOTM emerged, leading to an increasing internal human exposure to these new substances. The time trends provided by the ESB reveal strong increases in exposure to the substitute plasticizers DINCH (Schütze et al., 2014) and DETHP (Lessmann et al., 2019) since their market entry, resulting in a nowadays ubiquitous exposure of the population. These time trends are qualitatively mirrored by the comparison of data from two GerES cycles, GerES IV and GerES V. This is also apparent in Table 3, which shows the fraction of ESB and GerES participants with urinary plasticizer concentrations above the respective quantification limits. Since the market entry in 2002, exposure to DINCH steadily increased along with increasing production, resulting in quantifiable internal exposure levels in all GerES V participants. The same holds true for DEHTP. Rapidly increasing production volumes are reflected in increasing internal exposure levels, leading to quantifiable urinary DEHTP levels in each GerES V sample. While TOTM is not yet used widely and is consequently found in only few individuals (Murawski et al., 2021), the annual consumption of DPHP is clearly related to the detection rates of its main metabolite oxo-MPHP in human urine (Schmidtkunz et al., 2019).

6. Identification of subgroups of high exposure – GerES

A decrease of BBzP, DiBP, DnBP, and DEHP levels in ESB samples – and corresponding daily intake – after regulation (Apel et al., 2020a; Koch et al., 2017), as shown in Fig. 2, was similarly observed by

Table 2
Urinary excretion fractions (F_{UE}) and respective metabolites used for daily intake calculation of plasticizers.

Plasticizer	F_{UE}	Metabolites considered	Source
DMP	0.69	MMP	US Consumer Product Safety Commission (2014)
DEP	0.69	MEP	Koch et al. (2003a)
BBzP	0.73	MBzP	Anderson et al. (2001b)
DiBP	0.707	MiBP	Koch et al. (2012)
DnBP	0.691	MnBP	Anderson et al. (2001b)
DEHP	0.471	MEHP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP	Anderson et al. (2011), summarised by Apel et al. (2020a)
DiNP	0.189	OH-MiNP, oxo-MiNP	Anderson et al. (2011), summarised by Apel et al. (2020a)
DiDP	0.34	OH-MiDP, oxo-MiDP, cx-MiDP	summarised in Wittassek et al. (2011)
DEHTP	0.13	cx-MEPTP	summarised in Lessmann et al. (2019)
DPHP	0.1392	OH-MPHP, oxo-MPHP	average of Leng et al. (2014) and Klein et al. (2018), as also used by Lange et al. (2021)
DINCH	0.1276	OH-MINCH, cx-MINCH	Koch et al. (2013)

comparison of GerES IV and GerES V data (Schwedler et al., 2020c). Fig. 2 presents the GerES data for different age groups, showing a significant age gradient for most plasticizers with the highest exposure of young children (3–5 years) compared to older children and adolescents (15–17 years).

The daily intakes of children and adolescents were an order of magnitude higher than those of young adults. Apart from age-dependent metabolic differences and a relatively higher intake of food, drinks, and inhaled air per kg body weight by younger children compared to adults, strong associations between plasticizer concentrations in urine and house dust samples were observed in GerES V (Schwedler et al., 2020a, 2020b, 2020c). As young children tend to ingest dust due to hand-to-mouth contacts (Salthammer et al., 2018), exposure via house dust might additionally contribute to the observed age gradient. Furthermore, as many toys made of plastic contain plasticizers, and especially small children tend to put them in their mouth, further uptake of plasticizers by small children is likely.

For the interpretation of the results from ESB and GerES and a comparison of daily intakes methodological differences and some limitations have to be considered. The general sampling design differs, as described above (ESB: convenience sample of young adults, GerES: two-stage population-representative sample of children and adolescents in Germany), as well as the sampling method of the analysed urine samples (ESB: 24-h-urine, GerES: first-morning void urine). While the total

Table 3
Percentage of ESB and GerES participants with measurements above the limit of quantification (% > LOQ). Percentages for GerES refer to the full survey period. For ESB, percentages were derived for each individual sample year and displayed ranges refer to the range of annual values in the displayed time period.

Parent compound	BBzP	DEHP					DnBP		DiBP		DiNP		
Metabolite(s)	MBzP	MEHP	5OH-MEHP	5cx-MEPP	5oxo-MEHP	2cx-MMHP	MnBP	OH–MnBP	MiBP	OH-MiBP	7OH-MiNP	7oxo-MiNP	7cx-MiNP
LOQ (µg/L) [a]	0.5/0.2	0.5	0.5/0.2	0.5/0.2	0.5/0.2	0.5/–	2.0/1.0	–/0.25	2.0/1.0	–/0.25	0.5/0.2	0.5/0.2	0.5/0.2
GerES IV (2003–2006)	100	100	100	100	100	100	100	–	100	–	100	98	100
GerES V (2015–2017)	99	86	100	100	100	–	100	99	100	100	100	99	100
ESB (2003–2006)[b]	100	97–98	100	100	100	100	100	–	100	–	97–98	91–93	92–93
ESB (2015–2017)[b]	98	90	100	100	100	–	100	92	100	100	100	100	100
ESB before 2002	98–100	93–100	100	100	100	100	100	–	100	–	90–100	58–86	–
ESB from 2002	96–100	90–100	100	100	100	100	100	92–100	100	100	96–100	91–100	92–100

Parent compound	**DMP**	**DEP**	**DiDP**			**DCHP**	**DnPeP**	**DnOP**
Metabolite(s)	MMP	MEP	OH-MiDP	oxo-MiDP	cx-MiDP	MCHP	MnPeP	MnOP
LOQ (µg/L)	1.0	0.5	0.2	0.2	0.2	0.2	0.2	0.2
GerES V (2015–2017)	97	100	98	88	97	6	6	0
ESB (2015–2017)[b]	95	100	97	57	82	5	2	0
ESB from 2007	88–98	100	90–97	53–67	77–100	0–5	0–5	0–3

Parent compound	**DINCH**				**DPHP**			**DEHTP**				**TOTM**[c]
Metabolite(s)	MINCH	OH-MINCH	oxo-MINCH	cx-MINCH	OH-MPHP	oxo-MPHP	cx-MPHP	5OH-MEHTP	5oxo-MEHTP	5cx-MEPTP	2cx-MMHTP	
LOQ (µg/L)	0.1	0.05	0.05	0.05	0.3	0.25	0.15	0.3	0.2	0.2	0.4	0.09–0.26
GerES V (2015–2017)	–	100	97	99	50	62	1	67	79	100	20	0–3
ESB (2003–2006)[b]	0	0–7	0–5	0–3	0	0	0	0	0	0–3	0	–
ESB (2015–2017)[b]	–	100	95–97	80–87	2	18	0	22–47	18–40	98–100	2–3	–
ESB before 2002	0	0	0	0	0	0	0	0	0–2	3–10	0	–
ESB from 2002	0–5	0–100	0–100	0–88	0–3	0–23	0	0–47	0–40	0–100	0–3	–

[a] First values refer to ESB sampling years until 2006, 2008, and GerES IV; second values refer to ESB sampling years 2007, 2009–2015, and GerES V.

[b] Not every year in the given period is covered, for sample years of ESB see Fig. 2 or refer to the original publications.

[c] Six metabolites, for more information see Murawski et al. (2021).

Table 4
Percentage of ESB and GerES participants exceeding HBM values of the German HBM commission (HBM-I; Apel et al., 2017) or HBM guidance values derived within the framework of HBM4EU (HBM-GV; Lange et al., 2021). Percentages for GerES refer to the full survey period. For ESB, percentages were derived for each individual sample year and displayed ranges refer to the range of annual values in the displayed time period.

	HBM-I				HBM-GV						
Parent compound	DEHP	DEHTP	DINCH	DPHP	BBzP	DEHP	DEHP	DnBP	DiBP	DINCH	DPHP
Metabolite(s)	5oxo-MEHP + 5OH-MEHP	5cx-MEPTP	OH-MINCH + cx-MINCH	oxo-MPHP + OH-MPHP	MBzP	5cx-MEPP + 5OH-MEHP	5oxo-MEHP + 5OH-MEHP	MnBP	MiBP	OH-MINCH + cx-MINCH	oxo-MPHP + OH-MPHP
HBM value (mg/L)											
- children (<14 years)	0.5	1.8	3.0	1.0	2.0	0.38	0.34	0.12	0.16	3.0	0.33
- women/men	0.3/0.75	2.8	4.5	1.5	3.0	0.57	0.5	0.19	0.23	4.5	0.5
GerES IV (2003–2006)	1.55%	–	–	–	0%	4.23%	3.41%	34.10%	19.37%	–	–
GerES V (2015–2017)	0%	0%	0.04%	0%	0%	0.05%	0%	1.18%	1.76%	0.04%	0%
ESB (2003–2006)[a]	0%	0%	0%	0%	0%	0%	0%	1.72%–5.00%	0%–3.45%	0%	0%
ESB (2015–2017)[a]	0%	0%	0%	0%	0%	0%	0%	0%	1.67%	0%	0%
ESB before 2002[b]	0%–1.67%	0%	0%	0%	0%	0%–1.67%	0%–1.67%	10.00%–48.33%	0%–6.67%	0%	0%
ESB from 2002[c]	0%–1.67%	0%	0%	0%	0%	0%	0%	0%–5.00%	0%–3.45%	0%	0%

[a] Not every year in the given period is covered, for sample years of ESB see Fig. 2 or refer to the original publications.

[b] Earliest samples measured are from 1999 (DEHTP, DINCH, DPHP), and 1988 (BBzP, DEHP, DnBP, DiBP).

[c] Latest samples measured are from 2017 (DEHTP, DINCH, DPHP), and 2015 (BBzP, DEHP, DnBP, DiBP), respectively.

amount of daily metabolite excretion is known in ESB's 24-h-urine samples, daily intake calculation from GerES data requires various assumptions. Based on the first-morning void creatinine content, the excretion was extrapolated using generalised daily creatinine excretion totals given by Remer et al. (2002). Thus, only qualitative comparisons can be made between ESB and GerES results, while the comparability between the two cycles of GerES is mostly warranted.

The recent population representative study FLEHS IV from Belgium investigated the internal exposure of 14–15-year-olds to phthalates and compared geometric means (GM) of urinary concentrations of different studies (Bastiaensen et al., 2021). The internal exposure to various phthalate metabolites was in the same range as observed in GerES V and this was also true for studies from USA and Canada, which investigated 12–19-year-olds. These studies, investigating adolescents, had somewhat lower GM than observed for the GerES V population, which presumably is due to the difference in age. Studies from Sweden, Poland and Portugal which examined the internal exposure of younger children found considerably higher concentrations for some phthalates (summarised in Bastiaensen et al., 2021).

Given the reprotoxic properties of some phthalates and the higher exposure of young children to these substances, special attention is needed. Since highest exposure for all investigated phthalates and substitutes appears in small children, a cause for highest concern is given and indicates the necessity for further action.

7. Health-based risk assessment

HBM values derived by the German HBM Commission for DEHP, DEHTP, DINCH, and DPHP (Apel et al., 2017), as well as the HBM guidance values for DEHP, DINCH, DiBP, DnBP, BBzP, and DPHP derived within the framework of HBM4EU (Apel and Ougier, 2017; Lange et al., 2021) are well suited to evaluate possible health risks of the exposure. A limitation of this assessment lies in the approach to assess the risk only for a single substance and not for the real simultaneous exposure to a mixture. Table 4 summarises the percentage of participants exceeding the respective HBM-I value or HBM-GV. Urinary concentrations of BBzP, DEHTP, and DPHP were below the HBM-I values and HBM-GVs in ESB samples and samples of GerES IV and GerES V, which is in line with daily intakes being below the respective tolerable daily intakes (TDIs) (cf. Fig. 2). With a view on decreasing exposure levels to BBzP observed in the ESB data, the exposure to this phthalate can, according to today's knowledge, be classified as not of current concern, but is still found in nearly all samples. The current DEHTP and DINCH levels reach, up to now, only in exceptional cases the HBM-I value and can, therefore, be counted as below the level of health concern. However, the fact that their metabolites were found ubiquitously in the most recent samples of GerES V and increasing concentration trends are observed in ESB data require continuous attention and a further development of a mixture risk assessment, especially as the maximum exposure to DINCH has reached the range of mg/L in urine.

The HBM-I value for DEHP was exceeded by 1.55% of GerES IV and up to 1.67% of ESB participants, but not by GerES V participants. Up to 1.67% of ESB participants in sampling years before 2002 exceeded the HBM-GVs (adults: 500 μg/L for 5oxo-MEHP + 5OH-MEHP and 570 μg/L for 5cx-MEPP + 5OH-MEHP; Apel and Ougier, 2017) but none exceeded the HBM-GVs after 2002. The new HBM-GVs for DEHP for children, considering two different metabolite compositions, are lower (children: 380 μg/L for 5cx-MEPP + 5OH-MEHP and 340 μg/L for 5oxo-MEHP + 5OH-MEHP; Apel and Ougier, 2017) than the HBM-I values derived in 2007 (children: 500 μg/L for 5OH-MEHP + 5oxo-MEHP; Apel et al., 2017). The HBM-GVs were exceeded by 4.23% and 3.41% of GerES IV participants, but only by 0.05% and 0% of the GerES V population, respectively. In the GerES V sample, which was collected after several steps of regulation, still up to 0.05% exceeded the HBM-GV, which represents about 5690 children and adolescents in Germany. However, the decrease in exceedances also reflects the success of the increasingly

strict regulation. Yet, even more than a decade after the first regulatory steps all samples contained quantifiable amounts of DEHP and some individuals still had concerningly high exposure levels.

The HBM-GVs for DiBP and DnBP were exceeded by substantial fractions of the GerES IV population (DiBP: 19.37%, DnBP: 34.10%), but only by 1.76% (DiBP) and 1.18% (DnBP) of the participants of GerES V. This shows the considerable progress in reducing exposure to these reprotoxic and endocrine disrupting phthalates, but they are still present in all samples analysed. Again, the higher exposure of small children is still of concern as they represent a vulnerable group. ESB samples collected in the years of GerES IV sampling (2003–2006) exceeded the HBM-GVs in at most 3.45% and 5.00% of the participants. For comparison, in 1988–2001 DnBP concentrations exceeded the HBM-GV in 10.00%–48.33% and from 2002 in, at maximum, 5.00%. This again reflects a successful reduction of exposure, but also emphasises that young adults reached lower exposure levels much earlier than children.

Considering the differences between the physiology of children and adults, it is recommendable to apply the HBM-I values and HBM guidance values to children under 6 years of age only if the metabolite ratio in young children is the same as in adults. Therefore, the comparison with exposure data used here can only be used as an approximation for rough guidance (German HBM Commission, 2007).

Health based guidance values (HBM-I and HBM-GV) allow for assessing the exposure risk of single substances only, but many phthalates share similar effects and modes of action (Apel et al., 2020a). Therefore, mixture effects must be considered to account for the impact of the real-life exposure to multiple phthalates simultaneously, which for phthalates are mainly the antiandrogenic effects. Based on reproductive and liver effects, EFSA derived a group-TDI of 50 µg/kg_{bw}/day for DEHP, DnBP, BBzP, and DiNP expressed as DEHP equivalents (EFSA, 2019). While median exposure of ESB participants was below the group-TDI already from 1988 on (Fig. 3), virtually the whole GerES IV population of children was above the group-TDI with median values of individual age groups ranging between 150 and 300 µg/kg_{bw}/day. Despite the observed decrease of exposure, the youngest age groups (3-5- and 6-10-year-olds) of GerES V (2014–2017) were on average still exceeding the group-TDI of 50 µg/kg_{bw}/day. Within the framework of HBM4EU, a mixture risk assessment approach using HBM guidance values is currently developed, evaluating the exposure based on internal concentrations. Thus, avoiding the conversion of HBM exposure levels into daily intake estimates to compare to external health-based guidance values.

In summary, a remarkable, successful reduction is seen in the exposure to critical, regulated phthalates. However, the overall exposure to plasticizers (phthalates and their substitutes) remains high. As long as regulated substances are replaced by new ones with presumably better toxicological properties derived from animal studies, but no long-time knowledge in humans (epidemiological studies), and human exposure only shifts from high exposure levels of a few substances to medium exposure levels of a wider mixture of substances, the desired success in exposure reduction may not be gained. For some of the substitutes investigated (e.g. DEHTP), exposure amplitudes might even surpass those of their predecessors very soon, with no net effect on exposure reduction.

Such developments might not fulfil the demands of a precautionary use of chemicals as well as the zero pollution ambition of the EU (European Commission, 2019), considering that the plasticizers discussed in this paper are all anthropogenic, synthetic chemicals. Regular monitoring of exposure levels, their time trends and subsequent cumulative risk assessment are therefore the key instruments to achieve a further improvement of chemical policy, including improvement of use, safe substitution and potentially reduction measures for plasticizers if needed.

8. Conclusions

Germany has established a unique HBM system at federal level allowing for the multidimensional assessment of chemical exposure. Due to the complementary application of cross-sectional and time trend studies, combined health and HBM surveillance, and additionally environmental monitoring, evidence-based conclusions on the chemical burden of the population, sources of exposure, vulnerable groups, and the efficacy of regulations can be drawn. The bidirectional interaction between science and policy strengthens the appropriate regulatory initiatives on a national and European level. The development of new HBM methods, especially for substitute plasticizers, enabled a more complete assessment of the exposure to plasticizers after the market shift to non-phthalate alternatives. The achievements in reducing the exposure to single reprotoxic phthalates were put into perspective when considering the increasing exposure to substitute substances. EU-wide regulated phthalates are still detectable in every sample analysed. The overall exposure to plasticizers remains at a very high level and regulated compounds have only been substituted by new chemicals with net exposures not changing much, but exposure profiles becoming more complex regarding mixtures. The German HBM studies show that decreasing numbers of participants exceed HBM assessment values for single plasticizers. However, the cumulative risk assessment revealed toxicologically relevant mixture exposures, which are particularly concerning for small children.

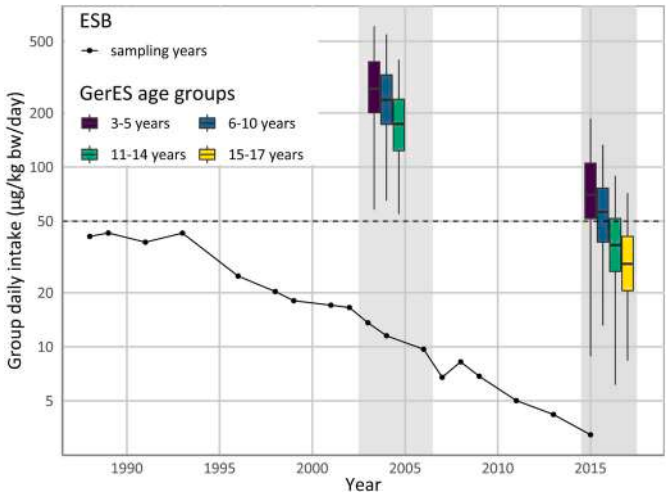

Fig. 3. Group-TDI for BBzP, DnBP, DEHP, and DiNP for ESB, GerES IV, and GerES V. Annual median values for ESB are presented by line-connected dots. GerES data are presented by colour-coded boxplots for each age group (whiskers spanning the 1.5-fold interquartile range at max, outliers are not shown). Grey shaded areas denote the sampling period of the respective GerES cycle (GerES IV – 2003–2006, GerES V – 2015–2017). The position of boxplots within the grey shaded area is for presentation purposes only; each boxplot represents the full sampling period of the respective GerES. Horizontal dashed line denotes the group-TDI of 50 µg/kg_{bw}/day. Note the logarithmic scale!

Based on current knowledge, the substitutes clearly have a preferable toxicological profile over the regulated phthalates which shows in lower risk estimates of their exposure levels. However, regarding exposure levels, overall plasticizer exposures seem stable, but the composition has changed and the complexity of mixtures has increased.

Therefore, in the future, investigations of the whole group of plasticizers are needed, not only single substance exposure and risk assessments. To sustainably reduce exposure to plasticizers, chemicals regulation yet in place might not be sufficient. The reduction of plasticizer use in consumer products, especially marketed for children, must be encouraged and policy makers, industry, and consumers have to reconsider their use of plastics and plasticizers.

HBM studies remain a key instrument in monitoring of the chemical market and controlling preventive and risk reduction measures within health and environmental policy programmes. However, the time between the detection of potentially critical levels of a substance in

humans and the respective regulatory actions is still too long. Regulation often starts much too late when harmful substances are already ubiquitously present in the population. To protect the population from harmful chemicals, a chemical policy in line with the precautionary principle and the zero pollution ambition (European Commission, 2019) is needed. Up to now, HBM has increasingly been established as an instrument to inform policy makers and the public about the exposure to environmental chemicals. Several European countries but also countries worldwide have set up national HBM programs (see e.g. WHO, 2015). A comprehensive HBM on a broader scale can only be achieved by burden sharing and channeling efforts into transnational projects. Therefore, HBM4EU, as a network of 30 countries, has been set up to accomplish this goal on a European level. The advances in HBM gained from the German HBM tools are shared within HBM4EU, which in return provides the broader expertise for harmonised risk assessment, hereby implementing a scientific base for chemical policy making in the EU.

Declaration of competing interest

The authors declare no conflict of interest related to this work.

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Corrigendum to "Substitutes mimic the exposure behaviour of REACH regulated phthalates – A review of the German HBM system on the example of plasticizers" [Int. J. Hyg. Environ. Health 236 (2021) 113780]

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The authors regret that some statements in chapter 3 "Human biomonitoring of phthalates and substitute plasticizers" are wrong due to missing text in the previously published version. Below is the corrected statement with changes in bold font.

For DPHP, **effects on the thyroid gland and pituitary gland, both observed in a subchronic toxicity study in rats, are considered critical (BfR, 2011; Bhat et al., 2014; German HBM Commission, 2015). DPHP is currently being under assessment for endocrine disruption (ECHA, 2021b). DEHTP is not considered toxic for reproduction and no warning for potential endocrine disrupting properties were identified under the regulatory management option analysis (ECHA, 2021a).** EFSA and also the German HBM Commission based their guidance values on the combined chronic and carcinogenicity dietary study in rats by Deyo (2008), in which effects on the retina were considered critical (Apel et al., 2017; Deyo, 2008; EFSA, 2008).

The authors would like to apologise for any inconvenience caused.

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Successes, challenges, and support for men versus women implementers in water, sanitation, and hygiene programs: A qualitative study in rural Nepal

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ABSTRACT

Introduction: Women's active participation is important for inclusive water, sanitation, and hygiene (WaSH) programs, yet gender roles that limit women's access to formal education and employment may reduce their skills, experience, and capacity for implementation. This paper explores differences between men and women implementers of rural WaSH programs in implementation approaches, challenges, and sources of support for implementation, and success in achieving program quality outcomes.

Methods: We interviewed 18 men and 13 women in community-based implementation roles in four districts of Nepal. We identified challenges and sources of support for implementation in four domains—informational, tangible, emotional, or companionship—following social support theory. We assessed successes at achieving intermediate implementation outcomes (e.g., adoption, appropriateness, sustainability) and long-term intervention outcomes (e.g., community cleanliness, health improvements).

Results: Women used relational approaches and leveraged social ties to encourage behavior change, while men used formative research to identify behavior drivers and sanctions to drive behavior change. Women experienced stigma for working outside the home, which was perceived as a traditionally male role. Companionship and emotional support from other women and male community leaders helped mitigate stigma and lack of informational support. Women were also more likely to receive no or low financial compensation for work and had fewer opportunities for feedback and training compared to men. Despite lack of support, women were motivated to work by a desire to build their social status, gain new knowledge, and break conventional gender roles.

Conclusions: Both men and women perceived that women were more effective than men at mobilizing widespread, sustained WaSH improvements, which was attributed to their successes using relational approaches and leveraging social ties to deliver acceptable and appropriate messages. Their skills for motivating collective action indicate that they can be highly effective WaSH implementers despite lack of technical experience and training, and that women's active participation is important for achieving transformative community change.

1. Introduction

Women's active participation is important for safe, effective, and inclusive water, sanitation, and hygiene (WaSH) programs. Women and girls are disproportionately affected by cultural norms for modesty and privacy during defecation and hygiene (Caruso et al., 2015). They are typically the duty-bearers for WaSH-related tasks within the household, such as water collection (Graham et al., 2016) and child feces management (Majorin et al., 2019). These duties expose women to additional health risks, such as exposure to pathogens or injury from carrying heavy water containers (Sevilimedu et al., 2017; Sorenson et al., 2011). The Sustainable Development Goals (SDGs) recognize the importance of women's participation in WaSH through Goal 5, which sets targets for increasing women's participation in leadership roles and decision making, and ending discrimination against women and girls, and Goal 6, which calls for universal access to WaSH "for all" and

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emphasizes "paying special attention to the needs of women and girls" (United Nations, 2015).

WaSH programs often rely on women for implementation at the local level. Community-based sanitation programs in low- and middle-income countries often emphasize recruitment of natural leaders from within the community and inclusion of women (WHO, 2018), and many countries have incorporated WaSH activities into the duties of female community health volunteers (female CHVs or FCHVs) (Perry et al., 2016). FCHV programs have extended basic health services to poor, rural areas that otherwise have little or no access (Bhutta et al., 2010; Perry et al., 2014). FCHVs are well suited to deliver health messaging to other women on sensitive topics such as menstruation that would be taboo for male healthcare providers (El Arifeen et al., 2013; Mumtaz et al., 2013; Panday et al., 2017). Mobilizing women's groups for participatory learning and action has been shown to reduce gender inequalities that impede access to proper care and improve maternal and neonatal health outcomes (Perry et al., 2014; Prost et al., 2013).

Women's active participation in WaSH and other natural resource management committees has been found to improve community trust in fee collection and financial management (Kelly et al., 2017), sustainable service delivery (Hoque et al., 1996; Westermann et al., 2005), collaboration and conflict management (Westermann et al., 2005), and recognition and resolution of problems in the community (Kelly et al., 2017). Women are often selected as implementers for community-based programming because they are familiar with the local context, well integrated into community social networks, and able to speak with other women on sensitive health topics (WHO, 2018).

However, recruiting women implementers from local communities has challenges. Communities targeted for health and development programs often have lower levels of formal education and other socioeconomic indicators (Kane et al., 2016). Gender norms that limit women's participation in formal education and employment can reduce their experience, skills, and capacity to effectively deliver health programming (Panday et al., 2017; Sarin and Lunsford, 2017). Furthermore, women may lack agency to participate in WaSH implementation (Wali et al., 2020) or be nominally included without valuing their contributions and concerns (Yerian et al., 2014). Although documentation is limited, some evidence suggests that meaningful inclusion of women in community-based implementation can improve the quality of programs, provided they receive appropriate support (Bhutta et al., 2010).

The purpose of this study was to explore how the gender of community implementers influences implementation at the local level for rural WaSH programs in Nepal. Specific objectives were to describe differences between men and women implementers in implementation approaches, challenges, and sources of support for implementation, and success in achieving program quality outcomes at the local level. We discuss the implications of these differences for improving program implementation, including opportunities to strengthen training and feedback systems, leverage women's social ties within communities, and reconsider compensation of unpaid workers.

2. Methods

2.1. Study design

We collected data through qualitative interviews with WaSH implementers in four districts in Nepal (Siraha, Mahottari, Surkhet, and Salyan) from June to August 2019. Interviews were conducted as part of a larger study on quality improvement and innovation in rural WaSH, which included implementers from the regional to community levels. Here, we analyzed a subset of interviews from men and women in community-based implementation roles to understand how implementers perceive approaches, challenges, support, and successes at the local level.

2.2. Conceptual frameworks

Implementation approaches are the activities and strategies used for program delivery. In implementing these approaches, men and women experience various challenges that hinder successful implementation and sources of support to mitigate those challenges. When challenges can be mitigated and adequate support is received, implementation approaches are expected to yield program success in terms of intermediate implementation outcomes (e.g., adoption and sustained practice of WaSH behaviors) and long-term impacts on target intervention outcomes (i.e., improvements in health and wellbeing). Fig. 1 depicts the conceptual model that we developed for this study.

We hypothesized that gender would influence the implementation approaches used by men versus women and the challenges and support systems experienced when implementing these approaches. In turn, we hypothesized that these approaches, challenges, and support systems would influence success at achieving implementation and intervention outcomes. We assessed implementation approaches, support and challenges, and implementation and program quality outcomes for all participants following the frameworks described below, then compared differences between men and women.

2.2.1. Implementation approaches

Activities conducted by village-level implementers primarily comprised behavior change messaging delivered in household and community settings. These activities in Nepal draw heavily from a community-led total sanitation (CLTS) approach (Department of Water Supply and Sewerage, 2011). CLTS uses participatory activities designed to elicit negative emotions such as shame and disgust at open defecation to "trigger" behavior change and collective action to make the community open defecation free (ODF) (Kar and Chambers, 2008). Similar approaches for behavior change triggering are also used to promote hygiene and other WaSH behaviors (e.g., food hygiene) post-ODF. Following triggering, local-level implementers conduct household and community-level follow up activities to give behavior change messages and create demand for WaSH.

For this study, we assessed which CLTS and related behavior change and demand creation activities were conducted by implementers, plus any alternative or additional activities delivered to households or communities. We did not assess implementation approaches related to capacity building or coordination of stakeholders, which were occurring at the local level but were rarely conducted by participants in our sample.

2.2.2. Challenges and support systems

We applied social support theory to categorize challenges and sources of support for implementation as either informational, tangible, emotional, or companionship. Informational support provides knowledge or guidance, typically to assist with problem solving. Tangible support provides physical goods, services, or money as a form of direct assistance. Emotional support provides encouragement through making an individual feel valued or self-confident, such as through expressions of empathy, concern, or caring. Companionship support provides a feeling of social belonging or presence of companions to engage in shared activities (Langford et al., 1997).

Social support theory has been used to explore determinants of successful implementation of health behaviors by lay individuals, households, and communities (Heaney and Israel, 2008; Kelly et al., 1991), similar to lay persons who serve as natural leaders under CLTS-style programs. Social support is particularly relevant for community-based WaSH implementers, who live in the community in which they work and have strong social ties to program recipients.

2.2.3. Program successes

We used outcomes defined by Proctor et al. (2011) as measures of program success: acceptability, adoption, appropriateness, cost,

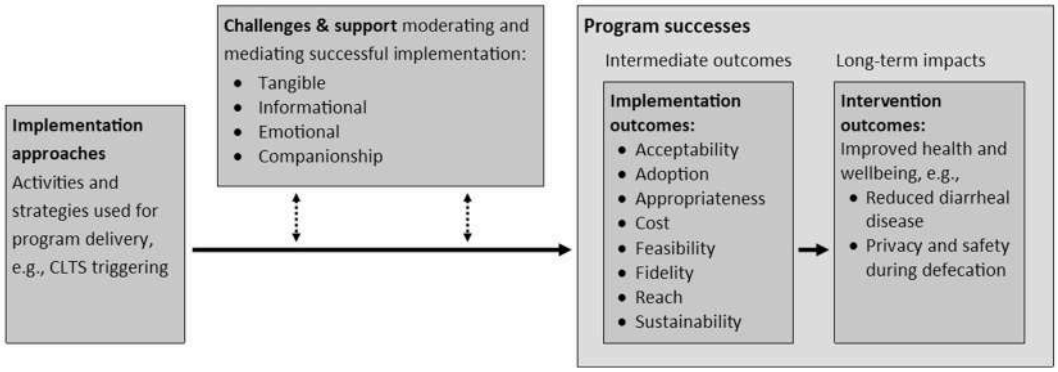

Fig. 1. Conceptual model of relationships between implementation approaches, challenges and sources of support for implementation, and program successes. Implementation approaches are expected to achieve program success measures where they are evidence-based, contextually appropriate, and receive appropriate support to overcome challenges. The model combines elements of social support theory (Langford et al., 1997) to describe challenges and support, and implementation outcomes and long-term impacts (Proctor et al., 2011) to describe successes.

feasibility, fidelity, penetration, and sustainability. Table 1 provides definitions. Proctor's framework proposes that implementation outcomes are precursors to achieving intervention outcomes, and that intervention outcomes may only be achieved if a program is implemented well. In the case of WaSH programs, relevant intervention outcomes include reductions in diarrheal disease or child mortality.

2.3. Study setting

WaSH implementers in Nepal include a diverse range of government, international and local non-governmental organizations (NGOs), multilateral organizations, private sector, and civil society organizations, such as women's, mothers', and journalist groups. We consider WaSH implementers to be anyone who participates in the delivery of program activities directly to beneficiary households (e.g., behavior change messaging) or the supervision, regulation, or technical support of these activities.

Sanitation and hygiene delivery in Nepal are governed at the federal level by the National Sanitation and Hygiene Master Plan. The Master Plan outlines responsibilities for different levels of government and defines "guiding principles"—such as representation of women on steering committees at a "minimum of 33% of the members, as appropriate." The details of specific activities or program plans are determined at the subnational level (Department of Water Supply and Sewerage, 2011).

District committees called "WaSH coordination committees" are responsible for supervising and harmonizing activities of implementers.

Table 1
Implementation outcomes as measures of program quality. Adapted from Proctor et al., (2011).

Outcome	Definition
Acceptability	Perception that a given WaSH program or its component parts is agreeable and satisfactory
Adoption	Intention, decision, or action to uptake a WaSH behavior or technology
Appropriateness	Perceived fit, relevance, or compatibility of the WaSH program with context, or perceived fit of the program to address a specific issue or problem
Cost	Cost of implementation efforts
Feasibility	The extent to which the WaSH program can be successfully delivered or used within a given context
Fidelity	The extent to which the WaSH program is delivered as originally developed and specified in program plans and protocols
Penetration	Coverage area and intensity of exposure to the WaSH program among the target population
Sustainability	The extent to which WaSH behaviors and technologies are maintained and institutionalized within the target population

Municipal committees plan and coordinate implementation activities in partnership with NGOs. Coordination committees are chaired by elected government officials, such as mayors, vice-mayors, and ward presidents. Elected government officials participate in the supervision and policy regulation but rarely deliver activities directly to beneficiary households. The majority of day-to-day behavior change programming at the local level is delivered to households by paid staff from local NGOs and unpaid community volunteers, including FCHVs and women's and mothers' groups. Technical support is provided by government employees, who are non-elected, hired specifically for their technical expertise. Multilateral agencies and international NGOs also provide technical support, including the World Health Organization (WHO), United Nations Children's Fund, United Nations Human Resettlement Program, and the SNV Netherlands Development Organization (Adhikari, 2012).

Men outnumber women in WaSH implementation particularly for roles in district, regional, and national supervision; private sector jobs for construction and small business owners; and engineering or technical jobs (Wali et al., 2020). Women more often fill unpaid roles at the local level as FCHVs and women's and mothers' group members. FCHVs, women's, and mothers' group members may receive reimbursement or a small stipend to cover travel and food allowances but otherwise do not receive formal wages (Khatri et al., 2017).

2.4. Study population and recruitment

Within each district, a program coordinator who had lived and worked in the district for at least the past five years assisted with recruitment. Program coordinators were employees of local or international NGOs. Their duties included supervising teams of NGO staff and unpaid volunteers (e.g., FCHVs, women's groups) across multiple villages and liaising with government and other partners.

Program coordinators were briefed on the research purpose and asked to identify a list of individuals who were highly involved in WaSH delivery at the district, municipal, and sub-municipal levels. From this list, we purposively sampled participants to represent a range of perspectives from government, multilateral organizations and NGOs (hereafter "development organizations"), and civil society organizations. When both men and women were available for interview for a particular stakeholder role, we preferentially recruited women to maintain a more equal gender balance.

For this study, we defined local-level as implementers at the sub-district level. We excluded regional- and national-level implementers, as their challenges, support, and successes differed substantially from those experienced at the local level. We also excluded participants from roles filled exclusively by men in the study sample—specifically WaSH

technicians and engineers, masons, journalists, and small business owners.

2.5. Data collection

A research team comprised of one interviewer and one note taker conducted interviews in private offices or meeting rooms at participants' workplace, or at the home or a nearby community center for participants without a formal office. During interviews, we asked participants to describe a novel WaSH solution or approach they used to improve program quality. We identified successes through questions that asked participants to describe whether they perceived their solution to be successful and how they defined and measured success, as well as questions that asked participants to describe successes and challenges for implementation and program improvement overall. We identified challenges and sources of support through questions that asked participants to describe barriers or facilitators to implementing and sustaining their solutions. Where participants described difficulties in implementing or sustaining solutions, we probed to explore sources of support for overcoming those difficulties.

Interview guides were developed in English, translated into Nepali, and pre-tested in a district bordering Siraha. Based on pre-testing, we reworded and reordered questions to improve the guide's ability to elicit information on key concepts and question flow. We also iteratively revised the interview guide throughout data collection to explore emergent themes. Interviews lasted approximately 1 h. We conducted interviews in English, Nepali, Hindi, or Maithili, following the preference of the participant. We audio recorded interviews where participants gave permission (n = 27, 87%) and transcribed recordings directly into English for analysis.

We also asked participants to complete a brief demographic questionnaire to identify their job title, years of work experience, and gender.

2.6. Analysis

We conducted template analysis using NVivo qualitative data analysis software (QSR International, Melbourne, Australia) for coding. We coded for *a priori* themes for implementation approaches as CLTS, behavior change, and demand creation activities. We coded challenges and sources of support in four domains (informational, tangible, emotional, and companionship) following social support theory (Langford et al., 1997). Finally, we coded successes at achieving the eight implementation outcomes described in Table 1 and a single code for intervention outcomes encompassing health improvements in WaSH-related diseases.

We developed an initial template for analysis using a subset of six transcripts, revising codes for *a priori* themes, and developing inductive codes for emergent themes, as necessary. We then applied the template to an additional six transcripts, iterating the reflection and revision process, before proceeding to coding the full dataset. After coding all interviews, we sorted codes by participants' self-identified gender to examine similarities and differences across men and women.

2.7. Ethics

This study was ruled as non-human subjects research by the Institutional Review Board of the University of North Carolina-Chapel Hill (IRB # 19–0945). Local approval for study activities was obtained from the Nepali Ministry of Water Supply. Participants were informed of the study purpose and provided written consent before enrollment.

3. Results

3.1. Sample characteristics

Our sample comprised 18 men and 13 women (Table 2). Participants were primarily elected officials (n = 6 men, 3 women) and development organization employees (n = 8 men, 3 women). Men ranged in age from 30 to 62 years (median 40.5) and had served an average of 3.0 years in their current implementation role. Women ranged in age from 23 to 46 years (median 38) and has served an average of 4.6 years in their current implementation role. Women more commonly held unpaid non-elected roles (n = 6, 46%) compared to men (n = 2, 11%).

Table 2
Participant demographics.

	Number of participants	
	Men (n = 18)	Women (n = 13)
Age (years)		
20-29	1	2
30-39	8	5
40-49	4	6
50+	5	–
Employment position		
Elected officials[a]	6	3
Government employees	1	–
NGO or multilateral employees	8	3
Teacher or school official	1	1
Social activist[a]	1	–
Disabled persons' organization representative[a]	1	–
Female community health volunteers[a]	–	3
Women's or mothers' group members[a]	–	3
Years in current position		
0-2	8	7
3-5	9	3
>5	1	3
Location		
Mahottari	5	3
Salyan	2	4
Siraha	8	1
Surkhet	3	5

[a] Denotes participants in unpaid roles.

We use the term "unpaid women" to describe women in non-elected, unpaid roles as FCHVs and women's and mothers' group members. We use the term "government implementers" to describe the combined efforts of government elected officials and employees, or differentiate between elected officials versus government employees as applicable.

3.2. Implementation approaches

Activities and messages used by community-based implementers were typically designed by development organizations. Content was similar to triggering techniques used under CLTS, designed to raise negative feelings about open defecation and awareness of its dangers. In some cases, content had been adapted to other settings, such as school-led total sanitation, where children were given similar messages and asked to pressure their parents to build toilets.

When WaSH activities were first implemented, implementers perceived strong community norms for open defecation and reported that households were often unwilling to construct toilets without a subsidy. Both men and women implementers perceived that messaging strategies were often initially unsuccessful at motivating behavior change and toilet construction, and WaSH activities did not meet participants' expectations for achieving rapid, community-wide behavior change. Responses to poor perceived success varied across government versus development organizations and men versus women.

Men from government in our sample commonly reported using "pressure" approaches. They insisted that individuals and households must comply with WaSH policies to meet government regulations. When these messages did not meet expectations for achieving rapid toilet construction, sanctions were the used, such as threatening to restrict or restricting government services (e.g., work permits and citizenship papers) for non-toilet owners or arresting and fining open defecators. Men

perceived that these approaches created tension with the community:

> "… Women and people used to curse me and abuse me. We have also abused them and dishonored them. We also seek the help of police to control them." -man, local government official

Technical advisors from development organization did not endorse sanctions approaches. Men in development organizations instead reported adjusting the content of messaging to more effectively motivate behavior change. For example, one man used radio jingles with voices of local people and two men developed new slogans to address locally relevant behavioral motivators.

In contrast to "pressure" approaches, women in all implementation roles used "convincing" approaches when they perceived households to be resistant to behavior change. Women did not demand that households must change behavior, but rather emphasized the benefits of WaSH for individuals, other members of the household, and community at-large. Women also recruited others in the community to show their support and deliver messages in groups:

> "When we say to the women, 'Sister, this toilet is for you,' they agreed and made the toilet. If the women do not understand, then we convince the mother in law by saying, 'Aunt, please make the toilet. If you made the toilet, it will be good for your daughter in law.' We convince the daughter in law by saying, 'It will be good for your son and daughters. Please look after the child and their health.'" -woman, development organization local implementer

3.3. Challenges and sources of support

Challenges for implementers arose due to differences in implementation approaches taken by men versus women, community and peer responses to these approaches, and the variance in support received to cope with challenges. The following sections categorize and describe these differences following the four domains of social support theory: informational, tangible, emotional, and companionship.

3.3.1. Informational support

3.3.1.1. Participation in program planning and training. Participants in all districts recognized women's participation in coordination committees as important. However, large committees, particularly at the district level, were perceived as unwieldy and inefficient, so smaller subcommittees were often formed for developing work plans. These committees included primarily government implementers and development organization technical advisors—roles typically filled by men. Our sample included three women elected government officials at the sub-municipal level, but they rarely were invited to participate in these subcommittee meetings at the municipal or district level.

No women reported participating in subcommittees where high-level planning occurred. Instead, they participated in meetings to receive training on how to deliver and monitor program activities that had already been designed. For women in unpaid roles, trainings typically lasted one day, with some lasting up to three days. One woman conducting training for local mothers' groups described how indicators for WaSH behaviors such as toilet use and handwashing were complex and difficult to understand with little training:

> "I took the training from the district and then give training to the mother groups. I have a banner of Total Sanitation [post-ODF WaSH promotion activities], and I used it to educate them. In the banner there are many pictures …. They could not understand all the indicators at once." -woman, elected official and former development organization implementer

Government implementers often overestimated FCHVs' knowledge and training. Government implementers relied on FCHVs to support developing program plans. However, FCHVs were not confident in their ability to design, deliver, and improve messages independently. In one area where development organization-supported programs had ended and no technical advisors were available, FCHVs and other community-based workers with little formal WaSH training were the primary source of technical expertise:

> "We have not yet given the technical knowledge to the *tole* [small municipal sub-unit] … [FCHVs and] health workers have the knowledge of sanitation and hygiene." -woman, local government representative

3.3.1.2. Systems for feedback and troubleshooting. Men and women employed by development organizations described the most robust communication and information sharing for addressing implementation challenges. They shared information between workers across geographic areas through phone and social media groups and met locally to discuss progress and challenges towards achieving program targets and to share good practices.

Outside of development organizations, men typically had more robust systems for feedback and troubleshooting. Men in government described participating in committees with representatives from other municipalities, where they would share progress and troubleshoot problems. Technical advisers and program supervisors from development organizations, who were predominately men, also participated in these committees.

Women in unpaid roles were less integrated into systems for feedback and troubleshooting. In some areas, women in unpaid roles reported challenges to municipal WaSH committees but were rarely engaged in developing solutions. In some cases, they received ongoing support for improving the content of program activities from a development organization or government adviser. However, this support was less stable and strongly linked to presence of funded programs, as technical advisors often left program areas or were reassigned to other projects when funding ended. One FCHV described how she was unsure of the quality of her work, because she had never received feedback:

> "I cannot say that all the works I have done are good because I have not seen some work output. I do my best, but there may be a chance that I am doing wrong. For example, if someone tells me that I have done wrong, then I can improve. Until now, no one has said that I have done anything wrong." -FCHV

3.3.1.3. Exposure to external learning and experience. Men had more prior employment experience prior to holding their current position and had worked more years in with their institution compared to women (6.6 versus 5.0 years). They described experiences working on previous programs or in other areas, which they applied for improving program quality in their current positions:

> "Our community was very backward. We used to go to Kathmandu, India, and other places. We have observed about the drainage system and other many things. And by seeing all these we have put the plan to the rural municipality." -man, social activist

In contrast, most women had little to no prior work experience before taking their current position. One woman, who was president of her local mothers' group, described how mobilizing women for WaSH campaigns was difficult because women had received little formal education, and some were "very young because of the early child marriage." Another woman described how opportunities to learn were limited because no other development organizations were working locally:

> "If they [other development organizations] came, we would get a chance to learn about new approaches. What is needed for the

community, only we cannot decide. If other organizations come, their recommendation and feedback will help." -woman, development organization local implementer

3.3.2. Tangible support

Insufficient funding and resources to deliver program activities was a challenge consistently reported across men and women. However, women were more likely to work as unpaid volunteers (46% of women versus 11% of men for non-elected positions in our sample). Programs relied heavily on FCHVs, women's and mothers' groups to conduct house-to-house behavior change activities and monitoring but did not offer formal wages to compensate their work. Unpaid women sometimes received allowances for snacks or travel, but two FCHVs indicated that these allowances were insufficient to cover the costs of their duties.

Four women in unpaid positions described challenges with balancing other responsibilities, such as managing domestic responsibilities in the home or field, or other income-generating activities. One FCHV indicated that with her time commitments to deliver non-WaSH programming under the FCHV curricula (e.g., contraceptive education) and hold paid employment, WaSH messages were only added to her work agenda seasonally, or as needed during water-borne disease outbreaks. One unpaid woman indicated that if she were paid, she could dedicate more time to WaSH work.

Unpaid women reported feeling less responsibility to improve program activities, because this was the responsibility of development organization workers who paid employees with more expertise. While unpaid women indicated that they would be willing to dedicate more time and effort to WaSH activities if they were paid, benefits such as increased opportunities for training and building social networks and status outside the home were still sufficient incentives to engage in WaSH work part-time.

3.3.3. Emotional support

Women implementers in all roles faced stigma for taking formal employment outside the home, which was perceived to violate traditional gender roles of women working only domestically. Stigma was particularly strong when programs were first implemented and decreased over time as communities perceived improvements to health and environmental cleanliness. This stigma was similar across women in both formal paid implementation roles and unpaid volunteers. Five women described receiving "abusive" words or confrontational reactions from men when delivering program activities. Particularly when programs were first implemented, these women reported criticism for attempting to educate and change behavior of men, which was perceived to overstep women's authority. One woman was told that her work disrespected her husband and family:

"Some say face-to-face that, 'You are rubbish, you only have the job to roam here and there. Your husband cooks the food by himself. You only roam here and there, and you do not respect your husband.' It was most challenging for me during the working period. All people laugh on me. Some in front, some in back. Many abuse me" -woman, development organization local implementer

In response to this criticism, women received support from family, development organizations, and progressive community leaders who publicly endorsed their work. One woman development organization implementer reported that her husband called a meeting of other men in the village to ask that they "at least hear her" before criticizing, which caused a positive response once men recognized the benefits of the program. For other women, development organizations spoke with community men to mitigate stigma:

"[Development organization workers] have said, 'You have done good work. We will look after those people who have abused you,' and after they have said something to them, and now there is no such issue." -FCHV

Women perceived that criticism decreased as communities observed improvements in community health and cleanliness, at which point women reported that they were recognized and praised for their work by community members. In lieu of compensation, unpaid women often received formal recognition for their work from local government, in the form of appreciation letters, ceremonies, and small gifts, such as shawls, which motivated them to continue working.

Themes related to emotional challenges and support were less commonly raised among men in our study sample. No man reported facing emotional challenges specifically related to their gender. Emotional challenges faced by men were typically experienced in relation to specific activities that they perceived to have low community acceptance, often sanctions-based approaches.

3.3.4. Companionship support

3.3.4.1. Companionship support through group messaging strategies. Companionship support was an important coping mechanism for women in response to many types of challenges. When faced with households resistant to behavior change messaging, the most common response from women was to recruit others to revisit households and deliver the same messages in a group. Groups were perceived to have more persuasive power than individuals. Group messaging strategies were particularly important for women in unpaid roles with less access to informational support. For these women, group messaging was the primary strategy for improving implementation, rather than refining the content of messages. Groups were commonly formed of women's peers from the community, particularly women's and mothers' group members:

"When the challenges came, they [women] do not move back. Their meeting was continuous, and unity was their strength. When a woman has problems, all the women move to that house and sort out that problem by explaining to them. They go in the groups and convince other people, that it is the benefit for the community, it is the benefit for us, it is for family. And this how people have gradually changed." -man, development organization local implementer

In some instances, women would also recruit support from men from development organizations, elected leaders, or informal opinion leaders, who were perceived to hold more power to enforce messages:

"We approach the community with the elected members, and the people follow them. If a ward-WaSH coordination committee makes any decision, the ward members have to follow …" -woman, development organization local implementer

Women proud of their achievements, and wanted to support other women to demonstrate that they could be skilled WaSH implementers. One woman who chaired a local all-women WaSH committee described the committee's accomplishments despite a man who questioned their ability to succeed:

"He does not want to support the women, and then we have decided that we will do anything to show this man that we are not so much weak. And we can run the committee without the men and make changes in the community. This is how we have done. And we are successful. You can ask the people that our drinking water committee is one of the best drinking water committees in this area. Now the women are supporting me very much." -woman, local elected official

Companionship support from peers was less important to men. None described using group messaging approaches as a strategy to cope with challenges.

3.3.4.2. Political support and rivalries. Men reported companionship challenges from political rivals who were unwilling to support or would actively undermine WaSH efforts. One development organization implementer reported that the locally elected leader refused to conduct activities with him and "create a pressure on [his] people" out of fear it would affect his re-election chances. Another man elected offical attributed WaSH budget shortfalls to political rivalries. In some cases, political affiliation aided implementation, where members of the same political group motivated each other:

> "I am supporter of the winning politician. He comes to monitor and says everything is good in my school. It motivates me to do the work. But to a non-supporter he always tries to find mistakes in his work. This can develop a negative attitude." -man, school headmaster, Siraha

Women rarely mentioned politics as a challenge or source of support. No women in non-government roles mentioned politics as a challenge. Our sample included only three women in elected government positions women in politics, and only one elected woman described political challenges when interacting with men. However, the same woman reported that politics did not influence her interactions with women:

> "The women are continuous supporting me. They do not have any concern with the political party. The men of opposite party are somehow angry with me. When I ask anything of them, like 'Brother, please help me in this,' they say 'You do in your time. We will do in our time when we win.'" -woman, elected official

3.4. Successes in achieving implementation and intervention outcomes

The following section describes men's and women's perceived successes at achieving implementation outcomes (i.e., acceptability, appropriateness, penetration, adoption, and sustainability) and intervention outcomes, as defined by Proctor et al. and outlined in Table 1. Implementation outcomes for feasibility, fidelity, and cost were not described by participants during interviews. We also describe how these perceived successes were driven by the implementation approaches men and women used, challenges encountered using those approaches, and the support received to mitigate those challenges, as shown in Fig. 1.

3.4.1. Acceptability

Implementers perceived that relational approaches more commonly used by women faced less resistance from the community. Women reported that their "convincing" style of messaging was more likely to be given with respect, asking that households please change rather than demanding change:

> "Women speak very humbly. They do not use any harsh word or speak tightly … If we have pressurized the people saying, 'Uncle!! You have to do it.' Then maybe they have not built the toilet. But when we respect with respective words, then they agree. This respect develops a good relation with us, and people get convinced easily." -woman, development organization implementer

In contrast, men using pressure approaches reported being "cursed" or "abused" by some members of the community. One woman attributed the difference in communication styles between men and women to social norms dictating that women give respect to men and household elders:

> "Women have habit of giving respect. After marriage they have to respect the mother- and father-in-law. Women have the habit of speaking calmly, humbly. If they speak harshly, it will be hard to change people. But from birth women and girls are reared in that manner that they have to speak humbly and respect the older ones." -woman, development organization implementer

3.4.2. Appropriateness

FCHVs and women's and mother's groups were recruited from local communities and were familiar with local WaSH norms and conditions. While not required to be hired from local communities, all the women working for development organization that we interviewed lived locally in the communities in which they worked. Because of their local ties, women reported that they well positioned to identify community needs and develop appropriate solutions. Women believed that they were more honest in identifying and addressing community needs with good intentions of improving program quality and community health, whereas men, particularly government implementers, were often perceived as acting for political gain:

> "In my view, the women understand [community needs] better. Because if men go, they behave like the [political] party workers. Some behave like officers, but the women think that because we are living here, our place should be clean. Many women understand the needs of the community better. Some people's works are biased with their political party. What the party says or where the voters are high, they work there. But the women live here. They have to live all day and night here. They know better what is needed. Women are honest for themselves, their husband and children." -woman, development organization local implementer

Men working for development organizations commonly worked in larger geographic areas or in communities where they did not live. Formative research and engagement with women's, mothers' and other local groups were some of the strategies used by men to overcome lack of local knowledge and to improve the appropriateness of their implementation approaches.

3.4.3. Penetration

Women's and mothers' groups included large proportions of the local population. Women reported using these groups to efficiently spread messages through the community, while men reported challenges with reaching all the households in their working area. Men and women participants also reported that women were the best population to spread WaSH messages, as WaSH duties within the home are traditionally assigned to women, and women were often home during the day to receive messages:

> "The message spreads fast through the women. In the home, the women are sanitation workers. Men do not do, how much you convince or fight with men, they do not do the sanitation work … That's why I thought how I clean my household, I will mobilize the women for the cleaning the village." -woman, local government representative

3.4.4. Adoption

Approaches that asked households to build a toilet simply to meet government targets were widely perceived by all study participants to be unsuccessful at prompting adoption of sanitation improvements. Poor success of this messaging led participants to try new approaches, including both pressure and convincing approaches. Of the approaches tried in response, both men and women perceived that their efforts led to improvement in adoption of toilets and other WaSH behaviors among the community.

Both men and women also perceived buy-in from community women as important for widespread adoption of WaSH, and that adoption of WaSH practices was faster and more widespread when women actively participated in implementation. One woman attributed this to ease of working with other women and women's shared understanding and experience of WaSH needs. She perceived that this shared experience and understanding promoted cooperation and collective action by women:

> "It is very easy to work through women, because all the work [related to WaSH] is done by the women. That's why if we can make the women understand about the sanitation, change comes very fast. If the women wills, then the program is completed fast … In my ward, I have achieved success in less time working with the women of the ward. What I have experienced in life, almost all women are also affected by those problems." – woman, elected official and former development organization implementer

3.4.5. Sustainability

Women-led activities were perceived by both men and women to be more sustainable, and four participants identified women's leadership specifically as a solution to improve sustainability:

> "In the women-led areas, the activities are still continued. When we have gone for follow up, we observed that they are doing follow up of their activities. If you go and monitor, you will find that they are continuing. And the area where there was pressure, or where we had to push for work …. In some places we have seen that people are going back." -man, development organization implementer

Participants proposed several reasons for the sustainability of women-led programs. Women's and mothers' groups were not formal organizations within government or development organizations, so their activities had been sustained through government restructuring and program closeouts. Continual presence of women allowed for sustained delivery of program activities which reinforced habits in the community. Two women reported raising funds in the community to support continuing activities after formal program funding ended.

Implementers perceived that convincing approaches used by women to genuinely change social norms and attitudes regarding WaSH practices. Pressure approaches used by men were perceived as less sustainable: households would construct toilets to avoid sanctions, but individuals had not meaningfully changed perceptions, norms, and behaviors surrounding open defecation. One development organization implementer described how households had built toilets only to receive a "sanitation card" that entitled them to government services but continued openly defecating.

3.4.6. Intervention outcomes

We found no meaningful differences between men and women for intervention outcomes in terms of perceived impact on health or community cleanliness. Both men and women perceived that their efforts and those of others resulted in cleaner communities (e.g., less visible feces, solid waste) and improved health, specifically reducing diarrheal disease and child deaths. Most participants attributed changes to the program overall, noting the importance of a collective movement towards sanitation rather than specific activities or actions by individuals.

3.4.7. Other outcomes—women's empowerment

Traditional gender norms in Nepal dictate that women should not participate in formal employment outside the home. However, women consistently reported that participating in WaSH programs gave them access to non-domestic employment opportunities, education and training, larger social networks, and power and status in society. Opportunities to access training, build skills, and gain status outside the home were significant motivators to work in WaSH programs, despite not being paid:

> "Why I involved with FCHV was because the women are generally limited in the home, and this job have took me out from the house. That's why I always chose FCHV in first priority. It was my first opportunity." -FCHV

WaSH work built women's confidence to voice their opinions in the community and work in other programs and sectors. They reported happiness and pride that their work improved the community and that they had been recognized and praised locally and, in some cases, nationally and internationally, for their work. One woman described her experience being featured in a video campaign:

> "I have been recognized in many places, sir … many VDCs [village development committees] of the west, because in that area the program was implemented. The sisters and friends from that area have recognized me easily. When they have recognized, it developed a type of excitement in me, I can also do something. I was afraid of the video earlier. I was not able to speak publicly. After the video, I thought that I can speak more confidently in future." -FCHV

Participation in WaSH programs encouraged women to participate in social development and employment in non-WaSH areas. After their experience working on WaSH programs, two women in our sample ran for local office, and one woman started a bio-sand filter business and planned to offer training to other women on how to construct the filters. Two program coordinators reported increases in women's participation in other social programs, such as anti-child marriage, following their engagement in WaSH campaigns. One woman described how participation in the local WaSH program led to changes in how women expressed their views more openly:

> "Due to the participation, women started going to the meetings and came outside. The women's participation was increased in every program. If any NGO comes, like for the women's violence, the women now go and take the participation. Previously, women did not want to participate. … Now women support in such programs. If any NGO comes or any other person comes, women give time and put their views openly." -woman, development organization implementer

4. Discussion

This study explored differences in implementation approaches, challenges and sources of support for implementation, and successes in achieving program quality outcomes among men versus women in community-based WaSH implementation roles in Nepal. We found that men and women used different approaches for implementation, and each approach leveraged different sources of support within the community and faced unique challenges. In turn, these approaches, challenges, and sources of support influenced perceived program quality, as assessed using self-reported implementation and intervention outcomes (Proctor et al., 2011).

Men who were government implementers applied sanctions-based approaches in response to low adoption, while men in development organizations typically refined activity content to target behavioral determinants. Implementation approaches varied less among women, with women in all roles applying relational approaches to convince households to adopt WaSH practices for community wellbeing rather than aggressively demanding change. Women implementers also mobilized other women in the community to deliver messages in groups for more persuasive power.

Women's approaches to implementation were influenced by power dynamics within households and communities and patriarchal norms in which men are the primary decision makers. Women reported that they were raised to give respect to others, and these respectful messages had higher acceptance within the community than more confrontational approaches used by some men. Power dynamics within Nepali households where women have reduced autonomy are well documented (Wali et al., 2020). Women's success with respectful messaging is consistent with literature indicating that confrontational messaging is less effective at changing behaviors than other approaches (Hornik, 2002), and that respect and familiarity with social norms are important for successful program delivery by FCHVs (Mohajer and Singh, 2018). We also found that both men and women perceived WaSH as women's work and

women as more appropriate messengers to discuss domestic WaSH-related information. This suggests that women may have greater influence for decision making in WaSH compared to other areas, though further research to is needed to clarify how gender roles impact WaSH related decision making within the home.

We applied social support theory to examine challenges and sources of support for implementation as informational, tangible, emotional, and companionship. Many frameworks that examine the factors supporting effective implementation have been developed in a healthcare context to examine implementation by medical and allied health professionals (Aarons et al., 2011; Damschroder et al., 2009). They consider the role of the community only in terms of patient needs and do not account for how relationships between implementers and beneficiaries may influence implementation. However, this research suggests that social relationships between implementers and beneficiaries are an important determinant of implementation success, which are lacking from many implementation science frameworks. Future studies may incorporate constructs from social support theory or other frameworks for lay implementation to more holistically evaluate determinants of implementation success.

Relationships between women and the community were an important source of support and a driver of their success in delivering acceptable and appropriate messages. Women typically lived in the communities where they worked and leveraged their social ties to mobilize other women to join WaSH campaigns. In contrast, men worked in more widespread geographic areas, which may reduce their ability to build social ties and mobilize companionship support. The importance of social support and social capital for women to practice WaSH behaviors within the home is well documented (Friedrich et al., 2018; House et al., 2013; Malolo et al., 2021; Sahoo et al., 2015). Women are more likely to be engaged in community social networks, and to both provide and seek social support from others (Taylor, 2011; Taylor et al., 2000). Our study indicates that these trends extend to the professional setting and approaches to seeking and providing social support between WaSH implementers.

Some differences in challenges and sources of support between men and women may be more proximally attributable to the implementation roles they hold. This is likely the case for information and tangible support, where differences occur as much within genders as across genders. For example, women in development organizations reported being well integrated into training and feedback systems, while unpaid women did not. Poor integration into systems for feedback and learning are likely experienced by unpaid women not because of their gender directly, but because unpaid roles are less integrated into organizational structures that support program implementation. In contrast, emotional challenges and coping strategies of companionship support were consistently experienced and used across all women, suggesting that implementation role was not an important moderator.

While some differences in informational and tangible challenges and support may be proximally driven by the implementation roles, gender bias remains an important distal cause of women's underrepresentation in paid and senior positions and decision-making processes. Disproportionate numbers of women in unpaid WaSH implementation roles aligns with evidence of gender bias in hiring, promotion, and compensation of women in Nepal (Adhikary, 2016; Wali et al., 2020) and worldwide (Stamarski and Son Hing, 2015; Weichselbaumer, 2004; Weichselbaumer and Winter-Ebmer, 2005). Women in this study reported that they were expected to be the primary duty-bearer for cooking, cleaning, childcare, and other domestic chores even while holding employment outside the home. Other studies have similarly found that domestic responsibilities impede women's participation in WaSH work, and that support and willingness to share domestic chores from husbands and in-laws is necessary to give women the time and permission needed to engage in WaSH work outside the home (Leder et al., 2017; Rautanen and Baaniya, 2008).

Some have questioned the sustainability, appropriateness, and ethics of relying heavily on unpaid labor from CHVs for program implementation (Kasteng et al., 2015; WHO et al., 2007) and argued that dependency on unpaid female labor reinforces gender inequality (Panday et al., 2017). The WHO recommends that all trained workers, including CHVs, receive "adequate wages and/or other appropriate and commensurate incentives" to ensure sustainability (WHO et al., 2007). Reliance by development organizations and government on unpaid women not only fosters economic inequality, but also reinforces programmatic structures that treat women differently from their paid counterparts and reduce their opportunities for participation in decision making, training and promotions, and other sources of informational support.

Evidence suggests that while altruism is one motivator for engaging in unpaid community health work, benefits such as opportunities for training and building reputation are also important (Government of Nepal, 2014; Kasteng et al., 2015). In this study, women expected to gain knowledge, skills, and social status through WaSH work. However, as gender equality increases in Nepal, these women may be less willing to engage in unpaid work, as the perceived value of opportunities for skill building and status may decrease.

Consensus on how to design appropriate compensation for CHVs is lacking. Some studies suggest that offering formal wages to CHVs may undermine their credibility if the community views them as working only for personal profit rather than altruistic motives (Vareilles et al., 2017). Some Nepali policy makers perceive that formal wages would decrease FCHVs' motivations to work and decrease community respect, suggesting alternatives to formal wages such as free healthcare and education (Glenton et al., 2010). However, the assertion that pay will decrease community respect is predicated on the perception that woman holding formal employment is improper, and there is little evidence to suggest that payment would reduce motivation to work. These assumptions reinforce regressive gender norms and may no longer hold as gender equality has increased over the past ten years and continues to do so. FCHV participants in this study indicated that wages would increase their motivation to work, a finding which has been supported by other studies in South Asia (Alam et al., 2012). Ultimately, the design of appropriate compensation packages for FCHVs and other unpaid WaSH implementers is likely context specific and will require further research to understand and balance perceptions of policy makers, community members, and implementers themselves regarding what is appropriate, desirable, and best achieves programmatic goals.

Overall, women had less formal education and experience and fewer opportunities for training and feedback, yet women-led programs were perceived as having higher adoption and sustainability. Women achieved these successes regardless of their level of training or access to supervision and feedback. Sustainability of women-led activities suggests that relational group messaging techniques used by women were more effective at changing underlying behavioral drivers of sanitation behavior. This suggests that technical knowledge and experience may be less important than social skills to persuade peers and mobilize social groups, familiarity with local communities, and ability to navigate and communicate appropriately within social dynamics and structures.

Community-level action and collaboration between women is particularly important in light of evidence that household-level WaSH interventions are insufficient to realize health benefits (Pickering et al., 2019a), and calls for "transformative WaSH" as a more holistic effort to eliminate fecal contamination in the household and broader community environment (Pickering et al., 2019b). Collective efficacy (i.e., a group's belief in their ability to work together to achieve common goals) is important for community change necessary for transformative WaSH (De Shay et al., 2020; Dickin et al., 2017; Salinger et al., 2020). Group messaging strategies and willingness to support each other during implementation demonstrate collective efficacy among women to improve the health and wellbeing of their communities. This translated into implementation successes such as rapid and far-reaching dissemination of messages among women's and mothers' groups and sustained

commitment of these groups to deliver WaSH activities even where formally funded programs had ended. In contrast, political rivalries among men demonstrated how a lack of collective efficacy can negatively impact program acceptability and adoption. This study suggests women's ability to mobilize companionship support among peers may make them more effective at widespread community transformation than men.

4.1. Limitations

We assessed successes and challenges from the perspective of local-level implementers only. Participants' perceptions of successes and challenges related to community attitudes and norms may not fully reflect the range of perspectives within the community. For example, in working to reach ODF, implementers will spend a disproportionate amount of time interacting with households who are resistant to constructing toilets. This may lead to a skewed perception of acceptance and appropriateness of WaSH activities among the community.

In selecting the study sample, we prioritized recruiting a diverse range of perspectives across government, NGO, and multilateral and civil society organizations. This allowed for identifying differences within genders, as well as between genders. However, as a result, the sample size of sub-groups within genders (e.g., paid versus unpaid women) is small. Where gender is strongly associated with the type of implementation role participants fill, disentangling the influence of gender and other contextual factors is challenging. For example, we found that men were more likely to use sanctions, but men are also more likely to hold political office with the power to enforce sanctions (Thapa, 2019). Our sample did not contain enough women in government to conclude whether sanctions approaches would be used by women if they had political power to enact them.

Further research with more senior program officials at the regional and national levels may help to determine the influence of broader contextual factors versus gender. Additionally, recruiting participants from a narrow range of implementation roles could better identify the range of experiences of men and women within that role and disentangle the influences of gender versus other factors associated with implementation role. Similarly, research recruiting participants of only a single gender could achieve a richer representation from different implementation roles to understand how factors such as level of training, political affiliation, or financial compensation affect performance within genders.

4.2. Programmatic implications & recommendations

This study illustrates several opportunities to address gender-related challenges and to better support and leverage the abilities of men and women as rural WaSH implementers. At the household and community level, activities to normalize and build acceptance among family and community members for women in non-domestic employment may reduce emotional challenges experienced by women. Frameworks for gender mainstreaming highlight the need to include men as role models and champions for gender equality (Morgan et al., 2016; Tolhurst et al., 2012). Studies have shown that women are more likely to participate in WaSH leadership positions when they have husbands and parents in-law who encourage their work and share childcare and other domestic duties (Leder et al., 2017; Rautanen and Baaniya, 2008). Various frameworks and guidelines exist that can support programs in identifying opportunities and integrating programming to promote gender equality in WaSH (Cortobius and Kjellen, 2014; Gosling, 2012; Halcrow et al., 2010; Panda, 2007).

At the program management level, deemphasizing technical knowledge and prior WaSH experience as key criteria for hiring and promotion may help improve women's representation in paid roles without sacrificing the quality of activities. We found that women implementers achieved high adoption and sustainability even where they had received little formal training, and ability to mobilize peer support, not technical skills, was critical to that success. Yet, perceived lack of technical proficiency and skills to perform maintenance and repair tasks are barriers to hiring women in WaSH in Nepal (Bhandari et al., 2005). Systematic reviews have identified a variety of interventions to mitigate gender bias in hiring (Isaac et al., 2009), and further research would help identify which are most appropriate for rural WaSH programs.

This study suggests opportunities to strengthen training and feedback systems for non-development organization implementers, particularly for unpaid women who experience challenges related lack of training and poor integration into feedback systems. Training and feedback systems have been found to increase productivity, motivation, satisfaction among CHVs (Scott et al., 2018; Whidden et al., 2018), which is consistent with women in this study reporting training opportunities as a motivator for work. Women expressed a desire for additional technical training (e.g., making reusable menstrual pads), as well as training to develop leadership skills, overcome stigma, and empower themselves and others in their community to challenge traditional gender roles. This study also suggests opportunities to build men's communication and social mobilization skills. Training to build facilitation skills among both men and women community-based CLTS implementers has been shown to improve sanitation adoption (Crocker et al., 2016).

In the long-term, programs may ultimately need to plan for the possibility that women's willingness to work in unpaid roles may decrease as gender equality increases, and to reconsider the ethics, appropriateness, and sustainability of relying on unpaid labor. Unpaid women in this study reported that their motivation to work would increase with a formal salary, and studies of CHVs in other contexts have found that satisfaction with both monetary and non-monetary incentives is important for motivation and retention (Kasteng et al., 2015; Kok et al., 2015). Payment of all trained CHVs aligns with WHO recommendations to ensure sustainability of health programs (WHO et al., 2007) and addresses structural inequalities associated with reliance on women's unpaid labor (Panday et al., 2017). However, coordination will be needed between government and external funders to ensure that payment systems linked to specific WaSH activities are not disruptive of long-term CHV programs.

5. Conclusions

Women were more likely to be omitted from high-level planning, face stigma for engaging in work outside the home, and hold unpaid roles that are not well integrated into feedback and troubleshooting systems. Women also had less formal experience, education, and training. Social ties between women were robust sources of companionship and emotional support to mitigate challenges, and opportunities to build job skills, social status, and overcome traditional gender norms were important motivators for women to engage in WaSH work.

Both men and women perceived that women were more effective than men at mobilizing widespread, sustained WaSH improvements, which was attributed to their success using relational approaches and leveraging social ties to deliver acceptable and appropriate messages. Women's skills for motivating collective action indicate that they can be highly effective WaSH implementers despite lack of technical experience and training, and that women's active participation is important for achieving transformative community change.

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Declaration of competing interest

None.

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Systematic review of biomonitoring data on occupational exposure to hexavalent chromium

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ABSTRACT

Occupational exposure to hexavalent chromium (Cr(VI)) can cause serious adverse health effects such as lung cancer and irritation of the skin and airways. Although assessment of chromium (Cr) in urine is not specific for Cr (VI) exposure, the total amount of Cr in urine is the most used marker of exposure for biomonitoring of Cr(VI). The purpose of this systematic review was fourfold: (1) to assess current and recent biomonitoring levels in subjects occupationally exposed to Cr(VI), with a focus on urinary Cr levels at the end of a working week, (2) to identify variables influencing these biomonitoring levels, (3) to identify how urinary Cr levels correlate with other Cr(VI) exposure markers and (4) to identify gaps in the current research. To address these purposes, unpublished and published biomonitoring data were consulted: (i) unpublished biomonitoring data comprised urinary Cr levels (n = 3799) of workers from different industries in Belgium collected during 1998–2018, in combination with expert scores indicating jobs with Cr exposure and (ii) published biomonitoring data was extracted by conducting a systematic literature review. A linear mixed effect model was applied on the unpublished biomonitoring data, showing a decreasing time trend of 30% in urinary Cr levels. Considering the observed decreasing time trend, only articles published between January 1, 2010 and September 30, 2020 were included in the systematic literature search to assess current and recent biomonitoring levels. Twenty-five studies focusing on human biomonitoring of exposure to Cr(VI) in occupational settings were included. Overall, the results showed a decreasing time trend in urinary Cr levels and the need for more specific Cr(VI) biomarkers. Furthermore, this review indicated the importance of improved working conditions, efficient use of personal protective equipment, better exposure control and increased risk awareness to reduce Cr levels in biological matrices. Further investigation of the contribution of the different exposure routes is needed, so that better guidance on the use of control measures can be provided. In addition, this review support the call for more harmonization of human biomonitoring.

1. Introduction

Chromium (Cr) is a transition element that exists in oxidation states ranging from −2 to +6. The common stable ones in the environment are trivalent (Cr(III)) and hexavalent (Cr(VI)) chromium (Lunk, 2015). Cr (III) mostly occurs in nature, whereas Cr(VI) is mostly released from industrial processes. The main properties of Cr(VI) compounds are corrosion-resistance, durability and hardness. Exposure to Cr(VI) may occur when Cr(VI) compounds are manufactured as end-product (e.g. chromate production), when Cr(VI) compounds are used as start-product (e.g. electroplating) or when Cr(VI) compounds are formed as by-product (e.g. welding) (NIOSH, 2013). Cr(III) has limited toxicological properties (Anderson, 1997), whereas Cr(VI) is more toxic due to its oxidizing ability and high solubility leading to increased membrane permeability (Saha et al., 2011). Occupational exposure to Cr (VI) can cause serious adverse health effects such as cancer and irritation of the eyes, skin and airways (OSHA, 2006).

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Abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists
BAR	Biologischen Arbeitsstoff-Referenzwerte
BEI	Biological Exposure Indices
BLUPs	Best Linear Unbiased Prediction
BLV	Biological Limit Value
BMGV	Biological Monitoring Guidance Value
BRV	Biological Reference Value
Ca	Circa
Cr	Chromium
Cr(0)	Elemental Chromium
Cr(III)	Trivalent Chromium
Cr(VI)	Hexavalent Chromium
Cr-Air	Chromium level in Air
Cr–B	Chromium level in whole Blood
Cr-EBC	Chromium level in Exhaled Breath Condensate
Cr(III)-EBC	trivalent Chromium levels in Exhaled Breath Condensate
Cr(VI)-EBC	Hexavalent Chromium levels in Exhaled Breath Condensate
Cr-Fingernails	Chromium level in Fingernails
Cr-Hair	Chromium level in Hair
Cr-Hand	Chromium level on Hands
Cr–P	Chromium level in Plasma
Cr-RBC	Chromium level in Red Blood Cells
Cr–S	Chromium level in Serum
Cr-Surface	Chromium level on Surface
Cr–U	Chromium level in Urine
Creat	Creatinine
EBC	Exhaled Breath Condensate
EC	European Commission
EQA	External Quality Assurance
FCAW	Flux-Cored Arc Welding
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
GM	Geometric Mean
GMAW	Gas Metal Arc Welding
HBM	Human BioMonitoring
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ILC	InterLaboratory Comparison
ILO	International Standard Classification of Occupations
IQR	InterQuartile Range
JEM	Job-Exposure Matrix
LEV	Local Exhaust Ventilation
LOD	Limit Of Detection
LOQ	Limit Of Quantification
N°	Number of
NACE	Nomenclature statistique des Activités économiques dans la Communauté Européenne
OEL	Occupational Exposure Limit
OSH	Occupational Safety and Health
P_0	0^{th} percentile
P_{25}	25th percentile
P_{75}	75th percentile
P_{90}	90th percentile
P_{100}	100th percentile
PPE	Personal Protective Equipment
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RBC	Red Blood Cells
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
REML	REstricted Maximum Likelihood
RMMs	Risk Management Measures
RPE	Respiratory Protection Equipment
SD	Standard Deviation
SMAW	Shielded Metal Arc Welding
TIG	Tungsten Inert Gas
URL	Upper Reference Limit

Human biomonitoring (HBM) can be used for assessment of occupational exposure and involves measurement of the chemical or metabolites of this chemical in a biological medium such as urine, blood, breath, nails or hair (Scheepers et al., 2014). The measurement of Cr in urine is often used in the workplace to monitor Cr(VI) exposure and gives an indication of total Cr exposure as Cr(VI) is reduced inside the body to Cr(III) (Ray, 2016). Depending on the route of exposure, reported elimination half-lives differ. After exposure by inhalation, the excretion of Cr in urine is thought to follow a two- or tri-phasic process with elimination half-lives of 7 h, 15–30 days and 3–5 years (ATSDR, 2012; Hoet, 2005). In order to better interpret the biomonitoring results, several guidance values have been set up by authorities or advisory organisations for occupationally exposed population and general population with no known occupational exposure to Cr. Examples of these guidance values are shown in Table 1. Guidance values for occupationally exposed population are either based on a relationship between concentrations in biological exposure markers and health effects, between concentrations in biological exposure markers and occupational exposure limits (OELs) or on data collected from a representative set of workplaces with good control of exposure. (Worksafe, 2020). At EU level, no biological guidance value have been set up for Cr(VI) compounds (Hartwig et al., 2017). In the USA, the American Conference of Governmental Industrial Hygienists (ACGIH) has established two health-based biological exposure indices (BEI): one for the total Cr concentration increase in urine during the shift and one for the total Cr concentration at the end of the shift at the end of the working week. The values are respectively 10 μg/l and 25 μg/l (ACGIH, 2020). France has established the most stringent health-based biological limit value (BLV) for European workers, this being a BLV of 2.5 μg/l from its occupational exposure limit (OEL) of 1 μg/m^3 for Cr(VI) (ANSES, 2017). The same BLV is applied in the Netherlands (SZW, 2016). Finland has derived a health-based BLV of 10 μg/l corresponding to its OEL of 5 μg/m^3 for Cr (VI) (MSAH, 2012). In the UK, an occupational hygiene-based biological monitoring guidance value (BMGV) of 10 μmol/mol creatinine (approximately 6.3 μg/l) in post-shift urine has been established (HSE, 2020). Besides these occupational limit values, reference values exist for the general population with no known occupational exposure to Cr. These values represents the upper reference concentration of a biomarker in the general adult population without occupational exposure to the agent. These reference values are influenced by environment, lifestyle and medical factors. Therefore, these values for the general population may differ between regions (Hoet et al., 2013). Examples of such reference values are the German Biological reference values for chemical compounds in the work area (Biologischen Arbeitsstoff-Referenzwerte (BAR)), the Biological Reference Value (BRV) in France and the Upper Reference Limit (URL) in Belgium.

Due to required authorization of Cr(VI) compounds under Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) and the proposal of the European Commission (EC) to set a BLV for Cr (VI) (EC, 2004, 2017), we aimed to gather existing and recent occupational biomonitoring data concerning Cr(VI) and to identify gaps in the current research. Therefore, the goal of this review article was fourfold: (1) to identify the current and recent biomonitoring levels in subjects occupationally exposed to Cr(VI), with a focus on urinary Cr levels at the

Table 1
Biological monitoring guidance values for total Cr concentration in urine.

Type of guidance value	Definition	Sampling time	Guidance value	Authorities or advisory organisations
Occupationally exposed population				
Biological Guidance Value (BGV)	Genotoxic mechanism of action is likely or cannot be excluded		None	SCOEL - European Commission (Hartwig et al., 2017)
Biological Limit Value (BLV)	Health-based guidance value Derived corresponding to French OEL for Cr(VI) of 1 µg/m^3	End of shift at end of working week	2.5 µg/l [1.8 µg/g creatinine]	ANSES - France (ANSES, 2017)
Biological Limit Value (BLV)	Health-based guidance value. Derived corresponding to Finish OEL for Cr(VI) of 5 µg/m^3.	End of shift at end of working week	10 µg/l	Finland - MSAH (STM, 2018)
Biological Monitoring Guidance Value (BMGV)	Occupational hygiene-based guidance value. 90th percentile of data from workplaces with good control of exposure.	End of shift	10 µmol/mol creatinine [4.6 µg/g creatinine] [ca. 6.3 µg/l]	HSE - UK (HSE, 2020)
Biological Exposure Index (BEI)	On the basis of six studies.	Increase during shift	10 µg/l	ACGIH - USA (ACGIH, 2020)
	Health-based guidance value Derived corresponding to TLV-TWA of 0.05 mg/m^3 for airborne soluble Cr(VI) of µg/m^3.	End of shift at end of working week	25 µg/l	
General population with no known occupational exposure to Cr				
Biological reference values for chemical compounds in the work area ("Biologischen Arbeitsstoff-Referenzwerte", BARs)	95th percentile in German general population		0.6 µg/l	DFG - Germany (DFG, 2018)
Biological Reference Value (BRV)	95th percentile in French general population		0.65 µg/l (0.54 µg/g creatinine)	ANSES - France (ANSES, 2017)
Upper Reference Limit (URL)	upper limit of the 90% confidence interval of 97.5th percentile in Belgian general population		0.55 µg/l	UCLouvain - Belgium (Hoet et al., 2013)

Abbreviations: HBM4EU, Human Biomonitoring for Europe; MSAH: Ministry of Social Affairs and Health; OEL, Occupational Exposure Limit; TLV-TWA, Threshold Limit Value - Time-Weighted Average.

end of a working week, (2) to identify variables influencing these biomonitoring levels, (3) to identify how urinary Cr levels correlate with other Cr(VI) exposure markers and (4) to identify gaps in the current research. To achieve these objectives, we firstly analysed an unpublished dataset comprising urinary Cr measurements of workers from different industries in Belgium, in combination with another dataset comprising expert scores indicating jobs with Cr exposure based on risk assessments and exposure data. By combining these two datasets, we aimed to reveal recent exposure levels and time trends in exposure levels. In addition, published biomonitoring data was extracted by conducting a systematic literature review to evaluate the extent of current and recent exposure levels reported in literature and to identify possible correlations between different Cr(VI) exposure markers. The time period of the search strategy used for the literature review was based on the time trend of exposure levels reported in the unpublished dataset.

2. Methodology

2.1. Unpublished biomonitoring data

Two types of datasets regarding occupational exposure to Cr were obtained through a Belgian external occupational safety and health (OSH) service.

- The first dataset comprised of measurements on urinary Cr levels of workers from different industries across Belgium collected during 1998–2018. Workers under risk of exposure to hazardous chemicals had been examined periodically by the external OSH service. Post-shift urine samples were taken in the context of periodical examinations at the end of a working week. The data of these examinations were stored pseudonymized on a computerized database. Jobs were coded into ISCO-08, the International Standard Classification of Occupations. ISCO-08 is a system used to classify jobs into a set of groups according to the tasks and duties related to a job and the relevant necessary skills (ILO, 2016). Jobs are classified into 436 unit groups. These unit groups are aggregated into 130 minor groups, 43 sub-major groups and 10 major groups. This aggregation or classification is done based on their similarity. Each group is clearly defined and is designated by a code number. Major groups are indicated by a one-digit code, sub-major groups by a two-digit code, minor groups by a three-digit code and unit groups by a four-digit code. Therefore, ISCO-08 has a hierarchical structure and is composed of four levels, going from general to specific. This hierarchical use of the ISCO codes allows the generation of summary data for 10 occupational groups, indicated by major-groups, at the highest level of aggregation as well as relatively detailed data at the lowest level of aggregation, indicated by unit-groups. Industries were coded into NACE (Nomenclature statistique des Activités économiques dans la Communauté Européenne), the European industry standard classification system (Eurostat, 2008). NACE also uses four hierarchical levels. Industries are classified into 21 sections, 88 divisions, 272 groups and 629 classes. This aggregation is done based on similarity in business activities. Sections are indicated by an alphabetical letter (A to U), divisions by a two-digit code (01–99), minor groups by a three-digit code (01.1–99.0) and unit groups by a four-digit code (01.11–99.00). Data on date of birth, gender, ISCO-08 code, NACE code, sampling date and unit of measurement were extracted for the time period 1998–2018.
- The second dataset comprised of expert scores. Experts, namely occupational physicians of the external OSH service, rated (0 = unexposed and 1 = exposed) jobs based on data obtained through risk

assessments and exposure data obtained through periodical examinations. This dataset covered a wide range of exposure profiles or health outcomes linked to ISCO-08 codes. Data extraction that solely focused on Cr resulted in the identification of 47 jobs with Cr exposure. This data was used as the expert-based qualitative assessment on Cr exposure and incorporated in the statistical model *a priori* to combine urine measurements with expert knowledge (Peters et al., 2012; Vested et al., 2019). This approach of developing a job-exposure matrix (JEM) by combining measurement data and expert knowledge is especially helpful in situations where a limited number of measurements with high variability are available for a job. Otherwise, the resulting estimate from the limited number of measurements is characterized by a high uncertainty (Vested et al., 2019). Furthermore, JEM allows to estimate exposure levels when no measurements are available for a job.

2.1.1. Descriptive analysis of unpublished biomonitoring data

First, descriptive statistics were applied to biomonitoring data in order to find the number and the proportion of workers exceeding the BEIs set up by the ACGIH (ACGIH, 2020) and URL described previously for healthy Belgian adults (Hoet et al., 2013).

2.1.2. Temporal analysis of unpublished biomonitoring data

Subsequently, an empirical linear mixed effect model that combines urinary Cr measurements and expert decisions was applied in order to predict job and industry specific exposure levels. Year of measurement and expert score (0 = unexposed, 1 = exposed) were included as the fixed effects while job, industry and worker were included as random effect terms in the model. Random effects were incorporated in order to evaluate the possible effects of the variances between jobs, between workers and between industries on exposure levels. "Variance components" is selected as the covariance structure. The variance between workers and between jobs were assumed to be equal across all fixed determinants. Restricted maximum likelihood (REML) was used to estimate variance components and fixed effects. Data analysis was done using IBM Statistical Package for the Social Sciences (SPSS) Version 25.

The empirical model used for the estimation of exposure based on biomonitoring results is shown in equation (1) (adapted from Vested et al., 2019):

$$Ln(Y) = \beta 0 + \beta eE_{0\text{-}1} + \beta tT + bj_{1-56}J + bi_{1-101}I + bw_{1-606}W + \varepsilon \quad (1)$$

Where:

Ln(Y): Natural log-transformed urine chromium level
β0: Model intercept
βeE$_{0\text{-}1}$: Fixed effect term for expert score (0–1)
βtT: Fixed effect term for year of measurement (continuous)
bj$_{1-56}$J: Random effect term for job (1–56)
bi$_{1-101}$I: Random effect term for industry (1–101)
bw$_{1-606}$W: Random effect term for workers (1–606)
ε: Residual error

The model used an algorithm to predict estimates of covariance parameters for the random terms and mean levels for the fixed effect terms. A job with a limited number of measurements or high variable exposure data will result in an estimate of covariance parameter that is equal to 0, whereas a job with a larger number of exposure data or less variable exposure data will lead to an estimate for the exposure level that differentiate from the intercept (Vested et al., 2019). Modeled predicted geometric means were used to identify the jobs with highest exposure levels.

2.2. Published biomonitoring data: systematic literature review

2.2.1. Study identification

The databases PubMed, Scopus and Web of Science were systematically searched according to PRISMA guidelines (Moher et al., 2009) for articles published between January 1, 2010 and September 30, 2020 focusing on HBM of Cr(VI) in occupational settings. Search terms were developed using the keywords "workplace", "worker" or "occupation" in combination with "hexavalent chromium". The full search string used in each database is provided in the Supplementary Material S2. Additional articles were found by scanning the list of references of original publications and review articles.

2.2.2. Study selection

Studies meeting the following criteria were included in the review: 1) reported in a peer-reviewed journal, 2) in English, 3) full text article, 4) publication date between 01/01/2010 to 30/09/2020, 5) occupational exposure, 6) age of study characteristics between 18 and 65 years, 7) exposure to Cr(VI), 8) HBM and 9) at least two markers of exposure are reported.

2.2.3. Data extraction

For each study, information on the following variables was extracted in an MS Excel template: 1) Industry, 2) Study population characteristics, 3) Sample size, 4) Biomonitoring data, 5) Correlations between exposure markers, 6) Sampling time, 7) Sampling strategy (e.g. spot urine sample) and matrix adjustment (e.g. creatinine-adjusted or/and non-adjusted urinary Cr concentrations), 8) Technique (e.g. Inductively coupled plasma mass spectrometry (ICP-MS), Graphite furnace atomic absorption spectroscopy (GFAAS)), 9) Method characteristics (limit of detection (LOD)/limit of quantification (LOQ), stability, contamination (-free) and quality assurance), 10) Tasks with higher exposure/variables influencing exposure, 11) Risk management measures (RMMs) in place/to be applied, 12) Environmental monitoring (e.g. air, surfaces), 13) Health surveillance, 14) Biomarkers of effects, 15) Co-exposure characteristics and 16) Exposure characteristics.

2.2.4. Quality scoring

The quality of each study was then scored according to the LaKind scoring criteria (LaKind et al., 2014), adapted for Cr(VI) exposure (Supplementary Material S1). The LaKind criteria are designed to assess quality issues of exposure assessment by biomonitoring or environmental epidemiology for short-lived and non-persistent chemicals. In this review article, the LaKind criteria have been refined specifically for biomonitoring of Cr(VI), with these adaptations also taking into consideration a more recent scientific report related to HBM data collection from occupational exposure to pesticides (RPA HSL IEH, 2017). This report highlighted factors for standardisation in HBM studies. The following two assessment components were added to the existing LaKind criteria: study records and background levels. Both criteria are helpful for the interpretation of biomonitoring data. Study records provide contextual information. Background levels provide knowledge about exposure levels arising from non-occupational exposure. So in this review, the studies are assessed based on their study design (population size and selection of study participants), biomarker selection and measurement (exposure biomarker specificity, analytical technique, method sensitivity, biomarker stability, sample contamination, quality assurance and matrix adjustment), contextual information (study records) and interpretation (background levels). The study classification for each assessment component has to be accompanied by a justification for each decision to increase transparency. More information about the refined LaKind criteria is provided in Supplementary Material S1. For each study included in this review, eleven study assessment components are assessed by giving a score from 1 (Tier 1 = highest quality) to 3 (Tier 3 = lowest quality). Therefore, the possible total scores range from 11 (highest quality) to 33 (lowest quality).

Industry	Study Population country N° workers N° controls N° companies	Biomonitoring data - directly exposed workers Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	Key findings exposure assessment	LaKind scoring strengths and/or weaknesses	Reference
Welding	Germany 50 workers 0 controls 14 companies	● Cr–U: *0.*90 μg/g creat. (P_{25}: 0.56, P_{75}: 1.55) *0.*96 μg/l (P_{25}: 0.60, P_{75}: 1.60)	Significant positive correlations between: • Pre- and post-shift Cr–U ($r_S = 0.78$) • Creatinine-adjusted pre- and post-shift Cr–U ($r_S = 0.83$) • Respirable Cr(VI)-Air and post-shift Cr–U ($r_S = 0.25$, $P = 0.0008$) • Respirable Cr-Air and post-shift Cr–U ($r_S = 0.44$, $P \leq 0.0001$)	17 EQA	Pesch et al. (2018)
	Poland 67 workers 52 controls 5 companies	● Cr–U: *3.*81 μg/g creat. (P_{25}: 2.22, P_{75}: 6.70) ca. *5.*18 μg/l ca. (P_{25}: 3.02, P_{75}: 9.11) ● Cr–S: 1.25 μg/l (P_{25}: 0.12, P_{75}: 2.68) ● Cr-RBC: 0.09 μg/g Hb (P_{25}: 0.05, P_{75}: 0.15)	Significant positive correlations between: • Cr–U and inhalable Cr-Air ($r_S = 0.59$, $p < 0.0001$) • Cr–U and inhalable Cr(VI)-Air ($r_S = 0.58$, $p < 0.0001$) • Cr–U and inhalable Cr(III)-Air ($r_S = 0.64$, $p < 0.0001$) • Cr–S and inhalable Cr-Air ($r_S = 0.68$, $p < 0.0001$) • Cr–S and inhalable Cr(VI)-Air ($r_S = 0.67$, $p < 0.0001$) • Cr–S and inhalable Cr(III)-Air ($r_S = 0.67$, $p < 0.0001$)	17 EQA	Stanislawska et al. (2020)

3. Results

3.1. Unpublished biomonitoring data

3799 measurements on Cr collected during 1998–2018 were available among 1824 workers representing 56 minor groups of jobs. Jobs were coded into ISCO-08 which is characterized by a hierarchical structure (1–4 digits) as mentioned before. Measurements were distributed over unique ISCO-08 codes as follows (the maximum amount of unique codes in the ISCO-08 system is given between brackets): 9 (out of 10) unique one-digit codes, 26 (out of 43) unique two-digit codes, 56 (out of 130) unique three-digit codes and 83 (out of 436) unique four-digit codes. 101 (out of 629) unique NACE codes were represented by the measurement data. The urinary Cr levels ranged from 0.1 to 70 μg/l with a geometric mean of 0.88 μg/l 54.9% of the measurements exceeded the URL (0.55 μg/l) for Belgian adults and 0.8% of the measurements exceeded the occupational limit (25 μg/l) recommended by ACGIH. An overview on the characteristics of the data is presented in Table 2.

The linear mixed effect model applied at sub-major ISCO levels (three-digit) showed that about 15% of the total variance in Cr data was explained by the variance between jobs and industries.

There was a decreasing time trend of 30% in urinary Cr levels. The parameters of the linear mixed effect model are available in Table 3.

According to observed and model predicted urine levels, “sheet and structural metal workers, moulders and welders, and related workers”, “electronics and telecommunications installers and repairers”, “blacksmiths, toolmakers and related trades workers”, “metal processing and finishing plant operators” were found to have the highest Cr exposure (Table 4).

Table 2
Characteristics of data on urinary Cr levels.

Exposure matrix	Urine
Unit	μg/l
Number of measurements	3799
Total number of workers	1824
Median age (min.-max.)	40 (13–73)
Sex (F/M)	104/3701
Unique ISCO codes	
ISCO One-digit	9
ISCO Two-digit	26
ISCO Three-digit	56
ISCO Four-digit	83
Unique NACE codes	101
Years covered	1998–2018
Geometric mean (μg/l)	0.88
Geometric standard deviation (μg/l)	3.02
Min.-max. levels (μg/l)	0.1–70
Percentiles (μg/l)	$P_5 = 0.3$, $P_{10} = 0.4$, $P_{25} = 0.4$, $P_{50} = 0.6$, $P_{75} = 1.5$, $P_{90} = 4.6$, $P_{95} = 9.01$
Measurements under LOD (n, %)	684 (18%)
Measurements exceeding reference limits for Belgian adults[a] (n, %)	2089 (54.9%)
Measurements exceeding occupational limit[b] (n, %)	33 (0.8%)

[a] Upper Reference Limit (Hoet et al., 2013): 0.55 μg/l.
[b] Biological Exposure Index (ACGIH, 2020): 25 μg/l.

Table 3
Mixed effect model parameters applied to unpublished biomonitoring dataset

Estimates of fixed effects				
	β	SE	P	95 % CI
Intercept	7.63	0.52	**< 0.001**	6.61-8.66
Year	**-0.30**	0.02	**< 0.001**	-0.35-0.25
Riskpro				
Unexposed	-0.78	0.89	0.93	-1.84-1.68
Exposed (ref.)	—	—	—	—
Estimates of covariance parameters*				
	Estimate*	**SE**	**P**	**95 % CI**
ISCO (3 digit)	0.43	0.29	0.13	0.11-1.60
NACE	**1.99**	0.70	**0.005**	0.99-3.99
Workers	**4.79**	0.69	**< 0.001**	3.60-6.37
Residual	7.80	0.54	< 0.001	6.80-8.96

Abbreviations: Rikspro, Risk profile; ISCO, International Standard Classification of Occupations; NACE, Nomenclature statistique des Activités économiques dans la Communauté Européenne; β, fixed effects terms; SE, Standard error; p, p-value or probability value; CI, confidence interval.
*Job (ISCO), sector (NACE) and individual workers are parametrized as categorical variable and added as random effects in the model.

Table 4
Jobs with the highest chromium exposure, according to observed and model predicted geometric mean (GM) levels.

ISCO-08	Observed GM (μg/l)	Range of observed values [P_0 – P_{100}]	Model predicted GM (μg/l)	95% CI	Measurements (N)
721 Sheet and structural metal workers, moulders and welders, and related workers	3.75	0.60–23.80	3.70	2.32–5.08	658
742 Electronics and telecommunications installers and repairers	2.54	0.70–13.60	2.79	1.13–4.45	17
722 Blacksmiths, toolmakers and related trades workers	2.55	0.70–12.80	2.18	0.54–3.82	50
812 Metal processing and finishing plant operators	2.30	0.60–23.80	2.16	0.67–3.65	101

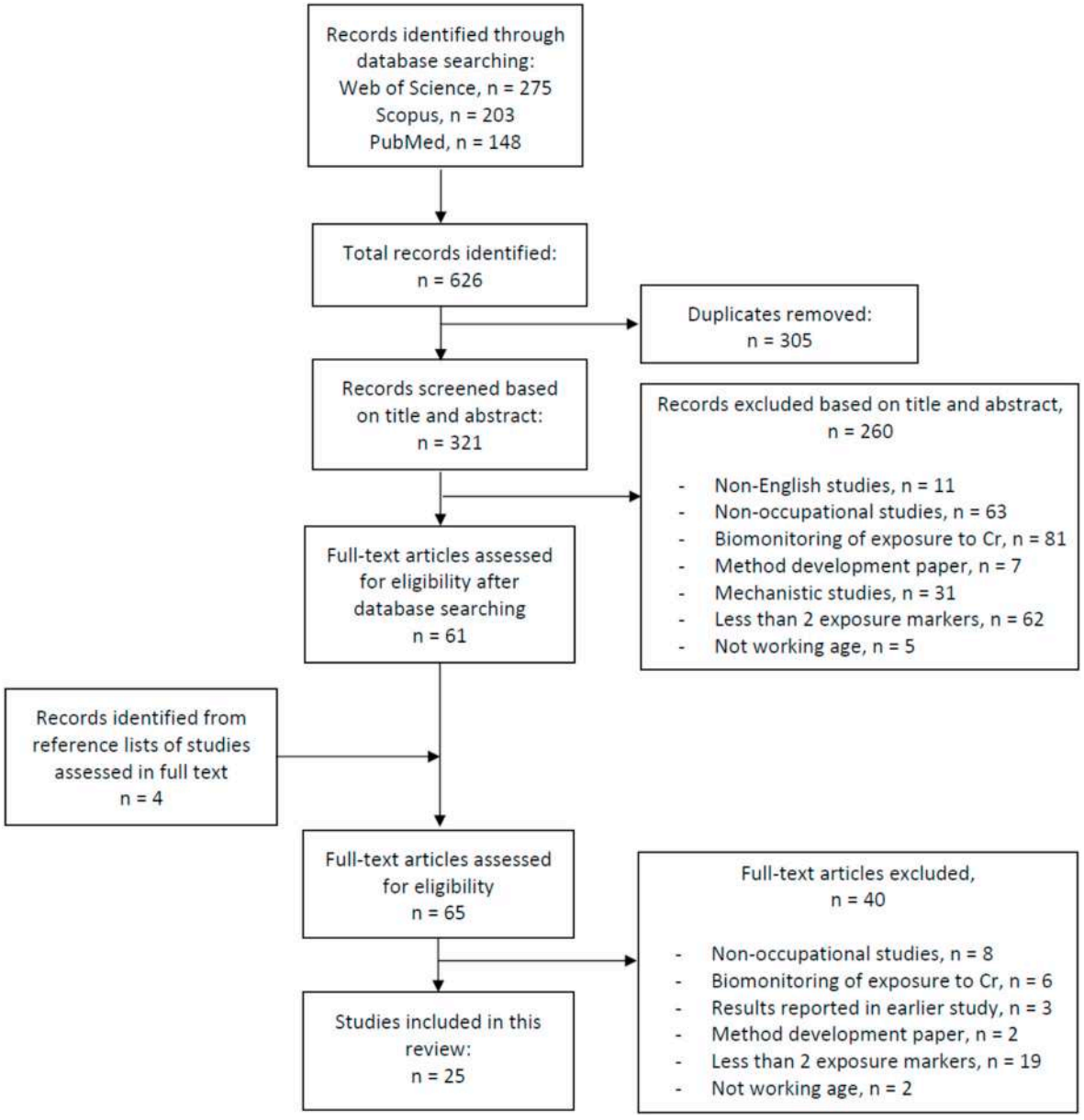

Fig. 1. PRISMA flowchart of the study inclusion process.

3.2. Published biomonitoring data: systematic literature review

Considering the observed decreasing time trend in the unpublished biomonitoring dataset, only articles published between January 1, 2010 and September 30, 2020 were included in the systematic literature search to assess current and recent biomonitoring levels. Based on our search strategy, a total of 630 articles were identified. Out of these, 626 articles were identified through database searching and 4 articles through scanning of the reference lists. After duplicate removal and an initial screening based on title and abstract, 65 studies were considered for further assessment. After full text screening, 40 studies were excluded. Out of these, 8 studies were performed in non-occupational settings (e.g. lab study and general population), 6 used biomonitoring for the assessment of exposure to total Cr (not explicitly indication of potential exposure to Cr(VI) by the authors), 3 displayed biomonitoring data of another study, 2 were considered as a method development, 19

Table 5
Summary of the studies for occupational exposure to Cr(VI).

Industry	Study Population country N° workers N° controls N° companies	Biomonitoring data - directly exposed workers Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	Key findings exposure assessment	LaKind scoring strengths and/or weaknesses	Reference
Welding	Germany 241 workers 0 controls 25 companies	**Welders (n=241)** ● Cr–U: *1.2* µg/l (P_{25}: <LOQ, P_{75}: 3.61) *<1.35* µg/g creat. (P_{25}: <0.74, P_{75}: <3.24) **High-exposure group (n=16):** ● Cr-RBC: *1.95* µg/l (P_{25}: <LOQ, P_{75}: 2.37) ● Cr–U: *13.53* µg/l (P_{25}: 5.21, P_{75}: 53.03) ca. *9.95* µg/g creat. ca. (P_{25}: 3.83, P_{75}: 38.99)	Significant positive correlation between: • Respirable Cr-Air and Cr–U ($r = 0.61$, $p < 0.0001$) High-exposure group: • gas metal arc welders with massive or flux-cored wire of stainless steel • Confined spaces or insufficient ventilation Detection frequency: • Cr-RBC was only detected in 15 out of 150 stainless-steel welders (n = 8 FCAW, n = 1 GMAW, n = 6 SMAW).	15 EQA	Weiss et al. (2013) *
	Italy 100 workers 0 controls 24 companies	● Cr-EBC: *0.08* µg/l (P_{25}: <LOD, P_{75}: 0.22) ● Cr–U: *0.74* µg/g creat. (P_{25}: 0.41, P_{75}: 1.21) ca. *1.01* µg/l ca. (P_{25}: 0.56, P_{75}: 1.65)	No correlation between: • Cr-EBC and Cr–U Detection frequency: • Cr(VI)-EBC was never detected.	22 No exclusion of contamination Stability Cr(VI)-EBC unknown	Riccelli et al. (2018)
	Iran 94 workers 25 controls Gas pipelines	**Welders (n=59)** ● Cr–U: 6.37 ± 3.74 µg/l (P_0: 1.00, P_{100}: 18.00) ca. 4.68 ± 2.75 µg/g creat. ca. (P_0: 0.73, P_{100}: 13.24) **High-exposure group (n=6):** ● Cr–U: 12.67 ± 4.50 µg/l (P_0: 6.00, P_{100}: 17.00) ca. 9.32 ± 3.31 µg/g creat. ca. (P_0: 4.41, P_{100}: 12.5)	Weak correlation between: • Cr-Air of the breathing zone and Cr–U (R^2_p=0.481, $P < 0.05$) High-exposure group: • back welders inside confined spaces	23 8h samples	Golbabaei et al. (2012)

Industry	Study Population country N° workers N° controls N° companies	Biomonitoring data - directly exposed workers Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	Key findings exposure assessment	LaKind scoring strengths and/or weaknesses	Reference
Welding	Kenya 40 workers 0 controls 1 company	Welding ● Cr–U: 24.7 ± 8.2 µg/g creat. ca. 33.6 ± 11.2 µg/l	Significant positive correlation between: • Cr-Air of the breathing zone and Cr–U in all 112 production workers ($r = 0.86$, $P < 0.01$) $PbCrO_4$ is commonly used to formulate decorative paint	18 ILC Spot morning urine	Were et al. (2013)
Paint manufacturing	Kenya 41 workers 0 controls 1 company	Paint manufacturing ● Cr–U: 9.8 ± 5.3 µg/g creat. ca. 13.3 ± 7.2 µg/l			
Leather tanning	Kenya 31 workers 0 controls 1 company	Leather tanning ● Cr–U: 35.2 ± 12.1 µg/g creat. ca. 47.9 ± 16.5 µg/l			
	India 36 workers 36 controls small-scale tanneries	● Cr–U: 2.11 ± 1.01 µg/l ca. 1.55 ± 0.74 µg/g creat.	Higher sensitivity of cytogenetic assays for human biomonitoring of an occupationally exposed population.	26 No exclusion of contamination 24h samples	Balachandar et al. (2010)
	Kenya 40 workers 40 controls 1 company	● Cr–U: 35.6 ± 7.4 µg/g creat. (P_0: 25, P_{100}: 51) ca. 48.4 ± 10.1 µg/l ca. (P_0: 34, P_{100} : 69)	Significant positive correlation between: • Cr–U and Cr-Air of the breathing zone ($R^2 = 0.76$, $P < 0.001$)	20 ILC	Were et al. (2014)
	Egypt 304 workers 304 controls 1 company	● Cr–S: 3.1 ± 2.2 µg/l (P_0: 0.01, P_{100}: 10.3) ● Cr–U: 2.6 ± 2.5 µg/l (P_0: 0.02, P_{100} : 8.6) ca. 1.9 ± 1.8 µg/g creat. ca. (P_0: 0.01, P_{100} : 6.3)	Significant positive correlations between: • Cr-Air of the breathing zone and Cr–S (regression coefficient $\beta = 0.339$; $P < 0.05$) • Cr-Air of the breathing zone and Cr–U (regression coefficient $\beta = 0.435$; $P < 0.05$) • Cr–S and duration of current employment ($r = 0.187$, $P < 0.001$) • Cr–U and duration of current employment ($r = 0.128$, $P < 0.05$)	22 No exclusion of contamination No methodology mentioned	Abdel Rasoul et al. (2017)

Industry	**Study Population** country N° workers N° controls N° companies	**Biomonitoring data - directly exposed workers** Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	**Key findings exposure assessment**	**LaKind scoring** strengths and/or weaknesses	**Reference**
Electroplating	Italy 14 workers 0 controls 1 company	Post-shift at end of 2nd working day ● Cr(VI)-EBC: *1.0* µg/l (P_0: nd, P_{25}: nd, P_{75} : 1.9, P_{100} : 2.9) ● Cr-EBC: *2.6* µg/l (P_0 : 0.2,	Significant positive correlations between: • Cr–P and Cr–U at the end of the working shift ($r_S = 0.77$, $p < 0.01$) • Cr-EBC and Cr-RBC ($r_S = 0.57$, $p < 0.05$) • Cr–U and Cr-RBC at the beginning of shift ($r_S = 0.86$, $p < 0.01$)	21 Stability Cr(VI)-EBC unknown Small sample size	Goldoni et al. (2010)

(*continued on next page*)

Table 5 (*continued*)

Industry	Study Population country N° workers N° controls N° companies	Biomonitoring data - directly exposed workers Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	Key findings exposure assessment	LaKind scoring strengths and/or weaknesses	Reference
		P_{25} : 0.8, P_{75} : 3.2, P_{100} : 6.1) ● Cr–P: *3.0* μg/l (P_0 : 1.4, P_{25} : 2.0, P_{75} : 3.4, P_{100} : 5.3) ● Cr-RBC: *3.4* μg/l (P_0 : 1.2, P_{25} : 2.0, P_{75} : 3.8, P_{100} : 5.8) ● Cr–U: *2.8* μg/g creat. (P_0 : 1.3, P_{25} : 2.3, P_{75} : 3.3, P_{100} : 5.5) ca. *3.8* μg/l ca. (P_0 : 1.8, P_{25} : 3.1, P_{75} : 4.5, P_{100} : 7.5) Post-shift at end of working week ● Cr(VI)-EBC: *0.5* μg/l (P_0: nd, P_{25} : 0.3, P_{75} : 3.6, P_{100} : 10.1) ● Cr-EBC: *2.2* μg/l (P_0 : 1.0, P_{25} : 1.3, P_{75} : 7.7, P_{100} : 21.2) ● Cr–U: *2.4* μg/g creat. (P_0 : 1.0, P_{25} : 1.8, P_{75} : 4.3, P_{100} : 7.5) ca. *3.3* μg/l ca. (P_0 : 1.4, P_{25} : 2.4, P_{75} : 5.8, P_{100} : 10.2)	• Cr-EBC and Cr(VI)-EBC at the beginning (r_S = 0.91, $p < 0.01$) and end ($r = 0.94$, $p < 0.01$) of the working shift		
	Egypt 41 workers 41 controls 1 company	**All chromium workers (n=41):** ● Cr–S: *3.30* μg/l (P_0 : 0.09, P_{100} : 7.20) **High-exposure group (n=27):** ● Cr–S: *3.90* μg/l (P_0 : 0.09, P_{100} : 7.20)	High-exposure group: • 34.1% of the workers wore PPE. Their Cr–S ranged from 0.13 to 4.40 with a median of 2.05 μg/l 65.9% of the workers didn't wear PPE. Their Cr–S ranged from 0.09 to 7.20 with a median of 3.9 μg/l.	23 No exclusion of contamination	El Safty et al. (2018)
	China 157 workers 93 controls 20 companies	● Cr-RBC: *4.41* μg/l (P_0 : 0.93, P_{100} : 14.98)	Cr-RBC in smokers were significantly higher than that in non-smokers ($P < 0.05$).	18	Zhang et al. (2011)
Industry	**Study Population** country N° workers N° controls N° companies	**Biomonitoring data - directly exposed workers** Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	**Key findings exposure assessment**	**LaKind scoring** strengths and/or weaknesses	**Reference**
Electroplating	Great Britain 354 workers 152 controls 53 companies	**All chromium workers (n=354):** ● Cr–U: 1.2 ± 1.2 μg/g creat. ca. 1.7 ± 1.7 μg/l *1.1* μg/g creat. (P_{90}: 4.9) ca. *1.5* μg/l ca. (P_{90}: 6.6) **High-exposure group (n=180):** ● Cr–U: 1.6 ± 1.3 μg/g creat. ca. 2.1 ± 1.8 μg/l *1.5* μg/g creat. (P_{90}: 6.0) ca. *2.0* μg/l ca. (P_{90}: 8.1)	Significant positive correlations between: • Cr–U and Cr-Hand for chromium electroplaters ($r = 0.71$, $P < 0.0001$) • Cr–U and Cr-Hand for all directly exposed chromium workers ($r = 0.63$, $P < 0.0001$) • Cr–U and inhalable Cr(VI)-Air exposure for chromium workers ($r = 0.62$, $P < 0.0001$) • Cr–U and inhalable Cr-Air exposure for chromium workers ($r = 0.52$, $P = 0.03$) High-exposure group: • chromium electroplaters	16 EQA ILC	Beattie et al. (2017)
	Taiwan 105 workers 125 controls 16 companies	● Cr–U: 2.3 ± 1.8 μg/g creat. ca. 3.1 ± 2.4 μg/l ● Cr-Hair: 7.2 ± 4.7 μg/g ● Cr-Fingernails: 12.7 ± 4.5 μg/g	Significant positive correlations between: • Cr–U and Cr-Air • Cr–U and Cr(VI)-Air • Cr–U and Cr-Hair • Cr–U and Cr-Fingernails	21	Pan et al. (2018)
	China 162 workers 87 controls Several companies	● Cr–B: *6.37* μg/l (P_0: 0.04, P_{100}: 58.92) ● Cr–U: *1.66* μg/l (P_0: 0.42, P_{100}: 94.27) ca. *1.02* μg/g creat. ca. (P_0: 0.26, P_{100}: 57.83)	Significant positive correlation between: • Cr–U and Cr–B ($r_S = 0.211$, $P < 0.01$) High-exposure group: • Two subgroups were identified using Cr–B and Cr–U. The cut-off values were 6.37 μg/l for Cr–B and 1.61 μg/l for Cr–U.	21	Xia et al. (2019)
Industry	Study Population country N° workers N° controls N° companies	Biomonitoring data - directly exposed workers Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	Key findings exposure assessment	LaKind scoring strengths and/or weaknesses	Reference
Electroplating	China 66 workers	● Cr–B: *7.81* μg/l (P_{25}: 4.69, P_{75}: 16.66)	Cr–U, exposure duration, and age were the major risk factors	20	Jia et al. (2020)

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Table 5 (*continued*)

Industry	Study Population country N° workers N° controls N° companies	Biomonitoring data - directly exposed workers Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	Key findings exposure assessment	LaKind scoring strengths and/or weaknesses	Reference
	66 controls Several companies	● Cr–U: *6*.87 μg/l (P_{25}: 2.33, P_{75}: 14.47) ca. *5*.05 μg/g creat. ca. (P_{25}: 1.71, P_{75}: 10.64)			
	Brazil 50 workers 50 controls 2 companies	● Cr–B: 2.02 ± 0.20 μg/l ● Cr–U: 10.65 ± 5.26 μg/g creat. ca. 14.48 ± 7.15 μg/l	Although internal exposure levels are below the exposure level recommended by ACGIH, a significantly increase in the effect biomarkers was observed.	21	Muller et al. (2020)
Mixed	Great Britain 58 workers 22 controls Several companies	● Cr(III)-EBC: *0*.54 μg/l (P_0 : 0.14, P_{90}: 4.21, P_{100} : 11.03) ● Cr(VI)-EBC: *0*.72 μg/l (P_0 : 0.01, P_{90}: 2.45, P_{100} : 27.3) ● Cr–U: *1*.5 μg/g creat. (P_0 : 0.3, P_{90}: 6.3, P_{100} : 17.1) ca. *2*.1 μg/l ca. (P_0 : 0.4, P_{90}: 8.6, P_{100} : 23.2)	Weak moderate correlation between: • post working week Cr–U and Cr(VI)-EBC (r = 0.33, p = 0.01)	16 EQA	Leese et al. (2017)
Aviation industry (assembling)	Italy 43 workers 23 controls 1 company (small & large hangar)	● Cr–U: 3.14 ± 0.69 μg/l ca. 2.31 ± 0.51 μg/g creat.	Pre-shift Cr–U at the beginning of the working week were higher than references from controls.	18	Genovese et al. (2015)
Printing	Thailand 75 workers 75 controls 16 companies	Cr–S: 1.24 ± 1.13 μg/l (P_0 : 0.1, P_{100} : 4.21) Cr–U: 6.86 ± 1.93 μg/g creat. (P_0 : 0.1, P_{100} : 9.5) ca. 9.33 ± 2.62 μg/l ca. (P_0 : 0.1, P_{100} : 12.9)	Significant positive correlations between: • Cr-Air of the breathing zone and Cr–U (r = 0.247, p = 0.032) • Cr-Air of the breathing zone and Cr–S (r = 0.166, p = 0.158)	19 ILC Spot morning urine	Decharat (2015)
Chromate production	China 100 workers 80 controls 1 company	● Cr–B: *15*.68 μg/l	Significant positive correlation between: • Cr–B and Cr-Air of the workplaces (r = 0.568, P < 0.001)	19 data is only presented graphically	Song et al. (2012)
Industry	**Study Population** country N° workers N° controls N° companies	**Biomonitoring data - directly exposed workers** Mean ± SD *Median* (P_0, P_{25}, P_{75}, P_{90}, P_{100}, IQR)	**Key findings exposure assessment**	**LaKind scoring** strengths and/or weaknesses	**Reference**
Chromate production	China 87 workers 30 controls 1 company	● Cr–B: 8.5 ± 1.3 μg/l *6*.4 μg/l (IQR: 7.2)	High-exposure group: • Two subgroups were identified using Cr–B. The cut-off value was 9.10 μg/l.	20 No exclusion of contamination	Hu et al. (2018)
	China 115 $workers_{2006}$ 60 $controls_{2006}$ 63 $workers_{2008}$ 45 $controls_{2008}$ 84 $workers_{2011}$ 30 $controls_{2011}$ 2 companies	● $Cr–B_{2006}$: *15*.6 μg/l (IQR: 13.7) ● $Cr–B_{2008}$: *7*.2 μg/l (IQR: 6.9) ● $Cr–B_{2011}$: *6*.6 μg/l (IQR: 6.8)	Significant positive correlation between: • Cr-Air of the workplaces and Cr–B in chromium exposed groups in the years 2006 and 2008 (r_{2006} = 0.60, r_{2008} = 0.35). High-exposure group: • Two subgroups were identified using Cr–B. The cut-off value was 20 μg/l.	20	Li et al. (2016)
	China 79 workers 112 controls 1 company 5 workshops	● Cr–B: 9.19 μg/l 6.90 μg/l (P_0: 1.17, P_{100}: 51.88) ● Cr–U: 17.03 μg/g creat. ca. 23.16 μg/l *12*.47 μg/g creat. (P_0: 2.78, P_{100}: 97.23) ca. *16*.96 μg/l ca. (P_0: 3.78, P_{100}: 123.23)	Significant positive correlation between: • Cr-Air of the breathing zone and Cr–B (r = 0.472, P < 0.001)	20 ILC	Xiaohua et al. (2012)
	China 115 workers 60 controls 1 company ** Extra contextual information and results reported in (Wang et al., 2011b) and (Wang et al., 2012)	● Cr-RBC: 12.45 ± 20.28 μg/l (P_0: 0.96, P_{100}: 115.01) ● Cr–U: 17.41 ± 14.67 μg/g creat. (P_0: 0.20, P_{100}: 83.30) ca. 23.68 ± 19.95 μg/l ca. (P_0: 0.27, P_{100}: 113.29) ● Cr–B: 23.49 μg/l *15*.52 μg/l (P_0: 5.95, P_{100}: 207.15)	Significant positive correlations between: • Cr-RBC and Cr–U in workers exposed to chromate (r = 0.205, p = 0.049) • Cr-Air of the workplaces and Cr–U in both workers and controls (r = 0.816, p < 0.000) • Cr-Air of the workplaces and Cr–B in both workers and controls (r = 0.842, p < 0.000) • Cr–U and Cr–B in both workers and controls (r = 0.824, p < 0.000)	19	Wang et al. (2011a)**

* Extra contextual information and results reported in (Pesch et al., 2015a).

Abbreviations: Cr-Air, Chromium level in air; Cr–B, Chromium level in whole blood; Cr-EBC, Chromium level in exhaled breath condensate; Cr(III)-EBC, trivalent chromium levels in exhaled breath condensate; Cr(VI)-EBC, hexavalent chromium levels in exhaled breath condensate; Cr-Fingernails, Chromium level in fingernails;

Cr-Hair, Chromium level in hair; Cr-Hand, Chromium level on hands; Cr–P, Chromium level in plasma; Cr-RBC, Chromium level in red blood cells; Cr–S, Chromium level in serum; Cr-Surface, Chromium level on surface; Cr–U, Chromium level in urine; Creat., creatinine; EQA, external quality assurance; ILC, interlaboratory comparison; IQR, interquartile range; N°, number of; P_0, 0^{th} percentile; P_{25}, 25th percentile; P_{75}, 75th percentile; P_{90}, 90th percentile; P_{100}, 100th percentile; r_s, Spearman's rank correlation coefficient; SD, standard deviation;

used less than 2 exposure markers and 2 did not fit within the age limit. Finally, 25 studies were included in this review. An overview of the steps in the literature search is given in Fig. 1.

3.2.1. Data extraction

Table 5 shows the studies included in this review. More information about other extracted data is provided in the Supplementary Material S5–S10. Twenty out of twenty-five studies report a total amount of Cr in urine for the biomonitoring of Cr(VI) exposure. The other five studies report a total amount of Cr in serum (El Safty et al., 2018), red blood cells (RBC) (Zhang et al., 2011) or whole blood (Li et al., 2016; Hu et al., 2018; Song et al., 2012). The different biomarkers (including sampling times) used in each study are summarized in Supplementary Material S5. Since the first elimination half-live of Cr(VI) in urine is relatively short (7–8h), urine samples are mostly collected at the end of the work shift. The presented results focus on post-shift urine samples (when this data is available) to allow comparison between studies. In order to compare creatinine-adjusted and non-adjusted urinary Cr concentrations, a typical creatinine urine concentration of 1.36 g creatinine/l is assumed (Cocker et al., 2011). An approximated urinary concentration is indicated by *circa* (i.e. 'ca.').

3.2.2. Reported biomonitoring levels across industries

As mentioned before, 25 studies were included in this review. Two out of these 25 studies, namely Were et al. (2013) and Leese et al. (2017), reported exposure levels for more than one industrial setting. Were et al. (2013) was performed in the welding, paint manufacturing and leather tanning industry. Leese et al. (2017) reported exposure levels for workers potentially exposed to occupational Cr(VI) compounds in various occupational settings. No detailed information about the number of workers recruited from each occupational setting was given by the authors. Therefore, the industrial setting in Leese et al. (2017) is considered as one 'mixed' occupational setting. Overall, the HBM studies included in this review have been performed in chromate production industries (n = 5), welding (n = 6), electroplating (n = 8), leather tanning (n = 4), paint manufacturing (n = 1), assembling (n = 1), printing (n = 1) and mixed occupational settings (n = 1). More details of the type of industry (e.g. welding on stainless steel or mild steel, hard or decorative chrome plating) is provided in the Supplementary Material S9 (Column "Study Characteristics"). Eight studies (Pesch et al., 2018; Stanislawska et al., 2020; Weiss et al., 2013; Riccelli et al., 2018; Goldoni et al., 2010; Beattie et al., 2017; Leese et al., 2017; Genovese et al., 2015) were performed in European countries, twelve studies (Golbabaei et al., 2012; Balachandar et al., 2010; Pan et al., 2018; Zhang et al., 2011; Xia et al., 2019; Jia et al., 2020; Song et al., 2012; Decharat, 2015; Hu et al., 2018; Li et al., 2016; Xiaohua et al., 2012; Wang et al., 2011a) in Asian countries, four studies (Were et al., 2013, 2014; Abdel Rasoul et al., 2017; El Safty et al., 2018) in African countries and one study (Muller et al., 2020) in a South-American country. Overall, the reported median or mean urinary Cr levels (20 out of 25 articles) were lower in European countries (ranging from 0.96 μg/l to ca. 5.81 μg/l) compared to non-European countries (ranging from 1.66 μg/l to 48.4 μg/l). More information about the published current and recent urinary Cr levels (creatinine-corrected and -uncorrected) in relation to the limit values is provided in Fig. 2. This figure shows that the studies included in this review reported a wide range of exposure levels (Fig. 2A and B). The reported exposure levels varies across countries (Fig. 2C and D) and across industries (Fig. 2E and F).

3.2.3. Reported biomonitoring levels across studies within each industry

3.2.3.1. Welding (n = 6). Four of the studies in the welding industry were conducted in European countries, more specifically in Germany (Weiss et al., 2013; Pesch et al., 2018), in Italy (Riccelli et al., 2018) and in Poland (Stanislawska et al., 2020). Riccelli et al. (2018) and Pesch et al. (2018) report urinary Cr levels that were in reasonable agreement. The median urinary Cr levels were respectively 0.96 μg/l and ca. 1.01 μg/l. Weiss et al. (2013) observed slightly higher urinary Cr levels with a median of 1.2 μg/l and a 75th percentile of 3.61 μg/l. The other European study (Stanislawska et al., 2020) reported higher Cr levels with a median value of 5.18 μg/l compared to the three European studies discussed before.

The non-European studies were performed in Iran (Golbabaei et al., 2012) and Kenya (Were et al., 2013). Golbabaei et al. (2012) observed a similar range of urinary Cr levels as those reported by Stanislawska et al. (2020). Were et al. (2013) reported a higher mean urinary Cr level (ca. 33.6 μg/l).

3.2.3.2. Electroplating (n = 8). Two of the studies in the electroplating industry were conducted in European countries, more specifically in Great Britain (Beattie et al., 2017) and in Italy (Goldoni et al., 2010), and report similar ranges of urinary Cr levels, despite the small sample size (n = 14) used in Goldoni et al. (2010) that may hamper the interpretation of the results. The median urinary Cr levels were respectively ca. 2.0 μg/l and 3.3 μg/l, with maximum levels up to ca. 10.2 μg/l.

The other 6 studies in the electroplating industry were performed in China (Xia et al., 2019; Zhang et al., 2011; Jia et al., 2020), Taiwan (Pan et al., 2018), Brazil (Muller et al., 2020) and Egypt (El Safty et al., 2018). Out of these 6 non-European studies, four studies (Pan et al., 2018; Muller et al., 2020; Xia et al., 2019; Jia et al., 2020) report a total amount of Cr in urine for the biomonitoring of Cr(VI) exposure. The other two non-European studies report a total amount of Cr in serum (El Safty et al., 2018) and RBC (Zhang et al., 2011). The median (or mean) urinary Cr levels ranged from 1.66 to ca. 14.48 μg/l. For Cr levels in serum, El Safty et al. (2018) observed a median value of 3.30 μg/l. For Cr levels in RBC, Zhang et al. (2011) observed a median value of 4.41 μg/l.

3.2.3.3. Leather tanning (n = 4). In the leather tanning industry, Cr(III) salts are mainly used as tanning agent (China et al., 2020). Under certain conditions (e.g. oxidizing agents, high T, pH) Cr (III) can oxidise to Cr (VI) (Zhao and Chen, 2019). The studies in the leather tanning industry are only included in this review if the authors explicitly indicated the presence of Cr(VI) exposure in the workplace (e.g. environmental air Cr (VI) measurements or raw products containing Cr(VI)). This is an inclusion criterion for all studies in this review.

All four studies in the leather tanning industry were performed in non-European countries, namely in Kenya (Were et al., 2013, 2014), in India (Balachandar et al., 2010) and in Egypt (Abdel Rasoul et al., 2017). Were et al. reported in 2013 and in 2014 high urinary Cr levels for the leather tanning industry in Kenya, respectively ca. 47.9 ± 16.5 μg/l and ca. 48.4 ± 10.1 μg/l. The two other studies, namely Balachandar et al. (2010) and Abdel Rasoul et al. (2017), report about 20 times lower urinary Cr levels.

3.2.3.4. Chromate production (n = 5). All five studies in the chromate production industry (Song et al., 2012; Hu et al., 2018; Li et al., 2016; Xiaohua et al., 2012; Wang et al., 2011a) were performed in China and report a total amount of Cr in whole blood for biomonitoring of Cr(VI) exposure. Out of these, one study (Wang et al., 2011a) used whole blood

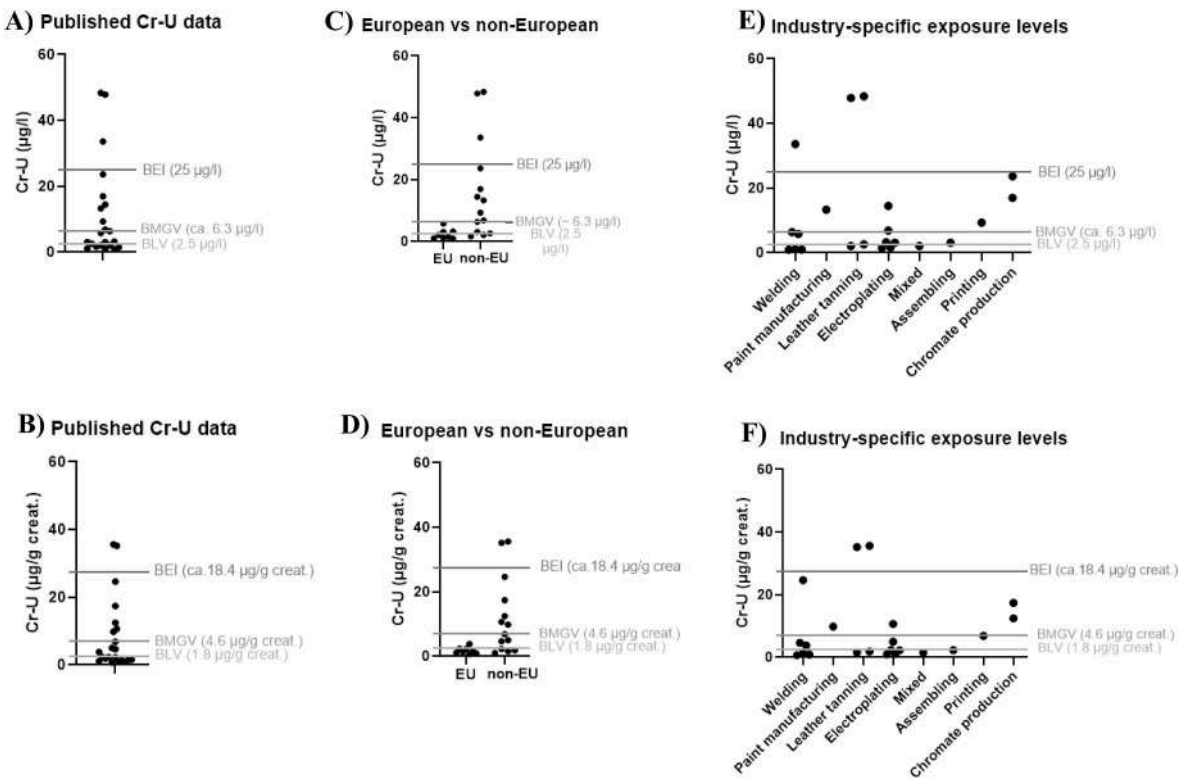

Fig. 2. Current and recent exposure levels in relation to the following occupational limit values: (i) Biological Limit Value (BLV = 2.5 µg/l or 1.8 µg/g creat.) (ANSES, 2017), (ii) Biological Monitoring Guidance Value (BMGV = ca. 6.3 µg/l or 4.6 µg/g creat.) (HSE, 2020), (iii) Biological Exposure Index (BEI = 25 µg/l or ca. 18.4 µg/g creat.) (ACGIH, 2020). Urinary Cr data (20 out of 25 articles) is shown as median or mean.

in combination with urine and RBC as exposure matrices and another study (Xiaohua et al., 2012) reported biomonitoring data for Cr whole blood and urine. The median Cr blood levels ranged from 6.4 to 15.68 µg/l. For Cr levels in RBC, Wang et al. (2011a) observed an average value of 12.45 µg/l with a standard deviation of 20.28 µg/l. For urinary Cr levels, Wang et al. (2011a) and Xiaohua et al. (2012) reported average levels of 17.41 µg/g creatinine and 17.03 µg/g creatinine.

3.2.4. Correlations between exposure markers

Studies included in this review observed significant positive correlations between exposure markers for occupational Cr(VI) exposure, as shown in Table 5 (Column "Key findings exposure assessment"). Different rules of thumb exist for interpreting the size of a correlation coefficient (SAGE, 2012; Mukaka, 2012; Overholser, Sowinski, 2008; Schober et al., 2018). The following rule of thumb is used in this review (SAGE, 2012):

- weak correlation: $-0.3 <$ correlation coefficient $< +0.3$
- moderate correlation: $-0.7 <$ correlation coefficient < -0.3 or $+0.3 <$ correlation coefficient $< +0.7$
- strong correlation: correlation coefficient < -0.7 or $+0.7 <$ correlation coefficient

3.2.4.1. Correlations between an external and an internal exposure marker. Nine studies (Pesch et al., 2018; Stanislawska et al., 2020; Weiss et al., 2013; Golbabaei et al., 2012; Abdel Rasoul et al., 2017; Decharat, 2015; Beattie et al., 2017; Were et al., 2013, 2014) observed weak to strong correlations between personal air Cr levels and urinary Cr levels. Pan et al. (2018) and Wang et al. (2011a) observed a correlation between ambient air Cr levels and urinary Cr levels as shown in Table 6a. Pan et al. (2018) did not report a value for the correlation and is therefore excluded from this table.

Four studies (Pesch et al., 2018; Stanislawska et al., 2020; Beattie et al., 2017; Pan et al., 2018) reported a correlation between air Cr(VI) levels and urinary Cr levels (Table 6b). Out of these, one study (Pan et al., 2018) used stationary air sampling and the others used personal air sampling. The correlations between airborne Cr or Cr(VI) levels and urinary Cr levels indicate that exposures to Cr(VI) may occur via inhalation. Pan et al. (2018) did not report a value for the correlation and is therefore excluded from this table.

Other studies also indicated the importance of the inhalation route by observing a correlation between air Cr levels and blood samples (Table 6c). Four studies (Song et al., 2012; Li et al., 2016; Wang et al., 2011a; Xiaohua et al., 2012) observed moderate to strong correlations between air Cr levels and Cr levels in whole blood. Out of these, one study (Xiaohua et al., 2012) used personal air sampling and the others used stationary air sampling. Three studies (Stanislawska et al., 2020; Abdel Rasoul et al., 2017; Decharat, 2015) observed moderate to strong correlations between personal air Cr levels and Cr levels in serum. Stanislawska et al. (2020) also observed a moderate correlation ($r_S = 0.67$) between personal air Cr(VI) levels and Cr levels in serum.

Beattie et al. (2017) observed a strong correlation between personal air Cr levels and urinary Cr levels ($r = 0.52$) and between Cr levels on the hands (dermal contamination) and urinary Cr levels ($r = 0.63$). This observation indicates that exposures to Cr(VI) may occur via a combination of inhalation, dermal and ingestion routes.

3.2.4.2. Correlations between internal exposure markers. Correlations were observed between urinary Cr at the end of the working week and the following exposure biomarkers: Cr(VI) levels in exhaled breath condensate (EBC) (Leese et al., 2017 [$r = 0.33$]), Cr levels in blood (Wang et al., 2011a [$r = 0.824$]; Xia et al., 2019 [$r_S = 0.211$]), Cr levels in erythrocytes (Wang et al., 2011a [$r = 0.205$]), plasma levels (Goldoni et al., 2010 [$r_s = 0.77$]) and Cr levels in hair and fingernails (Pan et al., 2018). Furthermore, Goldoni et al. (2010) observed strong correlations

Table 6a
Interpreting the size of correlation coefficient between air Cr levels and urinary Cr levels.

Size of correlation	Study: reported correlation	Industrial setting	Country	Type of air sample	Airborne dust fraction
Strong	Were et al. (2014): $R^2 = 0.76$	Leather tanning	Kenya	Personal	Inhalable
	Were et al. (2013): $r = 0.86$	Welding, paint manufacturing and leather tanning	Kenya	Personal	Inhalable
	Wang et al. (2011a): $r = 0.816$	Chromate production	China	Ambient	NM
Moderate	Stanislawska et al. (2020): $r_s = 0.59$	Welding	Poland	Personal	Inhalable
	Weiss et al. (2013): $r = 0.61$	Welding	Germany	Personal	Respirable
	Golbabaei et al. (2012): $R_p^2 = 0.481$	Welding	Iran	Personal	Inhalable
	Beattie et al. (2017): $r = 0.52$	Electroplating	Great Britain	Personal	Inhalable
	Pesch et al. (2018): $r_s = 0.44$	Welding	Germany	Personal	Respirable
	Abdel Rasoul et al. (2017): $\beta = 0.435$	Leather tanning	Egypt	Personal	NM
Weak	Decharat (2015): $r = 0.247$	Printing	Thailand	Personal	Inhalable

Abbreviation: NM: not mentioned.

Table 6b
Interpreting the size of correlation coefficient between air Cr(VI) levels and urinary Cr levels.

Size of correlation	Study: reported correlation	Industrial setting	Country	Type of air sample	Airborne dust fraction
Moderate	Stanislawska et al. (2020): $r_S = 0.58$	Welding	Poland	Personal	Inhalable
	Beattie et al. (2017): $r = 0.62$	Electroplating	Great Britain	Personal	Inhalable
Weak	Pesch et al. (2018): $r_S = 0.25$	Welding	Germany	Personal	Respirable

Abbreviation: NM: not mentioned.

between Cr levels in EBC and Cr levels in RBC [$r_S = 0.57$] and between Cr levels in EBC and Cr(VI) levels in EBC [$r = 0.94$].

3.2.5. *Quality scoring*

Supplementary Material S11 displays the quality scoring according to the adapted LaKind criteria for occupational exposure to Cr(VI). As mentioned before, each assessment component is assessed by giving a score from 1 (Tier 1 = highest quality) to 3 (Tier 3 = lowest quality). The justification for each classification is provided in Table 5 and in the Supplementary Material (S5–S10). Overall, the studies with the highest quality are those of Pesch et al. (2018), Weiss et al. (2013), Beattie et al. (2017) and Leese et al. (2017).

The adapted LaKind criteria are used in this review to consider the following quality issues:

- **Population size**

Small sample sizes may hamper the interpretation of the results. Only 5 studies (Balachandar et al., 2010; Goldoni et al., 2010; El Safty et al., 2018; Genovese et al., 2015; Were et al., 2014) recruited less than 50 occupationally exposed individuals (Tier-2 categorization). Three of these 4 studies had a sample size between 40 and 50 (cut-off value). One study (Goldoni et al., 2010) had a sample size of only 14 workers. The other studies were categorized as Tier 1 (≥50 occupationally exposed individuals). The justification for these classifications can be found in Table 5 (column "Study Population").

- **Study participants**

Another important criterion related to the study design of a HBM study is the selection of the study participants. Inclusion or exclusion criteria and recruitment strategies may influence the final biomonitoring results of the HBM study and may lead to selection bias. Only 3 studies (Beattie et al., 2017; Genovese et al., 2015; Goldoni et al., 2010) used surveillance measurements through an unbiased selection protocol with a high response rate (Tier-1 categorization). The remaining studies used a method of sample selection through considering exclusion criteria or through conduction special purpose or risk measurements (Tier-3 categorization). The justification for these classifications can be found in Supplementary Material S11.

- **Biomarker specificity and biomarker stability**

Although the measurement of Cr in urine is not specific for Cr(VI) exposure (Tier-3 categorization), the total amount of Cr in urine is the most used biomarker in this review (20 out of 25 studies). In contrast, Cr (VI) in EBC is considered a specific biomarker of Cr(VI) exposure (Tier-1 categorization). Three studies (Riccelli et al., 2018; Goldoni et al., 2010; Leese et al., 2017) report exposure levels for this specific biomarker. One of these three studies, namely Leese et al. (2017), documented the stability issues related to Cr(VI) in EBC (Tier-1 categorization). The justification for these classifications can be found in Supplementary Material S7 (column "Method characteristics - Stability history").

Another specific biomarker is Cr in RBC. This is considered as a specific biomarker, because Cr(VI) can easily enter the RBC through a nonspecific anion channel (EPA, 1998). In contrast, Cr(III) is taken up by the RBC with a very low efficiency (EPA, 1998). Five studies (Weiss et al., 2013; Goldoni et al., 2010; Zhang et al., 2011; Wang et al., 2011a;

Table 6c
Interpreting the size of correlation coefficient between airborne Cr levels and Cr levels in blood.

Size of correlation	Study: reported correlation	Industrial setting	Country	Type of air sample	Airborne dust fraction	Blood fraction
Strong	Wang et al. (2011a): $r = 0.842$	Chromate production	China	Ambient	NM	Whole blood
Moderate	Song et al. (2012): $r = 0.568$	Chromate production	China	Ambient	NM	Whole blood
	Li et al. (2016): $r_{2006} = 0.60/r_{2006} = 0.35$	Chromate production	China	Ambient	Inhalable	Whole blood
	Xiaohua et al. (2012): $r = 0.472$	Chromate production	China	Personal	NM	Whole blood
	Stanislawska et al. (2020): $r_S = 0.68$	Welding	Poland	Personal	Inhalable	Serum
	Abdel Rasoul et al. (2017): $\beta = 0.339$	Leather tanning	Egypt	Personal	NM	Serum
Weak	Decharat (2015): $r = 0.166$	Printing	Thailand	Personal	Inhalable	Serum

Abbreviation: NM: not mentioned.

Stanislawska et al., 2020) report levels for this specific biomarker (Tier-1 categorization). The justification for these classifications can be found in Supplementary Material S5 (column "Exposure matrix").

- **Technique and method sensitivity**

All the studies included in this review reported an acceptable LOQ (below 1 μg/l) and were therefore categorized as a Tier-1 study for the LaKind criteria method sensitivity. Only 4 studies mentioned how the authors calculated the LOD or LOQ. The justification for these classifications can be found in Supplementary Material S7 (columns "Technique" and "Method characteristics: LOD, LOQ).

- **Sample contamination**

A Tier-3 categorization might not always be a problem depending on the user's intent for the study data (e.g. not reporting background levels when the user is interested in correlations between exposure markers), but it would indicate a study of low utility in some cases such as the issue of contamination (LaKind et al., 2014). Low Cr levels in biological matrices must be interpreted with caution due to the ubiquitous presence of Cr (ATSDR, 2012). Twenty-one out of the twenty-five studies included in this review documented procedures to avoid contamination of the samples (Tier-1 categorization) while the other 4 studies (Riccelli et al., 2018; Balachandar et al., 2010; Abdel Rasoul et al., 2017; El Safty et al., 2018) did not report these procedures (Tier-2 categorization). The justification for these classifications can be found in Supplementary Material S7 (column "Method characteristics - Contamination-free").

- **Quality assurance**

Eleven studies (Abdel Rasoul et al., 2017; Balachandar et al., 2010; El Safty et al., 2018; Golbabaei et al., 2012; Hu et al., 2018; Li et al., 2016; Muller et al., 2020; Pan et al., 2018; Riccelli et al., 2018; Wang et al., 2011a; Zhang et al., 2011) did not use nor report procedures for quality assurance (Tier-3 categorization). Five studies (Pesch et al., 2018; Stanislawska et al., 2020; Weiss et al., 2013; Beattie et al., 2017; Leese et al., 2017) participated in an external quality scheme and five studies (Xiaohua et al., 2012; Were et al., 2013, 2014; Decharat, 2015; Beattie et al., 2017) participated in an interlaboratory comparison (Tier-1 categorization). The remaining studies used internal quality control (Tier-2 categorization). The justification for these classifications can be found in Supplementary Material S7 (column "Quality assurance").

- **Matrix adjustment, study records and background levels**

When restricted to the studies using Cr in urine for the biomonitoring of Cr(VI) exposure (n = 20), only 2 studies (Pesch et al., 2018; Weiss et al., 2013) include results for adjusted and non-adjusted urinary Cr concentrations (Tier-1 categorization). This might hamper the comparison of the biomonitoring results between studies. The justification for these classifications can be found in Supplementary Material S5 (column "Matrix adjustment and sampling strategy").

To help interpret the biomonitoring levels, detailed information about the subject's job and specific tasks in combination with exposure measurements might help. Only 5 studies (Pesch et al., 2018; Weiss et al., 2013; Were et al., 2013, 2014; Beattie et al., 2017) reported sufficient (according to OECD guidelines) study records (Tier-1 categorization). The justification for these classifications can be found in Supplementary Material S6 (columns "Task with higher exposure/variables influencing exposure" and "RMMs in place/to be applied") and Supplementary Material S9 (column "Study characteristics").

Reporting of background levels might also help to interpret the biomonitoring levels. Only 4 studies (Li et al., 2016; Beattie et al., 2017; Leese et al., 2017; Genovese et al., 2015) reported background levels for controls included in the study population and reference values for the general population, if available (Tier-1 categorization). The justification for these classifications can be found in Supplementary Material S7 (columns "Biomonitoring data - Controls" and "Reference values - General population").

4. Discussion

The goal of this review was fourfold: (1) to assess current and recent biomonitoring levels in workers occupationally exposed to Cr(VI) with a focus on urinary Cr levels at the end of a working week, (2) to identify variables influencing these biomonitoring levels, (3) to identify how urinary Cr levels correlate with other Cr(VI) exposure markers and (4) to identify gaps in the current research. Each of these goals is discussed in turn. Furthermore, the quality of the unpublished and the published biomonitoring dataset is discussed.

4.1. Current and recent biomonitoring levels in workers' occupational exposure to Cr(VI)

Although assessment of total urinary Cr is not specific for Cr(VI) exposure, it is the traditional biomarker for routine biomonitoring of occupational exposure to Cr(VI). The results of the analysis of the unpublished biomonitoring data from Belgian workers showed a decreasing time trend of 30% in urinary Cr levels between 1998 and 2018. Longer time span would increase the robustness of the time trend estimation. Analysis of the median or mean urinary Cr data reported in literature (20 out of 25 articles) from workers occupationally exposed to Cr(VI) showed that 3 median (or mean) values exceeded the BEI of 25 μg/l, recommended by ACGIH and 10 median (or mean) values exceeded the BMGV of ca. 6.3 μg/l, recommended by HSE. All of these 10 studies were performed in non-European countries. With regard to the most stringent BLV of 2.5 μg/l set in France and the Netherlands, 3 European and 11 non-European median (or mean) exposure levels exceeded this limit value. More information about the published current and recent urinary Cr levels (creatinine-corrected and -uncorrected) in relation to the limit values was already shown in Fig. 2.

The studies included in this review reported a wide range of exposure levels, namely in various countries and industries. This resulted in a heterogeneous biomonitoring dataset. Therefore there is a need for more measurement campaigns to establish current exposure levels in these industrial settings and countries.

4.2. Variability of biomonitoring levels

4.2.1. Variability of reported biomonitoring levels across industries

Overall, the reported median or mean urinary Cr levels (20 out of 25 articles) were lower in European countries (ranging from 0.96 μg/l to ca. 5.81 μg/l) compared to non-European countries (ranging from 1.66 μg/l to 48.4 μg/l). This also applies for the other internal exposure levels.

4.2.2. Variability of reported biomonitoring levels across studies within each industry

4.2.2.1. Variability of reported biomonitoring levels across studies (n = 6) within welding industry

4.2.2.1.1. European studies (n = 4). Riccelli et al. (2018) and Pesch et al. (2018) report urinary Cr levels for welders that were in reasonable agreement. The median urinary Cr levels were respectively 0.96 μg/l and ca. 1.01 μg/l. On one hand, Weiss et al. (2013) observed similar a median urinary Cr level, namely 1.2 μg/l. On the other hand, Weiss et al. (2013) observed higher urinary Cr levels for a sub-population of welders and reported a 75th percentile of 3.61 μg/l for urinary Cr levels. The higher levels detected in Weiss et al. (2013) could be explained by the different welding techniques in combination with the type of welding material included in the study population. Riccelli et al. (2018)

investigated the exposure to Cr(VI) in 100 stainless steel tungsten inert gas (TIG) welders and Pesch et al. (2018) in a study population consisting of 50 welders using TIG, gas metal arc welding (GMAW) and other welding techniques with GMAW predominantly applied to mild steel and TIG to stainless steel. Weiss et al. (2013) investigated a larger study population (n = 241), who used a larger variety of welding techniques. The welders used GMAW, TIG, shielded metal arc welding (SMAW), flux-cored arc welding (FCAW) and miscellaneous welding techniques during the shift. When restricted to stainless steel, Weiss et al. (2013) observed the following pattern for urinary Cr levels: FCAW > GMAW > SMAW > TIG. More information about the welding techniques and the type of welding material is provided in the Supplemental Information S9 (Column "Study Characteristics").

The other European study (Stanislawska et al., 2020) reported higher Cr levels with a median value of 5.18 μg/l compared to the three European studies discussed before. An explanation for the higher urinary Cr levels might be the higher air Cr levels observed in Stanislawska et al. (2020) in combination with the efficacy of local exhaust ventilation (LEV) and the use of respiratory protection equipment (RPE). Stanislawska et al. (2020) did not report anything about the efficiently use of LEV and no RPE was available for the welders, while the other European studies were equipped with efficiently LEV, a range of RPE was used and workers were aware of the importance of these preventive measures. More information about the air levels and the presence of RMM (LEV and RPE) is provided respectively in Supplementary Material S10 and in Supplementary Material S6.

4.2.2.1.2. Non-European studies (n = 2). Golbabaei et al. (2012) observed a similar range of urinary Cr levels as those reported by the European study of Stanislawska et al. (2020). There was a lack of RPE in both studies (Supplementary material S6, column "RMMs in place/to be applied"). Similar to the European study of Weiss et al. (2013), Golbabaei et al. (2012) identified welding in confined spaces as a high-exposure scenario for welders.

Were et al. (2013) reported a higher mean urinary Cr level (ca. 33.6 μg/l). A plausible explanation for these higher Cr levels compared to the Cr levels reported in the previously described studies, is the lack of PPE in combination with inadequate working conditions, no training and inadequate personal hygiene (Supplementary material S6, column "RMMs in place/to be applied").

4.2.2.2. Variability of reported biomonitoring levels across studies (n = 8) within electroplating industry

4.2.2.2.1. European studies (n = 2). Beattie et al. (2017) and Goldoni et al. (2010) report similar ranges of urinary Cr levels, despite the small sample size (n = 14) used in Goldoni et al. (2010) that may hamper the interpretation of the results. The median urinary Cr levels were respectively ca. 2.0 μg/l and 3.3 μg/l, with maximum levels up to ca. 10.2 μg/l.

4.2.2.2.2. Non-European studies (n = 6). Out of thee 6 non-European studies conducted in the electroplating industry, four studies (Pan et al., 2018; Muller et al., 2020; Xia et al., 2019; Jia et al., 2020) report a total amount of Cr in urine for the biomonitoring of Cr(VI) exposure. The other two non-European studies report a total amount of Cr in serum (El Safty et al., 2018) and RBC (Zhang et al., 2011). The median (or mean) urinary Cr levels ranged from 1.66 to ca. 14.48 μg/l.

The influence of PPE on exposure to Cr(VI) in the electroplating industry is a plausible explanation for the observed variance in the Cr levels reported in the studies included in this review. El Safty et al. (2018) observed that not wearing PPE led to an almost doubling of the median serum level. Also Beattie et al. (2017) indicated the importance of PPE for electroplaters. Sampling was carried out at three time points over the duration of that study. Additional urine samples were requested approximately 6 and 12 months after feedback had been provided from the initial visit. A reduction of 23% was observed in the urinary Cr levels of Cr electroplaters working in a subset of companies, characterized by control deficiencies. Increased risk awareness, including the use of PPE to minimize dermal exposure, throughout the study has contributed to the decreasing trend in urinary Cr levels. The urinary Cr levels reported by Beattie et al. (2017) are lower than those reported by Pan et al. (2018) (ca. 2.1 ± 1.8 μg/l vs ca. 3.1 ± 2.4 μg/l). A smaller fraction of workers (only 14.3%) used PPE in the study of Pan et al. (2018) compared to Beattie et al. (2017). Although PPE were made available to the workers in the study of Muller et al. (2020), they reported high urinary Cr levels (ca. 14.48 ± 7.15 μg/l). The authors (Muller et al., 2020) indicated that these workers did not use the available PPE. Zhang et al. (2011) reported Cr levels in RBC up to 14.98 μg/l with a median of 4.41 μg/l 52% of the workers in this study were provided with masks. More workers used gloves (95%) and protective clothes (63%). A plausible explanation for these higher Cr levels in RBC is the high environmental air Cr levels with a median of 60 μg/m^3 observed in this study, compared to other studies. The environmental data are provided in the Supplementary Material S10. With the protection of gloves and protective clothes, inhalation is the main exposure pathway in the lack of (efficient) RPE. More information about PPE is provided in the Supplementary Material S6, column "RMMs in place/to be applied".

No information was reported by Goldoni et al. (2010), Xia et al. (2019) and Jia et al. (2020) about RMMs or variables influencing exposure.

4.2.2.3. Variability of reported biomonitoring levels across studies (n = 4) within leather tanning industry

4.2.2.3.1. Non-European studies (n = 4). Were et al. reported in 2013 and in 2014 high urinary Cr levels for the leather tanning industry in Kenya, respectively ca. 47.9 ± 16.5 μg/l and ca. 48.4 ± 10.1 μg/l. Both studies were characterized by inadequate working conditions (Supplementary Material S6, column "RMMs in place/to be applied") leading to high mean urinary levels. The authors (Were et al., 2014) indicated the lack of environmental policies and enforcement of legislation in developing countries as the cause of inadequate working conditions and consequently higher urinary Cr levels. The two other studies, namely Balachandar et al. (2010) and Abdel Rasoul et al. (2017), report about 20 times lower urinary Cr levels. Also the personal Cr air levels in Abdel Rasoul et al. (2017) were 3–7 times lower compared to Were et al. (2013, 2014) . The environmental data are provided in the Supplementary Material S10. A plausible explanation for the discrepancy in reported urinary Cr levels in the leather tanning industry might be the significant positive correlation between Cr levels in the air of the breathing zone and urinary Cr levels reported by Were et al. (2013), Were et al. (2014) and Abdel Rasoul et al. (2017), in combination with the lack of RPE (Supplementary Material S6, column "RMMs in place/to be applied").

Balachandar et al. (2010) did not report information about the lack or presence of correlations between urinary and air Cr levels and used stationary air sampling instead of personal air sampling.

4.2.2.4. Variability of reported biomonitoring levels across studies (n = 5) within chromate production industry

4.2.2.4.1. Non-European studies (n = 5). All five studies in the chromate production industry report a total amount of Cr in whole blood for biomonitoring of Cr(VI) exposure. Out of these, one study (Wang et al., 2011a) used whole blood in combination with urine and RBC as exposure matrices and another study (Xiaohua et al., 2012) reported biomonitoring data for Cr whole blood and urine. The median Cr blood levels ranged from 6.4 to 15.68 μg/l. Although at least 90% of the workers was provided with gloves and masks in all these studies (no information about the effectiveness, suitability, and use of the PPEs was given by the authors), these studies reported remarkably higher Cr levels in the biological matrices of workers (and controls) compared to other European occupational studies in literature. Namely, Gil et al. (2011) observed a median value of 0.78 μg/l with a 95th percentile of 4.04 μg/l

for Cr levels in whole blood of 278 workers in the Italian iron and steel industry and Julander et al. (2014) reported a median blood Cr concentration of 1.4 μg/l with a maximum value of 5.0 μg/l for recycling workers in the Swedish e-waste industry. For residents of areas with a high density of industry, Bonberg et al. (2017) observed a median Cr blood level of 1.51 μg/l with a 95th percentile of 2.09 μg/l in 2821 residents of the German Ruhr area. Three other studies included in this review (Xia et al., 2019; Jia et al., 2020; Muller et al., 2020) also used whole blood for biomonitoring of exposure to Cr(VI) and were performed in the electroplating industry. Similar to the five studies in the chromate production industry, Xia et al. (2019) and Jia et al. (2020) also reported higher median values of 6.37 μg/l and 7.81 μg/l for workers in the Chinese electroplating industry. No information about the provision or use of PPEs was given by the authors of both studies. Muller et al. (2020) observed a lower mean blood Cr level of 2.02 μg/l for Cr electroplaters in Brazilian electroplating industry. As mentioned before, 2 out of 5 studies (Wang et al., 2011a; Xiaohua et al., 2012) conducted in the chromate production industry used whole blood in combination with another biological exposure matrices. The reported Cr levels for these matrices were also remarkably higher compared to other European occupational studies included in this review. Wang et al. (2011a) observed an average value of 12.45 μg/l with a standard deviation of 20.28 μg/l for Cr levels in RBC, while European occupational studies included in this review reported median levels of 3.4 μg/l, 0.09 μg/g Hb and 1.95 μg/l for respectively Italian Electroplating industry, Polish and German welding industry (Goldoni et al., 2010; Stanislawska et al., 2020; Weiss et al., 2013). Furthermore, Wang et al. (2011a) and Xiaohua et al. (2012) reported average urinary Cr levels of 17.41 μg/g creatinine and 17.03 μg/g creatinine, while lower median urinary Cr levels of 2.8 μg/g creatinine, 3.81 μg/g creatinine and <1.35 μg/g creatinine were reported by respectively Goldoni et al. (2010), Stanislawska et al. (2020) and Weiss et al. (2013).

In addition to biomonitoring data of Cr levels in whole blood for directly exposed workers in the Chinese chromate production industry, the 5 studies that used whole blood as exposure matrix also reported biomonitoring data for a control group. The background levels of controls ranged from 2.9 to 3.9 μg/l (Supplementary material S9, column "Biomonitoring data Controls") and also these values are remarkably higher compared to European reference values reported in literature. Minoia et al. (1990) observed a mean blood Cr level of 0.23 μg/l with a maximum value of 0.75 μg/l in 519 Italians subjects, Llobet et al. (1998) reported a mean value of 0.2 μg/l with a maximum value of 1.1 μg/l in 144 Spain subjects and Nisse et al. (2017) observed a mean value of 0.60 μg/l with a 95th percentile of 1.26 μg/l in 1130 French subjects. For the general population in China, Ding et al. (2012) observed a median value of 1.19 μg/l with a 95th percentile of 5.59 μg/l.

Depending on how well-controlled the occupational exposure is within the factory and on the selection criteria of the controls, elevated Cr levels for both workers and controls might be caused by (pre-) analytical factors, occupational exposure and environmental exposure. Each of these factors are briefly considered in turn:

- Though (pre-)analytical factors (e.g. needles, blood stoppers, anti-coagulants/additives) may be a contribution factor to the elevated Cr Levels in whole blood (Wood et al., 2010; Penny and Overgaard, 2010; Hodnett et al., 2012), exogenous contamination from Cr in pre-analytical factors was not always observed in literature (Bro et al., 1988; Sommer et al., 2021). Nevertheless, they all recommend to consider precautionary procedures (e.g. contamination control, testing sampling protocol, storage stability) to minimize or eliminate these sources of errors. One of the studies in this review with elevated Cr levels for controls, namely Song et al. (2012), indicated the difference in anti-coagulants as possible explanation for the elevated blood Cr levels in controls. Hu et al. (2018) used the same protocol as Song et al. (2012). More information about the method characteristics is provided in the Supplementary Material S7.
- Four studies (Wang et al., 2011a; Xiaohua et al., 2012; Li et al., 2016; Song et al., 2012) selected controls (farmers, salesmen, …) outside (>20 km) the factory. Hu et al. (2018) included controls from the administrative personnel. The authors explicitly indicated that the controls were not (directly) exposed to Cr. Therefore, it is unlikely that occupational exposure is the major relative contributing factor for the elevated background levels in all these 5 studies. More information about the selection of the controls is provided in the Supplementary Material S9 (column "Study characteristics").
- With respect to the relative contribution of environmental exposure, the median air Cr levels for controls ranged from 0.06 to 0.2 μg/m^3 in these 5 studies (Supplementary Material S10 - Environmental monitoring data). The environmental airborne Cr levels reported in these studies were higher than airborne Cr levels reported in European studies. The following ranges are reported in EU: 0–0.003 μg/m^3 (remote areas), 0.004–0.07 μg/m^3 (urban areas) and 0.005–0.2 μg/m^3 (industrial areas) (WHO, 2000). Concerning the environment policy of China, Gao and Xia (2011) indicated the presence of strict environmental regulations on Cr wastes and the presence of historical Cr contamination in some areas. Zhao et al. (2020) indicated that environmental Cr(VI) contamination in northeast China has been ongoing for over 60 years and they observed elevated urinary Cr levels with a median of 1.28 μg/l in 134 residents living in three Cr polluted villages. Therefore, environmental exposure might be a contributing factor for the elevated Cr levels in these studies.

4.2.3. *Variables influencing current and recent urinary Cr levels*

Repeating biological monitoring over time could drive sustainable improvements in exposure control, as suggested by the linear mixed effect model results of the unpublished urinary Cr data. Improved working conditions, better exposure control, feedback to workers and increased risk awareness over years (Beattie et al., 2017; Xiaohua et al., 2012; Li et al., 2016; El Safty et al., 2018; Riccelli et al., 2018) were all reported by authors of the included review articles to have decreased Cr levels in biological matrices.

Worker or workplace variability might also account for the variability in urinary Cr levels. The linear mixed effect model applied at sub-major ISCO levels (three-digit) showed that about 15% of the total variance in Cr data was explained by the variance between jobs (three-digit ISCO) and the industries. In the literature review, significant associations were found between urinary Cr levels and job characteristics such as work tasks and work duration (Decharat, 2015; Were et al., 2014). Some of the studies (Weiss et al., 2013; Pesch et al., 2018; Genovese et al., 2015; Decharat, 2015; Were et al., 2013) assessed for this review, included a (sub)group which multitasked a combination of different processes or (welding) techniques. Therefore, it was not always possible to assign workers to specific work tasks. Moreover, urinary Cr levels can also be affected by other individual variables such as hobbies, diet and individual capacity to reduce Cr(VI) (NIOSH, 2013).

The identification of high-exposure groups in the literature review showed the importance of the hierarchy of controls in different occupational settings to reduce exposure levels (El Safty et al., 2018; Golbabaei et al., 2012; Beattie et al., 2017). This highlights the need for (re) new(ed) sector-specific information, instruction and training targeted to this issue. Furthermore, one German (Weiss et al., 2013) and one Iranian study (Golbabaei et al., 2012) showed that welding in confined spaces is indicative of Cr(VI) exposure. If workers weld in confined spaces, airborne Cr(VI) levels can easily accumulate in the absence of precautionary measures. Currently, there is no specific limit value for welding in confined spaces, only a general limit value for welding fumes. Therefore, more attention is needed for welding in confined spaces.

4.3. *Correlation of urinary Cr levels with other Cr(VI) exposure markers*

The main limitation of the traditional biomonitoring method used for biomonitoring of exposure to Cr(V), namely urinary Cr levels, is that it

may overestimate the exposure to Cr(VI) since it measure the exposure to both Cr(III) and Cr(VI). Correlations between airborne Cr(VI) levels, Cr(VI) levels in EBC, Cr levels in RBC and urinary Cr levels allow to study the reduction of Cr(VI) to Cr(III) in the body. If the use of specific biomarkers is validated and correlations between these specific biomarkers and urinary Cr exist, the internal exposure to Cr(VI) may be calculated from urinary Cr levels.

4.3.1. Correlation of urinary Cr levels with air levels

The correlations between airborne Cr or Cr(VI) levels and urinary Cr levels indicate that exposures to Cr(VI) may occur via inhalation. Were et al. (2013) conducted two measurement campaigns one year apart and observed that personal air Cr levels were strongly correlated with urinary Cr levels in both of these (R^2 = 0.86 and 0.76 for the first and second campaign, respectively). A plausible explanation for these strong correlations might be the lack of RPE in combination with inadequate working conditions (Supplementary Material S6, column "RMMs in place/to be applied"). Wang et al. (2011a) also indicated inhalation exposure as a significant contributor to urinary levels, with a strong correlation (r = 0.816) being observed between Cr air levels of the workplaces and urinary levels in both workers and controls. Weak to moderate correlations between urinary and personal air Cr levels were observed by Pesch et al. (2018), Weiss et al. (2013), Golbabaei et al. (2012), Abdel Rasoul et al. (2017), Decharat (2015) and Beattie et al. (2017). Pan et al. (2018) observed a positive correlation between urinary and ambient air Cr levels. The association between Cr levels in the air and Cr levels in urine may be influenced by the physical and chemical properties of the inhaled Cr(VI) compounds. Properties such as size and solubility affect the behaviour of Cr(VI) deposited in the respiratory tract (Cena et al., 2014a, 2014b; Brand et al., 2013). These properties will probably account for the differences in absorption, retention and excretion. Other factors influencing these correlations might be individual variability of the workers, varying occupational settings, methodological and sampling factors (Balachandar et al., 2010).

Only three studies reported a correlation between Cr(VI) levels in air of the breathing zone and Cr levels in urine. Pesch et al. (2018) observed a weak correlation (r_S = 0.25) between respirable Cr(VI) levels and urinary levels for welders. Beattie et al. (2017) and Stanislawska et al. (2020) found a moderate correlation (r = 0.62 and r = 0.58) between inhalable Cr(VI) levels and urinary levels for chrome electroplaters. These differences in reported correlations are to be expected because of differences between the three studies in the type of exposure (welding vs electroplating vs "mixed"), the accompanying difference in physical and chemical properties of the inhaled Cr(VI) compounds and the different type of measurement to characterize the Cr(VI) levels in the air of the breathing zone (respirable vs inhalable). Furthermore, the differences in reported correlations may be due to the inaccuracies of assessing exposure to Cr(VI) resulting from the instability of Cr(VI) such as the difficulties in sampling and analysis of Cr(VI) (Unceta et al., 2010; KHADEM et al., 2017).

As mentioned before, total urinary Cr is the most common biomarker for routine biomonitoring of occupational exposure to Cr(VI). A validated equation showing a correlation between urinary Cr levels and airborne Cr(VI) levels allows a possible conversion of urinary Cr levels into corresponding airborne Cr(VI) levels. Such equations reported in literature are mainly derived from Cr plating activities (Lindberg and Vesterberg, 1983; Tola et al., 1977). The applicability of these equations to other exposure scenarios is questionable. For example, stainless steel welders may be exposed to different oxidation states of Cr. This is supported by Pesch et al. (2018) who concluded that airborne Cr(VI) levels cannot be precisely estimated from urinary Cr levels. Furthermore, the possibility to transform urinary Cr levels into airborne Cr(VI) levels depend on the quality of these equations and the experimental conditions (sampling time of urine sample, sampling duration of air measurements, number of workers, …). These equations are often derived from occupational studies with a limited number of workers and various experimental conditions such as collecting urine samples at the end of the 2nd working day (Lindberg and Vesterberg, 1983) or at the end of the working week (Tola et al., 1977).

4.3.2. Correlation of urinary Cr levels with other exposure biomarkers

Five studies included in this review observed correlations between urinary Cr at the end of the working week and the following exposure biomarkers: Cr(VI) levels in EBC (n = 1), Cr levels in blood (n = 2), Cr levels in erythrocytes (n = 2), plasma levels (n = 1) and Cr levels in hair and fingernails (n = 1). 13 out of 25 studies only used 1 internal exposure matrix (Supplementary Material S5 (Column "Exposure Matrix").

Even though urinary Cr levels are not specific for occupational exposure to Cr(VI), the sampling and analysing process for urine Cr levels is simpler than the potentially specific biomarkers Cr in RBC and Cr(VI) in EBC. Therefore, total urinary Cr is often used for routine biomonitoring of occupational Cr(VI) exposure. As mentioned before, the possible existence of correlations between validated specific biomarkers and urinary Cr allow to convert urinary Cr levels into internal Cr(VI) exposure levels. Therefore further measurement campaigns need to consider multiple internal exposure matrices in order to study the reduction of Cr(VI) to Cr(III) in the human body and to assess correlation between the different exposure biomarkers.

4.4. Gaps in the current research

4.4.1. Specific biomarkers

As mentioned before, the main limitation of using urinary Cr levels as biomonitoring method is that it cannot differentiate the exposure to Cr(VI) from the exposure to Cr(III). Furthermore, the most stringent BLV of 2.5 μg/l, set in France and the Netherlands, is close to the background level of the general population (e.g. BRV = 0.65 μg/l). Therefore, there is a need to validate specific biomarkers for exposure to Cr(VI) such as Cr(VI) in EBC and Cr in RBC.

Three studies (Goldoni et al., 2010; Leese et al., 2017; Riccelli et al., 2018) reported exposure levels for Cr(VI) in EBC. All three studies were conducted in European countries. Overall, low Cr levels or low number of detects were reported, which is in line with environmental measures and risk awareness. In the study of Riccelli et al. (2018), Cr(VI) was never detected (LOD = 0.2 μg/l) in the EBC samples, which is in line with the type of welding (TIG welding) and the risk awareness of the workers. The median levels of Cr(VI) in EBC reported by Goldoni et al. (2010) and Leese et al. (2017) were 1.0 μg/l and 0.72 μg/l, respectively. Both studies consist of a (sub)group of Cr electroplaters, for whom higher exposure levels are reported in literature, compared to TIG welders in European countries. As mentioned before, Cr(VI)-containing products are used during electroplating processes, whereas Cr(VI) is formed as a by-product during welding processes. Therefore, it is likely to expect lower levels for welding processes.

Five studies (Goldoni et al., 2010; Zhang et al., 2011; Wang et al., 2011a; Weiss et al., 2013; Stanislawska et al., 2020) included in this review report levels for Cr in RBC. Three studies (Weiss et al., 2013; Goldoni et al., 2010: Stanislawska et al., 2020) were conducted in European countries. Weiss et al. (2013) reported low number of detects (15 out of 150; LOQ = 1.5 μg/l), which is in line with the type of occupational setting, namely welding, and risk awareness (Supplementary Material S9, column "Study Characteristics"). Stanislawska et al. (2020) also observed low levels for Cr in RBC of welders. Goldoni et al. (2010) reported higher Cr levels in RBC for Cr electroplaters, which is in line with the type of occupational setting. The non-European studies (Zhang et al., 2011; Wang et al., 2011a) reported elevated Cr levels in RBC for both workers and controls in the electroplating and chromate production industry (Supplementary Material S9, column "Biomonitoring data - Controls"). The discrepancy between elevated levels for both workers and controls may be due to (pre-)analytical factors, occupational and environmental exposure.

Although urinary Cr levels are not specific for Cr(VI) exposure, a

limited number of studies included in this review used specific biomarkers to assess exposure to Cr(VI). In addition, these studies were conducted in distinct industries (welding, electroplating, chromate production and "mixed"). The difference in exposure scenarios limits the possibility to aggregate the biomonitoring data of the specific biomarkers and to make a general conclusion about the use of specific biomarkers. Nevertheless, the use of total urinary Cr is supposed to be appropriate in work tasks in which Cr(VI) is used as start-product and in which co-exposure to other oxidation states of Cr is negligible, like chrome plating. This may not be the case in work tasks in which co-exposure to different oxidation states of Cr may occur, like stainless steel welding. Therefore there is a need for further measurement campaigns to assess the appropriateness of specific biomarkers in distinct industries and tasks.

4.4.2. Occupational settings

From the literature review it is apparent that the available data do not report all the occupational settings where exposure to Cr(VI) can occur, such as wood preserving and spraying of Cr(VI)-containing paints. Furthermore, exposure to Cr(VI) is not limited solely to workers operating directly in areas of the workplace where Cr processes occur (Leese et al., 2017; Beattie et al., 2017; Balachandar et al., 2010; Genovese et al., 2015). Therefore there is a need for further measurement campaigns to establish current exposure levels in these settings (including the use of specific biomarkers) and to compare these with relevant limit values.

4.4.3. Exposure routes

Goldoni et al. (2010), Were et al. (2014), Beattie et al. (2017) and Leese et al. (2017) indicated that exposures to Cr(VI) may occur via a combination of inhalation, dermal and ingestion routes. The measurement of Cr in urine captures total exposure by all these routes. As mentioned before, 9 studies observed weak to strong correlations between personal air Cr levels and urinary Cr levels. Out of these 9 studies, only one study (Beattie et al., 2017) investigated the correlation between dermal and urinary Cr levels. Further investigation of the contribution of the different exposure routes is needed, so that better guidance on the use of control measures can be provided.

4.5. Quality of unpublished biomonitoring dataset

A low between-jobs variation of 15% can be related to the grouping strategy of jobs utilised in exposure assessment. When exposure is linked to individual ISCO codes, the workers merged under the same class are assumed to have the same level of exposure which results in a heterogeneous classification. This is an innate limitation of utilisation of ISCO classification system in exposure assessment studies. In order to obtain exposure classification closer to real life scenarios, tasks can be taken into consideration (Pesch et al., 2015a). Increasing resolution of ISCO codes would also provide more homogenous classification with regards to occupation. Nevertheless, this approach results in decrease in sample size per 4-digit ISCO code as study sample is allocated to a greater number of job classes (Sauvé et al., 2020).

The main limitation of our binary expert assessment method is the absence of validity and interrater reliability evaluation. Validity refers to how likely exposure categorization corresponds to real exposure classification and is statistically evaluated by sensitivity and specificity analysis estimated from gold standard. Reliability refers to the consistency of agreement among raters (assessors) or methods. The extent of the agreement is evaluated by the Kappa statistics (K). An association between K, sensitivity and specificity exists and true prevalence of exposure is further needed to calculate sensitivity and specificity. Testing for the credibility of an exposure assessment method is crucial as errors in exposure groupings generate bias in odds ratios or regression coefficients in studies investigating the link between exposure and disease. Validity assessment have been study of interest in occupational epidemiology because gold standard or information on actual prevalence of exposure is often lacking. In order to tackle the issue of missing validity information, Burstyn et al. has proposed a simulation based method to calculate the sensitivity and specificity by using K and exposure prevalence obtained through different exposure assessment methods (Burstyn et al., 2013). However, since a reliability analysis was not applied to our expert assessment approach, we were not able to determine validity through this approach. On the other hand, chromium exposure decisions of experts are based on risk assessment of the workplace they are responsible for and on periodical examinations of workers, thus assumed to be reflective of a first-hand knowledge of the worksite involved.

4.6. Quality of published biomonitoring dataset

In this review, adapted LaKind scoring criteria for exposure to Cr(VI) are used to consider study quality issues in a systematic way. The LaKind score gives an indication of the overall quality of the study. As mentioned before, a Tier-3 categorization might not always be problematic depending on the user's intent for the study data (e.g. not reporting background levels when the user is interested in correlations between exposure markers). However, it would indicate a study of low utility in some cases (e.g. inability to demonstrate samples were free of contamination). Hence only considering the overall LaKind score is insufficient to differentiate the quality of studies.

The quality assessment of the studies included in this review is based on the information in the article (and any supplemental information provided). The consequence of this is that if a study participated in an external quality scheme and did not report this, it would be categorized as Tier-3 instead of Tier-1. Only 4 studies (Hu et al., 2018; Pesch et al., 2018; Pan et al., 2018; Jia et al., 2020) included in this review provided supplemental information which was needed for a better interpretation of the results. Therefore, it is recommended that authors publishing biomonitoring data make more use of journals' supplemental information options to provide additional essential information, in order to allow a more thorough interpretation of biomonitoring results.

Besides insufficient reporting, there are other factors affecting the direct comparison and interpretation of data published in literature, such as different study characteristics (target population), different sampling years, different sampling strategies (morning, spot, 8h and 24h), different units (creatinine-adjusted or non-adjusted urinary Cr concentrations), different statistical parameters and analysis (mean, median, 25th-75th percentiles, range, LOD definition, representation of values below LOD, …). Therefore, this review reinforces the call (Berman et al., 2017; Latshaw et al., 2017; Kromerová and Bencko, 2019; Nakayama et al., 2019; Scholten et al., 2020; Fréry et al., 2020) for more harmonization in conducting HBM in future research and highlights the importance of the recent and current efforts (e.g. IPCHEM, DEMOCOPHES, COPHES, HBM4EU) to harmonize biomonitoring data across Europe. The chromates study protocol (Santonen et al., 2019) developed under HBM4EU presents harmonized methodologies for the collection and analysis of occupational hygiene and HBM samples and this protocol will contribute undoubtedly to the harmonization of biomonitoring data on occupational exposure to Cr(VI).

5. Conclusion

Due to required authorization of Cr(VI) compounds and the potential binding limit value for Cr(VI) compounds, it is important to gather existing occupational biomonitoring data concerning Cr(VI). We combined the findings from a linear mixed effect model, applied on unpublished biomonitoring data and a systematic literature review to investigate the Cr(VI) exposure levels. Specifically, our research focused on urinary biomonitoring data. Overall, the results showed a decreasing time trend in urinary Cr levels and reinforce the importance of preventive measures such as control the use of PPE, increased risk

awareness, improved working conditions, controlling Cr(VI) air levels at the workplace and minimizing exposure to Cr(VI) compounds during work shift (e.g. rotating jobs or maximum 8h shift duration). With regard to the most stringent BLV of 2.5 μg/l, which is close to the background level of the general population, set in France and the Netherlands, 3 European studies and 11 non-European studies exceeded this limit value. These results support the need for more specific Cr(VI) biomarkers. Furthermore, this review reinforces the call for more harmonization in conducting future HBM research. We identified the following gaps in current literature, which need to be further investigated: i) the available data do not report all the occupational settings where exposure to Cr(VI) can occur, ii) workers' exposure via dermal contact needs to be further investigated in occupational biomonitoring studies and iii) the specific biomarkers for Cr(VI) exposure were only used in a limited number of studies and need to be further validated in more occupational settings.

Disclaimer

The contents, including any opinions and/or conclusions expressed of this manuscript, are those of the authors alone and do not necessarily reflect the opinions or policy of the organisations to which they are employed.

Declaration of competing interest

No conflicts of interest are declared.

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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.2021.113799.

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The health effects of wearing facemasks on cardiopulmonary system of healthy young adults: A double-blinded, randomized crossover trial

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ABSTRACT

Background: Facemask had increasingly been utilized as a personal protective measure to reduce exposure to ambient particulate matter (PM) during heavily-polluted days and routine life. However, evidence on the potential effects on cardiovascular system by wearing particulate-filtering facemask was limited.

Methods: We conducted a double-blinded randomized crossover trial (RCT) to evaluate the effects of wearing N95 facemasks on the molecular responses of cardiopulmonary system among 52 healthy college students in Beijing, China. We measured cardiopulmonary health indicators and collected biological samples before and after (up to 5 h at multiple time points) a 2-h walk to examine the changes in lung function, biomarkers of respiratory and systemic oxidative stress/inflammation. We applied linear mixed-effect models to evaluate the effect of the facemask-intervention on the health of cardio-pulmonary system.

Results: In the trial wearing real facemasks, FEV_1 increased by 2.05% (95% CI: 0.27%–3.87%), 2.80% (95% CI: 1.00%–4.63%), and 2.87% (95% CI: 1.07%–4.70%) at V1 (30-min), V2 (3-h), and V3 (5-h) after the 2-h walk outsides, respectively. Compared with participants wearing the sham mask, the percentage change of nitrate in EBC was lower among those wearing the real mask. After the 2-h exposure, urinary MDA levels increased compared to the baseline in both trials. Real trial was lower than sham trial for 6 cytokines (i.e., IL-6, IL-10, IL-13, IL-17A, IFN-γ and TNF-α) in serum at 5-h post-exposure. Wearing facemasks on polluted days produced better improvement, however, on cleaner days, the improvement was weaker.

Conclusions: Short-term use of N95 facemasks appeared to effectively reduce the levels of lung function declines, the respiratory oxidative stress, and the systemic inflammation/oxidative stress which may be induced by short-term exposure to PM. Wearing facemasks on polluted days ($PM_{2.5} > 75$ μg/m^3) presented larger beneficial effects on the cardiopulmonary health than in clean days ($PM_{2.5} < 75$ μg/m^3).

1. Introduction

Ambient particulate matter (PM) had been well recognized as one of the leading risk factors of human health (Cohen et al., 2017). Currently, the concentrations of $PM_{2.5}$ (particles with aerodynamic diameters less than 2.5 μm) in most regions of the world exceed the guideline of the World Health Organization, especially in developing countries and regions. Therefore, it is very common to carry out personal protections to reduce the PM exposure. Wearing facemasks had been widely recommended by academic researchers and the department of public health as one of the practical solutions to minimize the adverse effects of air pollution (Allen and Barn, 2020; Cai and He, 2016; Carlsten et al., 2020; Rajagopalan Sanjay et al., 2020). While much attention had been put on the particle-removing efficiency of facemasks, the health effects of using facemasks on the cardiopulmonary health have not been fully evaluated.

There was evidence that wearing a N95 facemask for a few hours to days in real-world condition might improve the cardiopulmonary health, particularly in highly polluted circumstances. However, the results were inconsistent across different studies and only a few health outcomes have been investigated (Faridi et al., 2021; Guan et al., 2018;

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Langrish et al, 2009, 2012; Laumbach et al., 2014; Shakya et al., 2016; Shi et al., 2017; Yang et al., 2018). For example, two studies reported that wearing N95 facemasks for 48 h (worn as much as possible) was associated with reduced systolic blood pressure (SBP) (Langrish et al., 2009; Shi et al., 2017), but another study found no effect on SBP by wearing N95 facemasks for five days and 4 h per day (Yang et al., 2018). Only one interventional study on traffic policemen found that wearing a N95 facemask could offset potential decline in lung function caused by ambient air pollution exposure (Shakya et al., 2016). More evidence is needed regarding the effects on lung function of wearing facemasks, as consistent research reported a significant association between PM and lung function.

Inflammation and oxidative stress have been well recognized as the mechanisms that particles affected cardiopulmonary health, but the effects of wearing facemasks on pulmonary or systemic inflammation and oxidative stress were not fully investigated. In a double-blinded randomized crossover study, researchers found that wearing N95 facemasks for 2 h during peak traffic resulted in reductions in exhaled nitric oxide, multiple inflammatory cytokines in exhaled breath condensate (EBC), but no clear beneficial effects on endothelial function or systemic oxidative stress (Guan et al., 2018). Little evidence was provided about the effect of facemask-wearing on the systemic inflammation or oxidative stress.

Besides the beneficial effects of wearing facemasks during pollution days, there were some concerns about the potential adverse stress induced by wearing a facemask on cardiopulmonary system (Rajagopalan Sanjay et al., 2020). On one side, the available data to date suggested that N95 facemasks can effectively reduce $PM_{2.5}$ exposure in a manner that translates into cardiopulmonary health benefits. On the other side, wearing facemasks has some problems, including inducing heat, poor adherence, CO_2 build-up, discomfort, and respiratory resistance, resulting in some harmful impacts on cardiopulmonary health (Huang and Morawska, 2019). Study participants rated the N95 as the most protective due to sturdiness and fit, but also as uncomfortable and difficult to breathe through (Steinle et al., 2018). A study found that the N95 filtering facepiece respirator (FFR) dead-space partial pressure carbon dioxide levels were elevated (Roberge et al., 2010). Carbon dioxide can build up for a long time and cause drowsiness (Johnson, 2016). In addition, arguments had been made that these masks might worsen overall exposure by engendering a false sense of security (Huang and Morawska, 2019). Therefore, more data on the health responses deriving from the short-term facemask wearing are desiderated.

To comprehensively understand the health effects of facemasks on cardiopulmonary system, we conducted a double-blinded, randomized crossover trial (RCT) in a group of healthy young adults in Beijing China. We investigated the differences in the respiratory inflammation and oxidative stress, lung function, systemic inflammation and oxidative stress responses between participants wearing real and sham facemasks.

2. Methods

2.1. Study design and participants

The study was conducted at the campus of Peking University (PKU) in Beijing, China from December 2018 to April 2019. Fifty-two healthy, non-smoking volunteers were enrolled into the study. All subjects declared that they had no history of alcohol addiction nor diagnosed chronic cardiopulmonary diseases (including asthma, bronchitis, and rhinitis, etc.). None of them was on regular medication usage nor had symptoms of upper airway infection within 3 months. The volunteers lived in dormitories of two universities which were within 5 km. Thirty-one volunteers were from University of Science and Technology Beijing (USTB) and the others were from Peking University (PKU). The study was approved by the Institutional Review Board of the Ethics Committee of Peking University Health Sciences Center (IRB00001052-18071), and registered on Chinese Clinical Trial Registry (ChiCTR1800018628). Written informed consent was obtained from each of the participants during the enrollment.

To ensure the double-blinded and crossover design, each participant was asked to attend the study twice, with one time randomized to wear a reusable facemask (Respirator 3200; 3M, USA) installing a N95 filter (real trial) or not (sham trial). In the second trial, they wore a facemask with the filter installment opposite to the first trial (Guan et al., 2018). The facemask we chose consisted of a replaceable filter (3701CN; 3M, USA) which made the double-blind design feasible, and its filtration efficiency (92% for $PM_{2.5}$) had been tested in another study which was sufficient for our purpose. The body of the facemask was composed of soft silicone padding and louver-type enclosure that did not affect ventilation. The real facemask embedded filter membrane in the middle whereas the real facemask did not. The participants were instructed on how to wear the facemask in order to make the facemasks fitted their faces closely and comfortably. The appearance of the respirator and the material of the filter membrane were recorded in the supplementary material (Supplementary material: Fig. S8).

Fig. 1 showed the scheme of our study design. The trial visits were conducted in the Hospital of PKU, which was located where we conducted the 2-h outdoor exposure experiment. During each visit, the participants first went to the clinic for baseline measurement and biological sample collection (V0). Then, our investigators led them to the campus to walk slowly on a pre-defined (on-campus) road for 2 h wearing the customized facemask (real or sham). After the walking exposure, subjects returned to the clinic, took off the facemasks, and underwent the following three tests in 30-min (V1), 3-h (V2), and 5-h (V3) after the exposure (Fig. 1). All the clinical visits were implemented in a quiet exam room in the Hospital of PKU, and participants were not allowed to leave the room until all four visits had been completed. After a 2-week washout duration, each subject needed to go through the same study procedure but wore the facemask opposite to the first trial.

2.2. Air pollution measurement

Darta for air pollution and meteorological parameters were obtained from the Peking University Urban Atmosphere Environment Monitoring Station during the study period. The monitoring station was located on the roof of a six-story building on the campus of PKU where we conducted the exposure study. The station provided 1-min online data of $PM_{2.5}$ (TEOM, Model 1400, Thermo), sulfur dioxide (SO_2) (Model 43i-TL; Thermo), temperature, and relative humidity (Met One Instruments Inc., Grants Pass, OR, USA).

2.3. Lung function measurement

Lung function was measured using a hand-held portable spirometer (Spirolab New, MIR, Italy) following the ATS recommendation (Graham et al., 2019). The lung function parameters of interest for this study were forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC). Participants were instructed to inhale to maximum capacity (total lung capacity) in the standing position with nose clips, and then to exhale as fast and as long as possible. Calibrated reusable turbines and disposable mouthpieces were used for each participant to get the required parameters. Each participants competed at least five tests. The reproducibility criterion was that the difference between the two highest values was $\leq$150 mL for FEV_1 and $\leq$150 mL for FVC. We calculated the average values of eligible results for statistical analysis.

2.4. Biological samples collection

Exhaled breath condensate (EBC) was collected at all the four visits by using a commercially available device from RTube™ (Respiratory Research, Inc., US) following the recommended instruction (Horváth et al., 2005). Prior to each clinical visit, the aluminum cooling sleeves

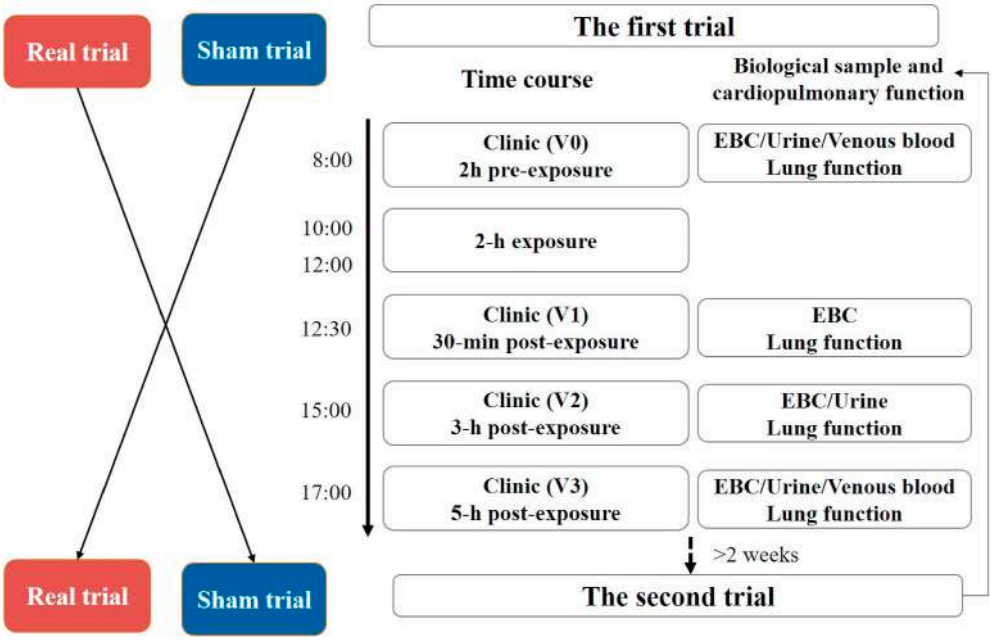

Fig. 1. Time course of health outcomes measurement.

were kept in a sealing bag and frozen in a −80 °C freezer for at least 2 h before transported to the hospital with dry ice in an insulation box. Subjects were asked to gargle for 3 times and wear nose clips before collection. During collection, the cooling sleeve was placed over the collection chamber and the subject was instructed to breath normally through the mouthpiece for 10 min. After each collection, around 1.5 mL EBC sample was collected using the plunger and sub-packed into centrifuge tubes instantly. At the start (V0) and the end (V3) of each trial, peripheral venous blood samples were drawn by a nurse using 6 mL evacuated and promoting coagulating tubes, and then centrifuged at 4000 r/min for 10 min to obtain serum samples after standing for 30-min. The serum samples were collected into 1.5 mL centrifuge tubes and stored at −80 °C within 30 min to minimize the in vitro changes. Urine samples (fractional) were collected in three times, i.e., V0, V2 and V3, and were stored at −80 °C for the future analysis.

2.5. Biomarkers analysis

EBC pH and nitrite/nitrate The pH value of EBC was measured on site using a pH meter (SevenCompact™, Mettler Toledo Inc.) after degassing the sample with argon stream. And the concentrations of nitrite in EBC were detected by an HPLC-UV (Model e2695, Waters, USA) with ION PAK Anion HC HPLC column (4.6*150 mm, WAT026770, Waters, US). Briefly, after the equilibration of the HPLC system with the Borate/Boric mobile phase (7.5 mM: 7.5 mM, pH 9.1), the concentrations of nitrite from the standards and the real EBC samples were detected at the wavelength of 214 nm by Photodiode Array (PDA) detector (Waters 2998, USA) with the flow rate of 0.8 mL/min. Besides, the concentrations of nitrate in EBC were determined by Ion Chromatography (Thermo Fisher, ICS-1100).

Urinary malondialdehyde (MDA) As the product of lipid peroxidation, we measured malondialdehyde from the urine samples as a biomarker of systemic oxidative stress. Malondialdehyde (MDA), released from its bound form(s) in urine by acid treatment, was measured as Thiobarbituric Acid derivative, using a High Performance Liquid Chromatography-UV detector (Model e2695, Waters, USA) according to a previously published paper (Lee et al., 2006). Briefly, 150 μL urine, 450 μL Thiobarbituric Acid (TBA) solutions, and 900 μL 0.5 mol/L phosphorous acid were added into a 1.5-mL centrifuge tube. The mixtures were incubated at 95 °C for 1-h, cooled in ice water for 5 min, followed by 5-min centrifugation (5000 g/min). The chromatographic column Nova-Pak (C18, 4 μm, 3.9*150 mm, Waters) was used to separate MDA in the mixtures, and the mobile phase was phosphate buffer (pH = 6.8) and methanol (60:40, V/V) with the flow rate of 0.8 mL/min. MDA was detected at the wavelength of 532 nm by Photodiode Array (PDA) detector (Waters 2998, USA). Concentrations of MDA were corrected by creatinine due to its highly potential influence by metabolism. Urinary creatinine levels were measured by a commercial kit (Jiancheng Bioengineering Institute, Nanjing, China).

Serum cytokines We used a commercially available analyzing kit (Human Cytokine/Chemokine Magnetic Bead Panel, Millipore Corporation, MA, USA) to detect 10 cytokines in serum samples (Knatten et al., 2014a). In short, 50 μl serum was firstly centrifuged at 13,000 g for 10 min. 25 μl of supernatant was collected to measure the concentrations of interleukin (IL)-1β, interleukin (IL)-2, interleukin (IL)-4, interleukin (IL)-6, interleukin (IL)-8, interleukin (IL)-10, interleukin (IL)-13, interleukin (IL)-17A, IFN-γ and TNF-α using Flex MAP 3D™ (Merck Millipore). Milliplex Analyst software (Merck Millipore, USA, version 5.1) was used to analyze median fluorescence intensity for each sample, and 3-parameter logistic regression and standard curve fitting methods were used to calculate the concentrations of cytokines in the samples (Hu et al., 2020; Knatten et al., 2014a). The limits of detection (LOD) for IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17A, IFN-γ and TNF-α were 0.8, 1.0, 4.5, 0.9, 0.4, 1.1, 1.3, 0.7, 0.8 and 0.7 pg/mL, respectively. For each cytokine, non-detectable values were replaced with half of the LOD. The detection rates for IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17A, IFN-γ and TNF-α were 10%, 15%, 70%, 59%, 99%, 98%, 94%, 99%, 99%. Due to the low detection rate for IL-1β and IL-2, they were excluded from the analysis.

2.6. Statistical analysis

We used linear mixed-effect model to examine the effects of N95 facemask on cardiopulmonary responses. The measured levels of all the health outcomes were logarithmic transformed due the skewed distribution. We used linear mixed-effects models to estimate the changes in health outcomes after the 2-h exposure at different post-exposure time points for two different trials (real and sham). We included in the model an interaction term between an indicator variable for wearing facemask ("1" for real mask and "0" for sham mask) and an indicator variable for

clinical visit ("V0", "V1", "V2", "V3"). Participants' age, sex, and body mass index were introduced as fixed effects. To account for the potential influence of clinical visit days, we also controlled for the 2-h average temperature and relative humidity. We included the urinary creatinine as a covariate when fitting the model for MDA to account for the dilution effect (Gong et al., 2013). Random intercepts for participants were included to account for the potential correlation in the repeated measurements of each participant. Based on the estimated coefficients of the interaction term, we calculated the percentage change (%) and 95% confidence intervals (CIs) from the baseline ("V0") at each of the following visit. We further evaluated the statistical significance of the difference, using the method of examining overlap, between the percentage (%) change at each visit in health outcome among those wearing real and sham masks (Schenker and Gentleman, 2001).

To examine whether the effects of wearing facemasks on cardiopulmonary responses depends on ambient air pollution, we classified all the person-visits into two trials based on the average concentration of $PM_{2.5}$ with a threshold of 75 μg/m^3, which is the 24-h mean concentration regulating level for $PM_{2.5}$ in China. We then conducted a sensitivity analysis using the same model to estimate the percent changes in health outcomes during polluted days ($PM_{2.5}$ > 75 μg/m^3) and clean days ($PM_{2.5}$ < 75 μg/m^3). All the analyses were conducted using R (Version 3.6.1; R Development Core Team).

3. Results

3.1. Characteristic information of the study participants

The characteristic information of the volunteers was shown in Table 1. We recruited 31 females and 21 males with a mean age of 20 ± 2 years. The overall average body mass index was 20 kg/m^2. According to the self-administrated questionnaire, all the participants remained healthy throughout the study period, and stayed within the central urban area of Beijing during the washout period. 48 out of the 52 participants completed two trials, and 4 participants finishing one trail (real trial). Therefore, we had 400 lung function measurements, 400 EBC samples, 300 urine samples, 200 blood samples.

3.2. Air pollution concentrations in all clinical visiting days

Fig. 2 showed the average air pollutant concentrations and meteorological parameters during the 2-h walks in all the clinic visiting days and in polluted and clean days. The average $PM_{2.5}$ concentrations were 75.8 μg/m^3 throughout the entire study period. The levels of $PM_{2.5}$ ranged from 4.9 to 254.8 μg/m^3. There were 8 days when the 2-h concentrations of $PM_{2.5}$ exceeded 75 μg/m^3 with an average of 127.7 ± 62.5 μg/m^3, and 9 days when the 2-h concentrations of $PM_{2.5}$ were below 75 μg/m^3 with an average of 21.70 ± 16.12 μg/m^3. During the whole study period, the mean environmental temperature and relative humidity were 9.84 °C and 26.14%, respectively, while in the 'polluted' days, the mean RH was 32.73% which was higher than that in the level of 'clean' days. The $PM_{2.5}$ and SO_2 concentrations 24 h before each visit were similar to those in the 2-h intervention-period (Supplementary material: Table S5). We conducted a paired *t*-test and found there was no significant differences in the concentrations of air pollutants between the 2-h period and the 24-h period in the previous day.

Table 1
Characteristics of the 52 study participants.

Gender	N	former smokers	Mean ± SD			
			Age (years)	Height (cm)	Weight (kg)	BMI(kg/m^2)
Male	21	1	19.71 ± 1.31	175.76 ± 4.65	67.33 ± 10.02	21.77 ± 2.95
Female	31	0	21.25 ± 2.34	162.23 ± 5.38	52.06 ± 6.43	19.75 ± 1.96
Total	52	1	20.63 ± 2.11	167.69 ± 8.39	58.23 ± 10.99	20.57 ± 2.58

3.3. Descriptive statistics of health outcomes

Table 2 showed the health endpoint levels of the four tests by different types of intervention, i.e., real trial versus sham. The trend of EBC nitrate was similar in both trials, increasing at 30-min post-exposure and then gradually decreasing to lower levels at 3-h, and 5-h post-exposure. Compared to the trial wearing sham facemasks, the mean concentration of nitrite was lower at 30-min, 3-h post-exposure in the trial of wearing real masks (120.37 vs 113.26, 172.62 vs 148.91, respectively). There were similar decreases at 5-h post-exposure in EBC pH values in two trials. There were slight increases in the levels of FVC and FEV_1 after 2-h walking. Compared to the baseline levels, the mean concentrations of serum IL-10, IL-13, IL-4, IL-17A became decreased at 5-h post-exposure in the trial wearing real masks, whereas the mean concentrations of those cytokines were higher in the trial wearing sham masks. The mean concentrations of IL-6, IL-8, TNF-α, IL-1β decreased at 5-h post-exposure in both trials.

3.4. Effects of facemasks on lung function

In the main analysis, we estimated the changes in each health endpoint after the 2-h walk at different post-exposure times by the intervention method (real vs sham). We found that FEV_1 was increased after the 2-h walk when wearing real facemasks, with increments by 2.05% (95%CI:0.27%–3.87%), 2.80% (95%CI:1.00%–4.63%) and 2.87% (95%CI: 1.07%–4.70%) at V1 (30-min), V2 (3-h) and V3 (5-h) after the exposure (Fig. 3). In contrast, there was no appreciable change in FEV_1 after the 2-h walk for the trial of wearing sham facemasks. It was notable that the increases in FEV_1 for the real-facemask trial at the three post-exposure times were larger than the changes for the sham one. Similar results were observed for FVC, i.e., FVC was increased from baseline by 2.40% (95%CI: 0.27%–4.58%), 3.21% (95%CI: 1.07%–5.4%) and 2.68% (95%CI: 0.55%–4.86%) in the real-facemask trial, whereas there were no notable differences in the percentage changes between the two trials at all the three post-exposure times.

We also performed the sensitivity analysis by dividing the observations into two trials, i.e., the 'polluted" days ($PM_{2.5}$ >75 μg/m^3) and the 'clean' days ($PM_{2.5}$ <75 μg/m^3). During 'clean' days, the improvements in FEV_1 were still appreciable at 30-min, 3-h, 5-h post-exposure in the trail wearing real facemasks, but the differences did not differ between two trials at 5-h post-exposure. (Supplementary material: Fig. S5). And during the 'polluted' days, there appeared to be different in the percentage changes in FVC between the two trials after the exposure (Supplementary material: Fig. S2).

3.5. Effects of facemasks on respiratory and systemic oxidative stress biomarkers

As shown in Fig. 4, EBC nitrate showed a downward trend at post-exposure compared to the baseline level in the trial wearing real facemasks, whereas EBC nitrate was gradually increased in the trial wearing sham facemasks. Differences in the changes of EBC nitrate were observed at 1-h post-exposure (−12.41%, 95%CI: −23.39% to −1.43%), 3-h post-exposure (−20.64%, 95%CI: −31.83% to −9.46%) and at 5-h post-exposure (−26.31%, 95%CI: −37.53% to −15.11%) between two trials. Regarding EBC nitrite and pH, there seemed no appreciable differences in their changes at three post-exposure times between the two trials (Fig. 4). After the 2-h exposure, the concentrations of urinary MDA increased compared to the baseline in both trials, but did not differ between the two trials (Fig. 4). During the 'polluted' days, we found that the increases in MDA in urinary from the baseline were higher in the real-facemask trial than the sham one at 3-h and 5-h post exposure

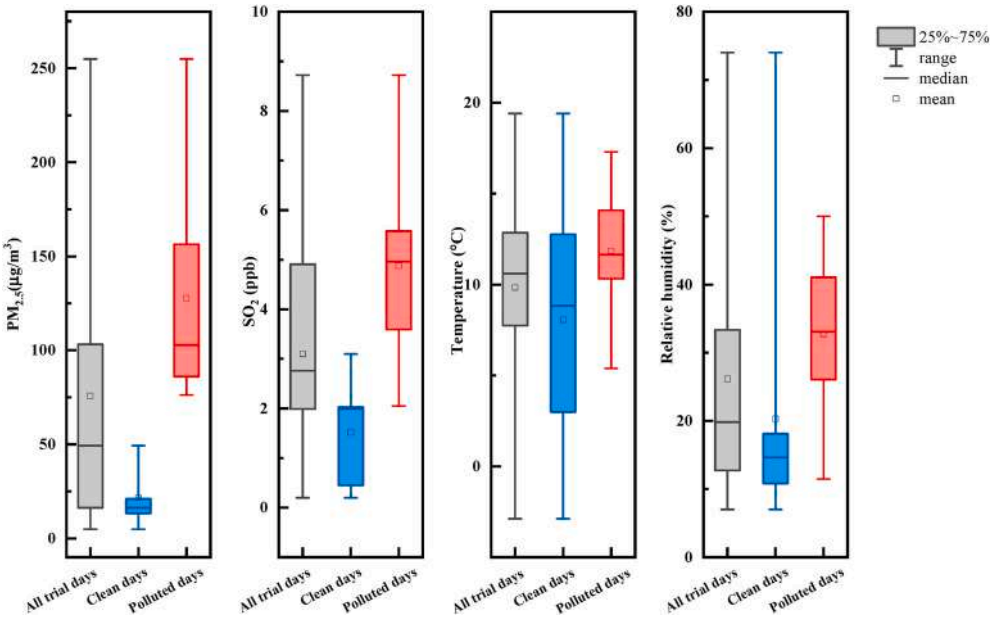

Fig. 2. Distribution of environmental $PM_{2.5}$, SO_2, temperature, and relative humidity during the 2-h exposure.

(Supplementary material: Fig. S3). And during the 'clean' days, the differences between the two trials were reversed, with the trial wearing sham facemasks showing higher increases from baseline (Supplementary material: Fig. S6).

3.6. Effects of facemasks on serum cytokines

At 5 h (V3) after the 2-h walk, we observed a clear decreasing trend in serum cytokines for the trial of wearing real facemasks, with eight of them showing reductions, namely, IL-6 (−18.96%, 95% CI: −34.28% to −0.06%), IL-8 (−7.08%, 95% CI: −16.31% to −3.17%), IL-10 (−27.61%, 95% CI: −37.30% to −16.43%), IL-13 (−12.29%, 95% CI: −22.11% to −1.22%), IL-17A (−17.12%, 95% CI: −23.17% to −10.6%) and TNF-α (−22.77%, 95% CI: −27.05% to −18.25%). In contrast, the levels of IL-13, IFN-γ for the group of wearing sham facemasks increased after the exposure. By comparing the changes in cytokines between the two trials, we found there were differences in percentage changes in 6 of the cytokines, including IL-6, IL-10, IL-13, IL-17A, IFN-γ and TNF-α (Fig. 5). In the sensitivity analysis of 'polluted' days, real trial was lower than sham trial for 6 cytokines (i.e., IL-6, IL-10, IL-13, IL-17A, TNF-α and IL-8) in serum at 5-h post-exposure and there was no notable difference of IFN-γ between two trials (Supplementary material: Table S2). The percentage change of IL-8 was higher in the trial wearing sham facemasks and there was no appreciable difference of IL-1β between two trials in the 'clean' days (Supplementary material: Table S3).

4. Discussion

A double-blinded randomized crossover trial was conducted to estimate the effects of wearing N95 facemasks on cardiopulmonary system of healthy young adults in China. After the 2-h walk wearing facemasks with real or sham filters, lung function parameters, and biomarkers for respiratory and systemic inflammation/oxidative stress have quadrupled from the baseline in 5-h after exposure. We observed differences in all the health outcomes between the two trials wearing sham and real facemasks.

We found notable increases in FEV_1 and FVC after 2-h walk wearing the real facemask, and differences in FEV_1 between the real and sham trials. Walking for 2 h with real facemasks was associated with an improvement in FEV_1 and FVC sustained up to 5 h. By contrast, in the trial wearing sham masks, there was no change in either parameter of lung function from the baseline. Although both trials experienced the beneficial effect of walking in terms of an improvement in FVC, the increase in the trial of wearing sham facemasks was lower than that in the real trial. This finding was consistent with another two RCT studies that were conducted in London. One reported attenuated increases in FEV_1 and FVC after walking along a busy street for 2 h compared to walking in the park for healthy elderly (Sinharay et al., 2018), and the other one reported reductions in FEV_1 by up to 6.1% and FVC by up to 5.4% after a 2-h walk on busy street (higher exposure), with reductions after walking in a park (lower exposure) smaller for the asthmatic subjects (McCreanor et al., 2007). In another interventional study, it was found that cyclists' FVC increased during riding in a low-traffic site (low level of air pollution), but decreased during riding in a high-traffic site (high level of air pollution) (Park et al., 2017). Another study conducted among children and adults demonstrated reducing indoor $PM_{2.5}$ may contribute to improved lung function by using an electrostatic air filter for 1 week (Weichenthal et al., 2013). The results mentioned above may suggest a beneficial effect on lung function induced by the reduction of particles exposed in a microenvironment, e.g., walking in a cleaner circumstance or using an indoor air purifier, while our study added more evidence to the beneficial effects on lung function when the inhaled PM was reduced by wearing N95 facemasks.

In addition, the beneficial effects associated with facemask intervention were strengthened when analyzing observations in the polluted days (Supplementary material: Figs. S2–S4). We found that the changes in FVC and FEV_1 in the trial of wearing sham facemasks at 30-min, 3-h and 5-h post-exposure were substantially attenuated, whereas the percentage change of FEV_1 in the trial wearing real facemask still increased at 30-min and 3-h post-exposure (Supplementary material: Fig. S2). Moreover, difference in the changes of FVC was observed between the two trials. We also found that, in days with the 2-h mean concentration of $PM_{2.5}$ smaller than 75 μg/m^3, the difference in lung function between the real facemask wearing trial and the sham facemask wearing trial narrowed, with differences in FEV_1 only occurring at 1-h and 5-h post-exposure (Supplementary material: Fig. S5). The results may suggest that a more beneficial effects on the lung function from the facemask wearing during heavily polluted days.

We observed differences in EBC nitrate, a biomarker of respiratory oxidative stress between the trials of sham and real facemask interventions. After the 2-h walk, the subjects wearing real facemasks showed a decreasing trend in EBC nitrate, while the subjects wearing

Table 2
Summary of health endpoints (mean ± SD) in sham-facemask group and real-facemask group during the intervention periods.

Endpoints	Trial	V0	V1	V2	V3
Respiratory inflammation					
EBC_nitrate (ng/mL)	Sham	358.99 ± 172.68	378.95 ± 374.94	332.54 ± 268.57	308.13 ± 239.91
	Real	328.17 ± 182.94	532.30 ± 951.70	439.18 ± 669.34	360.42 ± 292.35
EBC_nitrite (ng/mL)	Sham	123.32 ± 32.95	120.37 ± 54.22	172.62 ± 98.09	132.54 ± 42.66
	Real	127.65 ± 50.82	113.26 ± 34.06	148.91 ± 71.92	144.12 ± 72.62
EBC_pH	Sham	7.60 ± 0.48	7.49 ± 0.54	7.65 ± 0.43	7.79 ± 0.42
	Real	7.57 ± 0.50	7.56 ± 0.47	7.55 ± 0.51	7.74 ± 0.42
Lung function					
FVC (L)	Sham	3.63 ± 0.92	3.62 ± 0.84	3.66 ± 0.84	3.65 ± 0.82
	Real	3.56 ± 0.86	3.65 ± 0.87	3.67 ± 0.86	3.64 ± 0.83
FEV_1 (L)	Sham	3.25 ± 0.71	3.22 ± 0.66	3.26 ± 0.68	3.27 ± 0.65
	Real	3.17 ± 0.64	3.23 ± 0.66	3.25 ± 0.66	3.26 ± 0.66
Oxidative damage					
Urinary_MDA (μmol/L)	Sham	0.49 ± 0.30	–	0.41 ± 0.27	0.48 ± 0.41
	Real	0.50 ± 0.39	–	0.41 ± 0.29	0.39 ± 0.31
Creatinine (mol/L)	Sham	17.30 ± 9.04	–	8.25 ± 5.18	6.87 ± 5.66
	Real	15.08 ± 9.72	–	7.38 ± 4.87	5.40 ± 4.16
Blood Cytokines					
IFN-γ (pg/mL)	Sham	23.59 ± 14.59	–	–	28.10 ± 15.38
	Real	24.87 ± 15.49	–	–	25.85 ± 15.11
IL-10 (pg/mL)	Sham	4.98 ± 3.39	–	–	6.14 ± 0.80
	Real	7.56 ± 5.71	–	–	6.14 ± 0.80
IL-13 (pg/mL)	Sham	6.89 ± 9.95	–	–	8.20 ± 11.55
	Real	8.03 ± 10.86	–	–	7.62 ± 11.38
IL-17A (pg/mL)	Sham	6.87 ± 6.52	–	–	6.94 ± 6.72
	Real	7.95 ± 6.03	–	–	6.48 ± 4.47
IL-1β (pg/mL)	Sham	0.53 ± 0.52	–	–	0.43 ± 0.29
	Real	0.57 ± 0.45	–	–	0.43 ± 0.29
IL-2 (pg/mL)	Sham	0.47 ± 0.30	–	–	0.61 ± 0.35
	Real	0.99 ± 1.12	–	–	0.50 ± 0.32
IL-4 (pg/mL)	Sham	12.69 ± 14.17	–	–	12.95 ± 15.66
	Real	14.86 ± 21.59	–	–	12.95 ± 15.66
IL-6 (pg/mL)	Sham	4.63 ± 13.31	–	–	4.17 ± 11.51
	Real	4.99 ± 12.34	–	–	4.78 ± 12.97
IL-8 (pg/mL)	Sham	8.57 ± 6.13	–	–	7.64 ± 4.69
	Real	8.31 ± 5.25	–	–	7.87 ± 6.12
TNF-α(pg/mL)	Sham	4.41 ± 1.16	–	–	4.20 ± 1.18
	Real	4.95 ± 1.63	–	–	3.87 ± 1.3

Abbreviations:EBC_nitrite: nitrite in Exhaled Breath Condensate; EBC_nitrate: nitrate in Exhaled Breath Condensate; FVC: Forced Vital Capacity; FEV_1: Forced Expiratory Volume in 1s; Urinary_MDA: malondialdehyde in urinary.
V0: pre-exposure; V1: 30-min post-exposure; V2: 3-h post-exposure; V3: 5-h post-exposure.

sham facemasks showed an increasing trend (Fig. 4). Even though the changes in both trials at three post-exposure time points were not different from zero at $\alpha = 0.05$, we found there were notable differences between the two trials at 3- and 5-h post-exposure, with a higher level of EBC nitrate in the trial of wearing sham facemasks. These results may indicate that wearing a facemask while walking may be beneficial to human's respiratory oxidative stress condition since EBC nitrate has been treated as a relatively stable biomarker of oxidative stress, which had been positively associated with levels of air pollution in healthy adults (Zhang et al., 2013) and reflected the oxidative stress states of asthma and COPD patients (Corradi et al., 2003; Gessner et al., 2007). Unfortunately, we did not find appreciable difference in EBC nitrite between the two trials at either of the three post-exposure time points, even though we found the increases at the 3- and 5-h post-exposure points in both trials increased. The inconsistency in the results of EBC nitrate and nitrite may reflect the complexity of the health effects induced by the facemask. A study of subjects with and without tracheostomy showed that oropharyngeal bacteria chemically reduce salivary nitrates to nitrite, which contributes significantly to the concentration of nitrite in EBC collected by oral respiration (Marteus et al., 2005; Zetterquist et al., 2009). In our study, subjects fasted at baseline and before post-exposure EBC collections, and then all subjects were asked to eat a similar diet during the post-fasting meal. Controlling for food consumption and RCT designs may have mitigated any effects of oropharyngeal contamination, which may make our results more credible.

Results of the urinary MDA were consistent with EBC nitrate, which confirmed that wearing facemasks during walking would help to attenuate the oxidative stress induced by PM exposure. For all the visits after the intervention, we observed increases in urinary MDA from the baseline for both trials, but no notable differences between them. However, if we only considered the 'polluted' days ($PM_{2.5}$ >75 μg/m^3), there were differences between the two trial, with subjects wearing sham facemasks showed a higher level of urinary MDA increase. Notably, the increases in MDA post-exposure were higher for the real-intervention trial than the sham one during the 'clean' days ($PM_{2.5}$ <75 μg/m^3), which may suggest that the protective effect of the masks may be prominent on 'polluted' days as hinted in the results of lung function. This assumption may make sense since the particles exposed in the heavily 'polluted' days would generate considerable health effects, and if we take a facemask in these days, the removal of the particles would also be considerable enough to make health improvement to some extent. However, during a 'clean' day, since there were fewer effects induced by particle exposure, the removal of them would generate a less amount of benefit acquisition than in the 'polluted' days.

The current study also provided us evidence on the beneficial effects of wearing facemasks on the inflammatory response of cardiovascular system. We found that at 5-h after the 2-h walk outdoors, the serum levels of IL-13, TNF-α and IFN-γ in the trial of wearing sham facemasks increased, while IL-13, IL-6, IL-8, IL-10, IL-17A and TNF-α decreased for the trial of wearing real facemasks. And the changes in, IL-6, IL-10, IL-13, IL-17A, IFN-γand TNF-α were different between the two trials (Fig. 5). Similar results were observed in both 'polluted' and 'clean' days (Supplementary material: Fig. S2 -S7). Systemic inflammation has been proposed to be mainly responsible for the biological mechanism of $PM_{2.5}$ exposure on the cardiovascular health. Particles inhaled into the lung would cause pulmonary inflammatory response, produce a variety of cytokines and oxidative stress products into the circulatory system and trigger systemic inflammatory and oxidative stress (Brook Robert D. et al., 2010). And it was possible that the decreases in serum cytokines in the real-facemask trial and the differences between the two trials in the

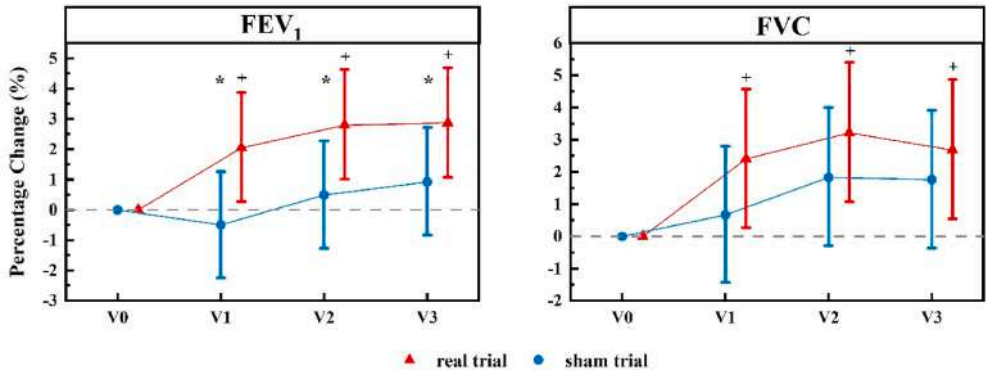

Fig. 3. Percentage Change in Lung Function from the Baseline at 30-min, 3-h and 5-h Post-exposure in the Sham Trial or Real Trial. +: Significantly different from baseline; *: Significantly different between the trial wearing real and sham facemasks.

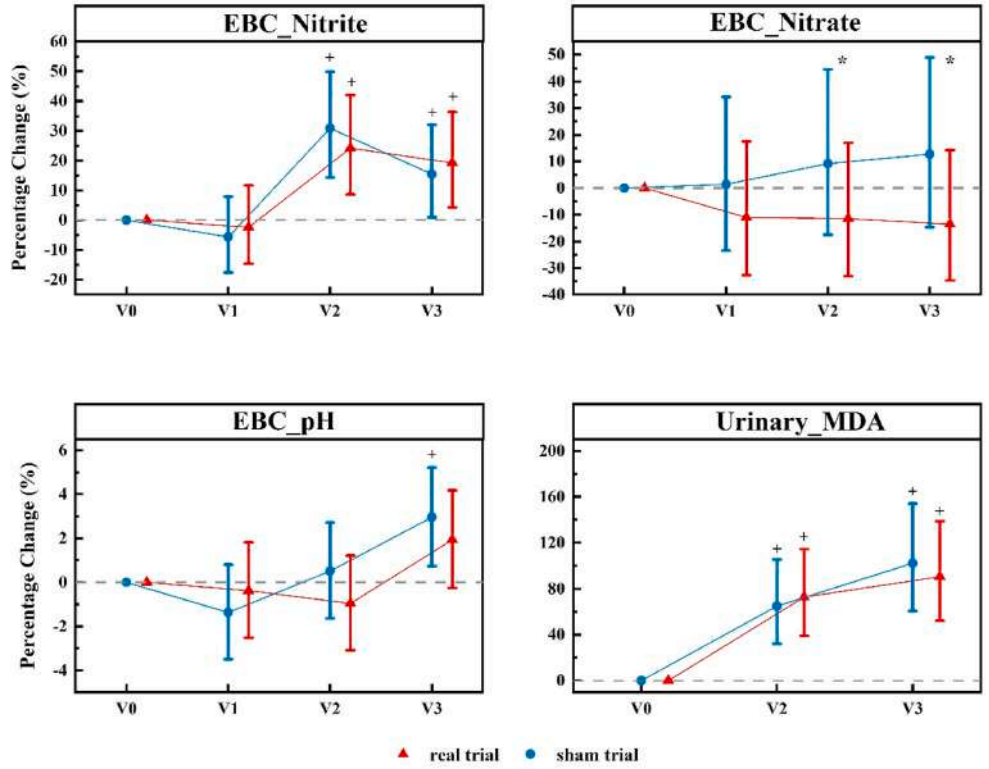

Fig. 4. Percentage Change in Respiratory and Systemic Oxidative Stress Biomarkers from the Baseline at 30-min, 3-h and 5-h Post-exposure in the Sham Trial or Real Trial, +: Significantly different from baseline; *: Significantly different between the trial wearing real and sham facemasks.

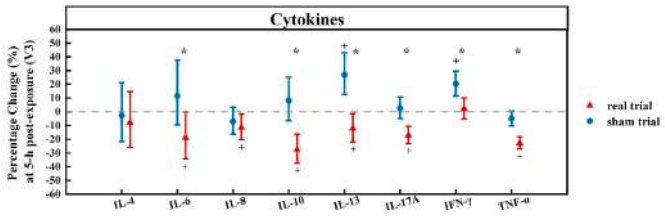

Fig. 5. Percentage Change in Serum Cytokines from the Baseline at 5-h post-exposure in the Sham Trial or Real Trial, +: Significantly different from baseline; *: Significantly different between the trial wearing real and sham facemasks.

current study were due to, at least partially, the intervention of facemask. Immunity and inflammation are closely related. Immunoregulation can be divided into pro-inflammatory regulation by Th17 cells and anti-inflammatory regulation by Treg cells, which play a role of secreting pro-inflammatory factors and anti-inflammatory factors respectively (Lane et al., 2010). Multiple inflammatory factors are needed to characterize the association between $PM_{2.5}$ exposure and inflammation. Many reports had shown that short-term exposure to PM increased the levels of pro-inflammatory cytokines including IL-6, TNF-α, IL-γ, and IL-8, IL-1, IL-4 and IL-6 in bronchial fluid, EBC, and blood (Corradi et al., 2010; Hu et al., 2020; Knatten et al., 2014b). Given the complex and intertwined nature of the inflammatory network (Sammour et al., 2010), the higher IL-10 and IL-13 (both anti-inflammatory cytokines) levels may be interpreted as increased immunosuppression or increased inflammatory activation by PM and facemask wearing. In another RCT study of facemask intervention, the

researchers observed that wearing N95 facemasks for 2 h during peak traffic resulted in reductions of multiple cytokines in exhaled breath condensate (Guan et al., 2018). Some evidence suggests that cytokine levels change only after days or weeks, not hours (Thompson et al., 2010; Törnqvist et al., 2007; van Eeden et al., 2001). Taken together, the current study provided evidence that a short-term wearing of a N95 facemask could generate beneficial effects on the systemic inflammation for at least 5-h after the exposure.

In summary, the current study utilized a double-blinded RCT design to provide molecular-level evidence on the health effects of wearing facemasks on cardiopulmonary system of young healthy adults in China. We may conclude that the facemasks appeared to reduce short-term PM-induced respiratory oxidative stress, the decline of lung function, and systemic inflammation/oxidative stress, and these beneficial effects of wearing facemasks on heavily 'polluted' days ($PM_{2.5}$ >75 μg/m^3) appeared to be more pronounced than in 'clean' days on lung function, systemic oxidative stress, and respiratory inflammation. Taken together, results from this interventional study demonstrated clear, albeit modest, cardiopulmonary benefits of short-term using facemasks in healthy adults. The use of facemasks offers individuals a feasible and affordable way to reduce exposure to hazardous air pollution in a highly-polluted circumstance, leading to significant public health benefits. Because our study participants were healthy young adults, one could reasonably expect different or even smaller cardiopulmonary benefits of air filtration among vulnerable populations, such as young children or elderly people. In addition, it is plausible that increased respiratory resistance and discomfort due to the wearing of facemasks might mitigate the potential health benefits resulting from the filtration of particles. Furthermore, the potential benefits from a longer intervention period could be expected and should be investigated. Additional trials are warranted to confirm or refute the protective effect of wearing facemasks, and healthcare providers may need to be cautious when recommending N95 facemasks to some susceptible subjects.

Some limitations of our study should be discussed. First, we did not consider potential face-seal leaks of the facemasks, which could be a penetration pathway for aerosol particles. Second, in part due to our participants were young healthy adults, the observed changes in certain biomarkers were fairly small. Thus, our results may not be applicable to other populations with different ages or disease status. More studies are needed to further evaluate potential health effects associated with wearing a personal protective equipment.

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CRediT authorship contribution statement

Meijie Jiang: Writing – original draft, Formal analysis, Investigation, Software, Visualization. **Xueling Meng:** Visualization. **Liang Qi:** Project administration. **Xinyan Hu:** Investigation. **Ruiwei Xu:** Investigation. **Meilin Yan:** Software. **Yunxiu Shi:** Investigation. **Xin Meng:** Investigation. **Weiju Li:** Project administration. **Yifan Xu:** Investigation. **Shiyi Chen:** Investigation. **Tong Zhu:** Conceptualization, Methodology. **Jicheng Gong:** Conceptualization, Methodology, Supervision, Project administration, Writing – review & editing.

Declaration of competing interest

All the authors declared no conflict of 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.2021.113806.

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Thyroid hormones in relation to polybrominated diphenyl ether and metals exposure among rural adult residents along the Yangtze River, China

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ABSTRACT

Although several studies indicate that exposure to polybrominated diphenyl ethers (PBDEs) and metals may influence thyroid function, the evidence is limited and inconsistent in general population. The current study was conducted to determine the levels of plasma PBDEs and urinary metals and evaluate the associations of co-exposure to both with thyroid hormones (THs) among rural adult residents along the Yangtze River, China. A total of 329 subjects were included in current analyses, and 8 PBDEs congeners and 14 urinary metals were measured to reflect the levels of environmental exposure. Multiple linear regression models were used to evaluate the association between PBDEs, metals and THs levels. Bayesian Kernel Machine Regression (BKMR) was used to examine PBDEs and metals mixtures in relation to THs. The geometric mean (GM) and 95% confidence interval (CI) of total measured PBDEs was 65.10 (59.96, 70.68) ng/g lipid weights (lw). BDE-209 was the most abundant congener, with a GM (95% CI) of 47.91 (42.95, 53.26) ng/g lw, accounting for 73.6% of the total PBDEs. Free thyroxine (FT4) was significantly negatively associated with BDE-28, 47, 99, 100, 154, and 183, and urinary strontium [β (95% CI): −0.04 (−0.07, −0.02)], but positively associated with selenium [β (95% CI): 0.04 (0.02, 0.06)]. Free triiodothyronine (FT3) was negatively associated with BDE-28 [β (95% CI): −0.03 (−0.05, −0.01)] and urinary arsenic [β (95% CI): −0.01 (−0.02, −0.001)]. The current study did not observe a statistically significant association of thyroid-stimulating hormone (TSH) with PBDEs and urinary metals. BKMR analyses showed similar trends when these chemicals were taken into consideration simultaneously. We found no significant interaction in the association between individual chemical at the 25th versus 75th percentiles and THs estimates, comparing the results when other chemicals were set at their 10th, 50th, and 90th percentile levels. Further study is required to confirm these findings and determine potential mechanisms.

1. Introduction

In hypothalamus–pituitary–thyroid (HPT) axis, the neuroendocrine cells of hypothalamus produce thyrotropin releasing hormone, and then promote the release and synthesis of thyroid stimulating hormone (TSH) and THs (i.e., thyroxine [T4] and triiodothyronine [T3]). Free thyroxine (FT4) and free triiodothyronine (FT3) are the free form of T4 and T3 respectively, accounting for less than 1% of T4 and T3, which have been used to assess thyroid function. It is a complicated regulation system of negative feedback that attempts to maintain the normal thyroid function and body metabolic homeostasis, including many proteins, enzymes, and carbohydrates metabolism (Foster et al., 2021; O'Kane et al., 2018). The disruption of HPT axis may result in various clinical or subclinical manifestations (Cooper and Biondi, 2012). It is clear that the HPT axis can be disrupted by exogenous environmental factors such as some persistent organic pollutants (e.g., polychlorinated biphenyls) and heavy metals (e.g., mercury) (Chen et al., 2013; Maervoet et al., 2007).

Polybrominated diphenyl ethers (PBDEs) are a class of synthetic

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flame retardants with similar chemical structure and properties to polychlorinated biphenyls, which were often used in various kinds of furniture, textiles, electronic products, and construction material. Historically, three PBDEs commercial products (i.e., penta-, octa- and deca-BDE) have been manufactured as major flame retardant mixtures (Bloom et al., 2008). Since they are semi-volatile and are not covalently bound to the consumer products in which they were incorporated, PBDEs were easily released into the surrounding environment. PBDEs are strongly lipophilic, previous studies showed that PBDEs were persistent and widely distributed in aquatic environment and bioaccumulated in various fish species (Kang et al., 2017). In addition, PBDEs may be a global pollutant that can be measured in samples taken from multiple sources such as indoor and outdoor air, marine sediments, human serum, urine, hair, nail, and breast milk (Ding et al., 2016; Kelly et al., 2008; Shi et al., 2013; Zhao et al., 2020a). Given that the potential environmental pollution and health effects of PBDEs, penta- and octa-BDE have been removed from the market. As for deca-BDE, dominated by BDE-209, its production and use remain fairly common in China. Unavoidably, because of the physical properties of the chemical, no matter how quickly PBDEs are phased out of use, PBDEs will continue to exist in the living animals and the environment for some time into the future.

The long term health effects of PBDEs exposure are an important potential concern. Several studies have investigated the potential association between PBDEs exposure and thyroid hormones (THs) during pregnancy, but there was no consistent association between THs and any BDE exposures (Chevrier et al., 2010; Makey et al., 2016; Stapleton et al., 2011; Vuong et al., 2015). Chen et al. suggested that BDE-209 exposure was positively associated with an increase in total thyroxine ($r = 0.270$, $p = 0.029$) among occupational workers from deca-BDE manufacturing plant (Chen et al., 2018). The result also showed that a 10-fold increase in the BDE-209 levels was related to an increase of 8.63 nmol/L (95% confidence interval: 0.93–16.30) in total thyroxine. However, a cross-sectional study conducted in 85 Alaska Native people found no significant association between serum PBDEs (including BDE-28/33, 47, 99, 100, 153, and 209) and either free or total thyroxine (Byrne et al., 2018). Another study conducted in 36 New York anglers, who represent a population with potentially increased dietary exposure to PBDEs, showed that there was no significant relationship of PBDEs (including BDE-28, 47, 66, 85, 99, 100, 138, 153, and 154) and thyroid function (including TSH, FT4, total thyroxine, and total triiodothyronine) (Bloom et al., 2008). However, Bloom et al. suggested that the sum of these PBDEs might be positively associated with FT4 when there was an approximately ninefold increase in sample size. It is still unclear whether PBDEs cause thyroid function disorders among general population, including those living in surrounding areas of the aquatic environment.

On the other hand, with the dramatic economic growth and increased industrialization in China, metal pollution has gradually become a health issue of great concern (Han et al., 2021; Li et al., 2013). Metals are ubiquitously dispersed in all natural environmental media, and its exposure will likely continue for a long time. People are exposed to metals mainly via intake of contaminated water and food, inhalation of polluted air, and direct contact with the skin. Cadmium and mercury have been extensively studied, because they are widely distributed in the environment and have been shown to be environmental endocrine disruptors (Castiello et al., 2020; Chen et al., 2013; Chung et al., 2019; Kim et al., 2021). However, the available evidence about the association between other metals and THs in general population is sparse and inconsistent, including arsenic and lead. For example, in National Health and Nutrition Examination Survey, there was no association between blood lead and TSH (Krieg, 2019). But another study included 5628 Chinese general population indicated that blood lead level was associated with increased TSH and hypothyroid status only in women (Nie et al., 2017). The potential mechanisms of THs inhibition might involve inhibiting the deiodination of T4, or competitive inhibition of binding of the hormones to their carrier protein (Rana, 2014). Of especial complexity with regard to metals is that their toxicity is not only related to the dose, but also related to their ability to change between their different chemical valency. Because the different metals are frequently found together in the environment, it is necessary to further investigate the potential impact of co-exposure to multiple metals on thyroid function.

PBDEs and metals are the industrial and technical grade products, whose pollution may coexist in the same environment. Nevertheless, whether simultaneous exposure to PBDEs and metals has a joint effect on thyroid function is unclear. In this study, we simultaneously investigated the association of PBDEs and metals exposure with THs in the general population living in rural areas along the Yangtze River, because they have more possibilities to be exposed to these chemicals bioaccumulated in aquatic products. Another aim was to investigate the interactions of PBDEs and metals exposure on THs levels.

2. Materials and methods

2.1. Study population

This study population forms part of the Wanjiang Cohort, which is located in sectional rural area along the Yangtze River. This cohort is designed to investigate the effect of long-term or chronic exposure to environmental pollutants on the health of adult residents along the Yangtze River (Cui et al., 2017). All participants were randomly recruited by local village doctors over the phone in 2019. All participants were required to be free of thyroid disease and thyroid medication use (based on self-report). The participants with a history of severe diseases in liver, kidney and gastrointestinal system were also excluded from current study. We also exclude those women that were in pregnancy and lactation because they had a higher basal metabolic rate and higher basal concentrations of THs during this period.

All study participants were invited to complete a face-to-face questionnaire once they agreed to participate in this study. The questionnaire information including demographic characteristics, lifestyle and behavioral habits (including smoking status, current alcohol-drinking, physical activity), personal disease history (including thyroid gland disease, head and neck tumors) and other medical history. The height and weight of participants was measured by a trained investigator and who was asked to report the weight of each participant to one decimal place. Then, all subjects were asked to provide a fasting blood and urine specimens in the second morning (between 7:00 a.m. and 8:30 a.m.) to minimize the influence of daily fluctuations in THs secretion. Finally, 329 participants were included in present analyses. This study was approved by the Ethics Committee of Anhui Medical University. All participants gave their informed consent.

2.2. Sample collection

All subjects were asked to fast for at least 8 h prior to collecting blood and urine specimens. All fasting samples were collected by medical staff from the local village clinic. Approximately 5 mL of blood was collected using two vacuum tubes (containing dipotassium ethylene diamine tetraacetate (EDTA-K_2) and anticoagulant-free), respectively. The serum and plasma were immediately separated after centrifugation. Approximately 0.5 mL of serum was sent to the laboratory within 1h for blood glucose and lipid testing. First morning urine specimen was collected from each participant by using a clean polyethylene centrifuge tube (approximately 50 mL). All biological specimens were stored at −80 °C within 3 h of collection until further analysis.

2.3. Plasma PBDEs measurement

The plasma specimens were measured for 8 PBDEs congeners (BDE-28, 47, 99, 100, 153, 154, 183 and 209), which are the flame retardant

mixtures mainly used commercially, using gas chromatograph-mass spectrometer at Anhui Medical University. The detailed detection method has been introduced elsewhere (Zhang et al., 2021). Briefly, we used internal standard substances (BDE-77 and 13C12-BDE-209, obtained respectively from Accustandard, USA and Cambridge Isotope Laboratories) and the mixture of formic acid and acetonitrile to pre-process plasma specimens. After the protein in the plasma had been denatured, this liquid system was extracted through a solid phase extraction column (Oasis© HLB-columns, 60mg/3 cc, Waters Corporation, USA) to remove the remaining lipids and other impurities. The PBDEs extracted from the column was eluted with dichloromethane, dried with nitrogen, and reconstituted with n-hexane. Finally, a quantified 50 μL of reconstituted solution containing PBDEs was prepared for instrumental testing.

All performance parameters of the instrumental testing had been successfully established in the preliminary test, including ion source, column flow, temperature programming, and scanning time. We established a standard substance (obtained from Accustandard, USA) curve in each experiment, and measured a blank specimen every 15 specimens, and used a spiked recovery method to ensure the stability of the experiment performance. The spiked recovery values ranged from 85.3% to 114.3%. The limit of detection (LOD) for PBDEs ranged from 0.03 mg/L (BDE-47) to 0.75 mg/L (BDE-209). The total lipid levels were calculated as described previous study for standardizing PBDEs concentrations in circulating blood (Guo et al., 2018b).

2.4. Urinary metals measurement

In this study, we measured 14 urinary metals levels and used them as predictive variables. Urinary levels of 13 metals [i.e., arsenic (As), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), lead (Pb), selenium (Se), strontium (Sr), zinc (Zn)] were determined using an inductively coupled plasma optical emission spectrometry (PerkinElmer Optima 7000DV, USA) at Anhui Medical University. The measurements of 13 metals were based on accordingly 13 sets of calibration standards in nitric acid (HNO_3) with internal standards. In order to carry out the chemical analysis on the urine samples, 3 mL of urine from each participant was diluted with 9 mL of 5% HNO_3 (guarantee reagent, GR). Following mixing, the resulting solution was microwave-digested at 90 °C for 1 h. After digestion, the resulting solution was centrifuged with 4500 r/min for 8 min and the liquid supernatant was extracted for subsequent analysis. Metallic cadmium (Cd) level was measured using a graphite furnace atomic absorption spectrometry (GFAAS, Analytik Jena AG ZEEnit®700P, Germany). The HNO_3 was added to raw urine specimens in order to acidify the sample. 1% diammonium hydrogen phosphate was added to the urine sample as a matrix modifier. In the conduct of test, one reference standard was used for every 10 test samples analyzed and the spiked recovery method was used to ensure the instrument performance. The spiked recovery values for 14 metals ranged from 92.4% to 105.2%. The LOD for 14 metals ranged from 0.04 μg/L (Mg) to 4 μg/L(Se). Urinary creatinine concentrations were measured using alkaline picric acid spectrophotometric for standardizing urinary metals. The detection reagent was using a commercial kit (Jiancheng 135 Bioengineering Ltd. Nanjing, China).

2.5. Serum thyroid hormones and urinary iodine measurement

Serum concentrations of FT3, FT4, and TSH were determined at Anhui Medical University using Roche Cobas e 411 analyzer and its manufacturer's reagents and calibrators (Roche Diagnostics GmbH, Mannheim, Germany). This was a fully automated electrochemiluminescence immunoassay process. It was required to establish an accurate standard curve and quality control testing based on the kit instructions before specimen measurement. The assay reference ranges of normal value for FT3, FT4, and TSH were (3.1–6.8) pmol/L, (12–22) pmol/L, and (0.27–4.20) uIU/mL, respectively. In addition, we also determined the urinary iodine levels because it is an important covariate in affecting thyroid function. Urinary iodine was determined by As^{3+}–Ce^{4+} catalytic spectrophotometry using a commercial kit (Wuhan Zhongsheng Biochemical Technique Co., Ltd, Wuhan, China).

2.6. Statistical analysis

Descriptive statistics were used to present the characteristics of study population, individual PBDE congeners and urinary metals, THs concentration. The specimen concentration below the LOD was assigned a value of LOD divided by the square root of two. Σ_8PBDEs was defined as the sum of eight congeners of PBDEs. The concentrations of PBDEs congeners, urinary metals and THs were natural-log (ln) transformed to decrease the effect of outliers and meet normal distribution. Spearman rank-order correlation was used to evaluate the correlations among PBDEs, urinary metals, and THs.

Multiple linear regression models were used to evaluate the association of THs concentrations with PBDEs and urinary metals, adjusted for covariates of age, gender, body mass index (BMI), education, smoking status, current alcohol-drinking, and urinary iodine/creatinine ratio, in which urinary iodine levels were natural-log (ln) transformed. For metals exposure, each metal was included in the single-metal models separately. We additionally conducted multiple-metal models considering all statistically significant metal in single-metal models to explore the simultaneous effects of co-exposure to multiple metals on THs. Partial correlation analyses for relationships of THs with PBDEs and metals were performed with the same covariates used in multiple linear regression models. In order to explore the potential threshold for effect or dose response relationship, we categorized exposures into tertiles, with first tertile as the referent group, to conduct a linear regression. Based on the β-coefficient in regression models, we calculated the percent change in THs levels between different tertiles by [exp (β)-1] × 100%. According to the normal reference ranges of TSH, logistic regression was used to evaluate odds ratios (OR) and 95% confidence intervals (CI) for high TSH (>4.20 uIU/mL) in relation with PBDEs and metals exposure. The trend test was performed in regression models using exposure tertiles as continuous variables.

Further, we used Bayesian Kernel Machine Regression (BKMR) to estimate the association of PBDEs and metals mixtures with THs, exploring the potential relationships and interactions between mixtures components (Bobb et al., 2015, 2018). BKMR facilitates exposure–response functions by using Bayesian variable selection and improves inference of correlated chemical mixture, and permits the visualization of exposure–response association. To avoid the data might not be able to distinguish among multiple chemicals in the mixture that were highly correlated, we included those PBDEs and metals with significant *p*-values in single models into BKMR models. In the analyses, the PBDEs and metals levels were natural-log (ln) transformed and then z-score normalized. Exposure–response functions were applied to examine the association between each individual chemical concentration and THs while holding other chemicals at median levels. Potential interactions within mixtures through evaluating the change in FT3 and FT4 estimate comparing individual chemical at its 25th to 75th percentiles levels, while setting other chemical at their 10th, 50th or 90th percentile levels.

In subgroup analysis, we ran the models of correlation analysis among men and women respectively. Given that thyroid function may be influenced by menopausal status among women, we additionally adjusted the menopausal status in the analysis of female subgroup. All analyses were performed using R software (version 3.3.1). All *p* values were tested in two-sided, $p < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of study participants

The characteristics of 329 study participants were shown in Table 1. The age of all participants ranged from 18 to 83, with mean age of 52.5 years. Among these participants, the majority of them were female (78.7%), were low educational level (66.3%), and were not smoking (85.1%) and not drinking (79.6%). Based on the calculation formula of total lipid, the median of total lipid was 5.5 g/L, with the interquartile range of 2.0 g/L. According to the reference ranges of normal value, there were 43 participants had abnormal concentrations of FT4 (6 participants with FT4 < 12 pmol/L and 37 participants with FT4 > 22 pmol/L), but only one participants had FT3 concentrations > 6.8 pmol/L, and 79 participants had TSH concentrations > 4.20 uIU/mL. The comparison of thyroid hormone levels according to general characteristics was showed in Supplementary Table S1.

3.2. Plasma PBDEs, urinary metals, and THs concentrations

The concentrations of plasma PBDEs, urinary metals, and THs were presented in Table 2. The geometric mean (GM) and 95% CI of $\sum_8$PBDEs was 65.10 (59.96, 70.68) ng/g lipid, where BDE-209 accounted for 73.6%. The congener with highest concentration in plasma was BDE-209 [GM (95% CI): 47.91 (42.95, 53.26) ng/g lipid]. Current PBDEs congeners were significantly correlated with each other, and the Spearman rank-order correlation was presented in Supplementary Table S2. The weakly significant correlation was observed between 14 urinary metals, correlation coefficients ranged from 0.115 to 0.480 (Supplementary Table S3). In addition, FT3 was statistically significantly associated with BMI (r = 0.115), education (r = 0.174) and alcohol-drinking (r = 0.152). FT4 was statistically significantly associated with education (r = 0.138) and alcohol-drinking (r = 0.160).

Table 1
Description of general characteristics for study population.

Variables	Mean ± SD or n (%) or IQR
Age, years	52.5 ± 12.3
Gender	
Male	70 (21.3)
Female	259 (78.7)
Body mass index, kg/m^2	
<24.0	173 (52.6)
24.0–27.9	120 (36.5)
≥28.0	36 (10.9)
Education	
Primary school	218 (66.3)
Junior high school	84 (25.5)
High school or above	27 (8.2)
Average annual household income, RMB	
< 30000	121 (36.8)
30000-59999	118 (35.9)
≥ 60000	90 (27.4)
Sleeping time, hours/day	
< 6	39 (11.9)
6-8	156 (47.4)
≥ 8	134 (40.7)
Smoking status	
Never	280 (85.1)
Ever	49 (14.9)
Current alcohol-drinking	
No	262 (79.6)
Yes	67 (20.4)
Physical activity	
Inactive	233 (70.8)
Active	96 (29.2)
Fasting blood-glucose, mmol/L	5.2 (4.8–5.6)
Total cholesterol, mmol/L	4.1 (2.4–5.0)
Triglycerides, mmol/L	2.0 (1.2–4.0)
Urinary iodine/creatinine ratio, μg/g	227.1 (153.9–309.5)

SD, standard deviation; IQR, inter-quartile range.

Table 2
The content of PBDEs and urinary metals and thyroid hormone levels from study population.

	Geometric mean (95% CI)	Percentile				
		5th	25th	50th	75th	95th
PBDEs (ng/g lipid)						
BDE-28	1.49 (1.39, 1.60)	0.47	0.98	1.55	2.24	4.08
BDE-47	0.96 (0.85, 1.07)	0.13	0.54	1.13	1.95	3.40
BDE-99	1.16 (1.05, 1.29)	0.27	0.59	1.03	1.90	6.76
BDE-100	2.04 (1.89, 2.20)	0.54	1.42	2.21	3.25	5.25
BDE-153	1.20 (1.11, 1.31)	0.35	0.75	1.19	1.82	4.69
BDE-154	1.73 (1.56, 1.93)	0.25	0.89	1.80	3.81	7.02
BDE-183	2.15 (2.00, 2.32)	0.65	1.39	2.07	3.42	6.87
BDE-209	47.91 (42.95, 53.26)	6.14	29.66	55.03	95.14	201.96
$\sum_8$PBDEs[a]	65.10 (59.96, 70.68)	16.52	40.39	66.50	111.40	228.92
Metals[b]						
As	12.62 (10.38, 15.35)	0.94	2.09	22.43	43.75	166.71
Cd	1.62 (1.45, 1.81)	0.21	0.94	1.91	2.98	6.45
Co	1.79 (1.43, 2.25)	0.09	0.23	2.05	10.76	32.37
Cr	35.06 (30.05, 40.91)	1.32	28.14	48.79	65.78	125.55
Cu	9.17 (8.38, 10.04)	1.74	6.78	11.07	13.72	23.62
Fe	24.89 (19.15, 32.37)	0.08	23.32	50.20	93.97	299.71
Li	14.56 (13.31, 15.93)	2.22	12.11	17.83	20.89	37.96
Mg	39.69 (30.37, 51.89)	0.04	39.91	78.14	134.66	270.47
Mn	1.38 (1.16, 1.65)	0.07	0.50	2.10	4.35	13.52
Mo	95.34 (85.41, 106.42)	23.35	56.41	106.13	158.77	433.28
Pb	22.38 (18.77, 26.68)	0.79	10.92	36.84	65.56	148.65
Se	111.03 (99.73, 123.61)	12.23	83.67	139.38	195.54	374.28
Sr	138.90 (125.96, 152.78)	35.87	92.97	165.48	198.92	493.40
Zn	184.93 (143.37, 238.56)	0.20	231.07	380.10	523.32	924.92
Thyroid hormones						
FT3, pmol/L	5.09 (5.02, 5.17)	4.19	4.64	5.05	5.56	6.56
FT4, pmol/L	17.46 (17.11, 17.83)	13.19	15.44	17.16	19.26	25.41
TSH, μIU/mL	2.78 (2.61, 2.96)	1.10	1.90	2.81	4.09	7.19

CI, confidence interval.
[a] Sum of 8 congeners of PBDEs, including BDE-28, -47, −99, −100, −153, −154, −183, and −209.
[b] The concentration of Mg was presented as mg/g creatinine and others were presented as ug/g creatinine.

3.3. Chemical exposures and thyroid hormones

3.3.1. Plasma PBDEs and thyroid hormones

As shown in Supplementary Table S4, the inverse associations were

observed between FT3 and BDE-28, as well as between FT4 and BDE-28, 47, 99, 100, and 183. There was no significant association between TSH and PBDEs. In multiple linear regression analyses, after adjusting for age, gender, BMI, education, smoking status, current alcohol-drinking, and urinary iodine/creatinine ratio, only BDE-28 was still negatively associated with FT3 [β (95% CI): −0.03 (−0.05, −0.01)] (Table 3). The reduced FT4 was associated with increased BDE-28, 47, 99, 100, and 183. In another dose-response relationship pattern, the decreased percent changes in FT3 and FT4 still had significant linear trend based on increased levels of these PBDEs congeners (all $p_{-trend} < 0.05$) (Fig. 1). According to the normal reference ranges of TSH, logistic regression was used to estimate the OR (95% CI) for high TSH (>4.20 uIU/mL) in relation with PBDEs and metals exposure. However, there was still no significant association between plasma PBDEs and TSH levels (Supplementary Table S5).

3.3.2. Urinary metals and thyroid hormones

In single-metal models, we observed an inverse association of FT3 with metal As, Cr, Fe, and Sr levels, while no association between urinary metals and TSH (Table 4). The result of partial correlation analyses showed similar trends (Supplementary Table S4). In multiple-metal models, we only observed As was associated with decreased FT3 [adjusted β (95% CI): −0.01 (−0.02, −0.001)] in all study population (Table 5). FT4 was positively associated with Se levels [adjusted β (95% CI): 0.04 (0.02, 0.06)] and negatively associated with Sr levels [adjusted β (95% CI): −0.04 (−0.07, −0.02)]. Similarly, a significantly reduced percent changes in FT3 and FT4 were presented between FT3 and the highest tertile of As [percent changes (95% CI): −3.92% (−7.60%, −0.20%), $p_{-trend} = 0.023$]; and between FT4 and the highest tertile of Sr [percent changes (95% CI): −8.33% (−12.98%, −3.54%), $p_{-trend} = 0.001$] (Fig. 1). However, there was still no significant association between urinary metals and TSH levels in logistic regression model (Supplementary Table S5).

3.4. Interaction and subgroup analysis

In BKMR analyses, univariate exposure–response function still observed an inverse association of FT3 with As and BDE-28 (Supplementary Fig. S1), the relationships of FT4 with Se, Sr, and several PBDEs were presented in Supplementary Fig. S2. In exploring the figures for potential interactions, bivariate exposure-response functions for every two chemicals showed no apparent differences in relationships of analyzed chemical with FT3 and FT4 estimates, where all of the other chemicals were fixed at median levels (Supplementary Fig. S3 and Supplementary Fig. S4). Similarly, no significant interactions were observed for any individual chemical with the remaining chemicals on FT3 and FT4 estimates (Fig. 2).

In subgroup analysis, there still were significant associations between the presence of some PBDEs congeners and FT3 and FT4 among women (Supplementary Fig. S5). Men in the highest tertiles of BDE-99 and BDE-100 had significantly reduced percent changes in FT3 [percent changes (95% CI): −6.67% (−12.37%, −0.70%), $p_{-trend} = 0.030$] and FT4 [percent changes (95% CI): −11.57% (−19.83%, −2.37%), $p_{-trend} = 0.017$] compared to men in first tertile, respectively (data not shown).

For urinary metals analysis, male FT3 and FT4 were inversely associated with metal Mn [Tertile 3 (T3) *vs*. Tertile 1 (T1), percent changes (95% CI): −8.79% (−14.96%, −2.18%), $p_{-trend} = 0.011$] and Sr [T3 *vs*. T1, percent changes (95% CI): −12.63% (−20.86%, −3.54%), $p_{-trend} = 0.008$), respectively. In addition, we found a significantly inverse association between metal Li and FT3 [T3 *vs*. T1, percent changes (95% CI): −4.3% (−8.06%, −0.3%), $p_{-trend} = 0.045$], and FT4 [T3 *vs*. T1, percent changes (95% CI): −7.5% (−12.8%, −1.88%), $p_{-trend} = 0.013$] among women (Supplementary Fig. S6). When we performed multiple-metal models only among female population, a significantly positive association was presented between FT3 and Se levels ($p_{-trend} = 0.001$). FT4 was negatively associated with Fe ($p_{-trend} = 0.039$) and Sr ($p_{-trend} = 0.006$), and simultaneously positively associated with Se ($p_{-trend} = 0.001$) among women (data not shown).

4. Discussion

In order to compare with other similar studies, the PBDEs congeners and urinary metals levels and its association with THs from general populations around the world were summarized in Supplementary Table S6 and Supplementary Table S7. However, in previously published studies, we only found limited information on the association of blood PBDEs and urinary metals levels with THs in the general population. A recent review presented that the concentrations of PBDEs in soil elsewhere in the world were similar to that in sectional remote areas in China (Jiang et al., 2019). This similar result was also observed in our study population. Obviously, a comparison with the study published by Huang et al. (2014) suggested that the current PBDEs concentrations in rural adult residents along the Yangtze River were likely higher than that of other general population in China during 2010–2011. This means that PBDEs pollution in China started late, but it becomes a significant concern. For metals burden, it will be a persistent health problem as long as there is industrial development. In Supplementary Table S7, we observed that the levels of Cd, As, and Pb in our study population were higher than those in other regions.

After adjusting for age, gender, BMI and so on, FT3 was found to be significantly negatively associated with As and BDE-28; and FT4 was negatively associated with Sr and most PBDEs congeners (including BDE-28, 47, 99, 100, 154, and 183), but positively associated with Se in our current study. Although these low-brominated PBDEs have been

Table 3
Associations between PBDE (ng/g lipid) and thyroid hormones levels among all study population [β (95% CI)].

PBDEs[a]	ln FT3		ln FT4		ln TSH	
	Unadjusted	Adjusted[b]	Unadjusted	Adjusted[b]	Unadjusted	Adjusted[b]
BDE-28	−0.03 (−0.05, −0.01)*	−0.03 (−0.05, −0.01)*	−0.05 (−0.08, −0.02)*	−0.05 (−0.08, −0.02)*	0.02 (−0.09,0.10)	0.01 (−0.09, 0.11)
BDE-47	−0.02 (−0.03, −0.01)*	−0.01 (−0.03, 0.01)	−0.02 (−0.04, −0.01)*	−0.02 (−0.04, −0.01)*	0.01 (−0.06,0.07)	0.01 (−0.05, 0.07)
BDE-99	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.01)	−0.02 (−0.04, 0.01)	−0.02 (−0.05, −0.01)*	−0.02 (−0.08, 0.05)	−0.04 (−0.07, 0.06)
BDE-100	−0.01 (−0.02, 0.02)	0.01 (−0.02, 0.02)	−0.03 (−0.06, −0.01)*	−0.03 (−0.06, −0.01)*	0.02 (−0.07, 0.11)	0.02 (−0.07, 0.11)
BDE-153	0.02 (−0.01, 0.04)	0.01 (−0.01, 0.03)	0.01 (−0.01, 0.04)	0.01 (−0.02, 0.03)	−0.04 (−0.12, 0.04)	−0.01 (−0.10, 0.07)
BDE-154	−0.01 (−0.03, 0.01)	−0.01 (−0.02, 0.01)	−0.02 (−0.04, 0.01)	−0.01 (−0.04, 0.01)	−0.05 (−0.11, 0.02)	−0.04 (−0.11, 0.02)
BDE-183	−0.02 (−0.04, 0.01)	−0.02 (−0.04, 0.01)	−0.03 (−0.06, 0.01)	−0.03 (−0.06, −0.01)*	−0.02 (−0.11, 0.08)	−0.01 (−0.10, 0.10)
BDE-209	−0.01 (−0.03, 0.01)	−0.01 (−0.03, 0.01)	−0.02 (−0.04, 0.01)	−0.01 (−0.04, 0.01)	−0.01 (−0.08, 0.05)	−0.02 (−0.08, 0.05)
$\sum_8$PBDEs[c]	−0.02 (−0.04, −0.01)*	−0.02 (−0.03, 0.01)	−0.03 (−0.06, −0.01)*	−0.03 (−0.05, 0.01)	−0.02 (−0.10, 0.06)	−0.02 (−0.11, 0.06)

*$p < 0.05$.
[a] PBDE concentrations were natural-log (ln) transformed.
[b] Adjusted for age, gender, body mass index, education, smoking status, current alcohol-drinking, and urinary iodine/creatinine ratio.
[c] Sum of 8 congeners of PBDEs, including BDE-28, -47, −99, −100, −153, −154, −183, and −209.

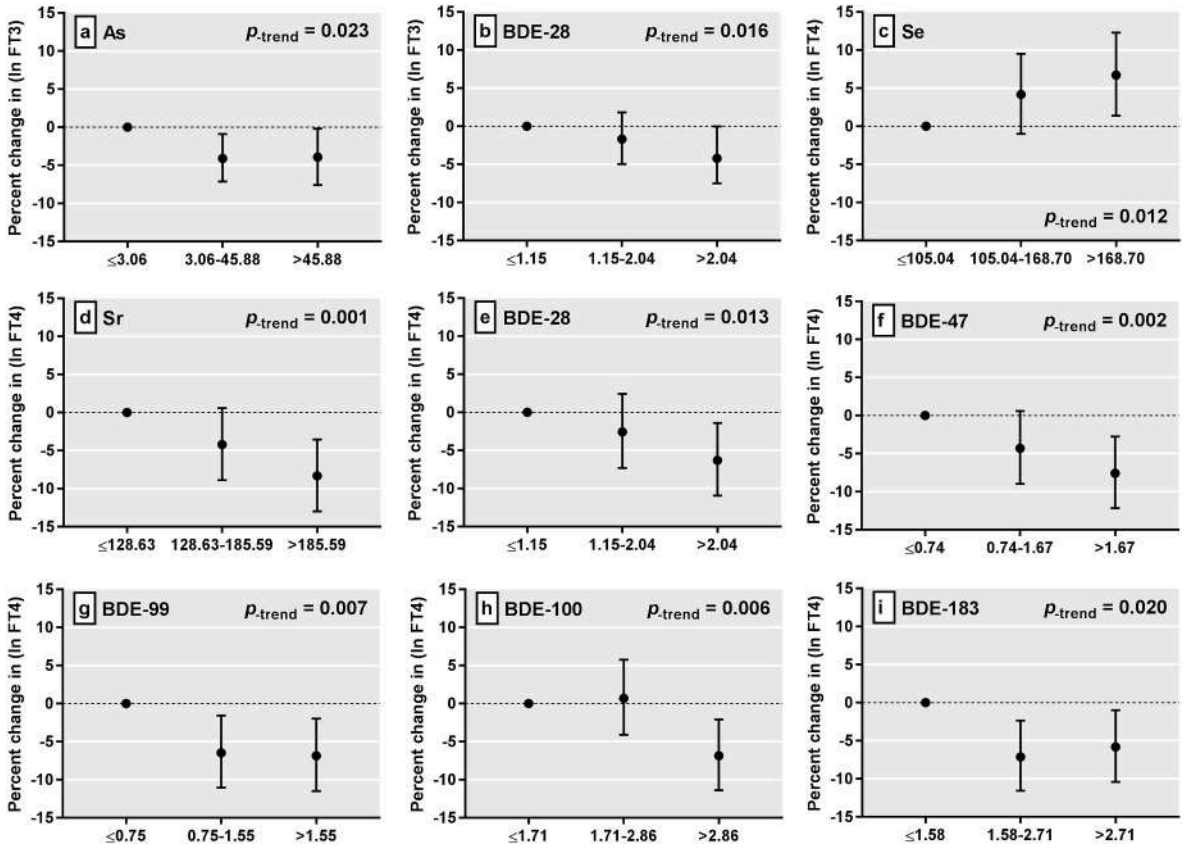

Fig. 1. Adjusted percent changes (%) and 95% confidence intervals for associations of urinary metals and PBDEs tertiles with thyroid hormones levels among all study population. The tertile of As (a), BDE-28 (b), Se (c), Sr (d), BDE-28 (e), BDE-47 (f), BDE-99 (g), BDE-100 (h), and BDE-183 (i) was presented on the x–coordinate (using the lowest tertile as reference). Units: metals (ug/g creatinine), PBDEs (ng/g lipid), FT3 (pmol/L), FT4 (pmol/L), and TSH (uIU/mL). All models adjusted for age, gender, body mass index, education, smoking status, current alcohol-drinking, and urinary iodine/creatinine ratio.

Table 4
Associations between urinary metals and thyroid hormones levels from all study population in single-metal models [β (95% CI)].

Metals[a]	ln FT3		ln FT4		ln TSH	
	Unadjusted	Adjusted[b]	Unadjusted	Adjusted[b]	Unadjusted	Adjusted[b]
As	−0.01 (−0.02, −0.003)*	−0.01 (−0.02, −0.001)*	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.01)	−0.02 (−0.05, 0.02)	−0.02 (−0.05, 0.02)
Cd	0.01 (−0.01, 0.02)	0.01 (−0.01, 0.02)	0.01 (−0.01, 0.03)	0.02 (−0.01, 0.04)	−0.03 (−0.09, 0.03)	−0.02 (−0.09, 0.04)
Co	−0.007 (−0.01, −0.01)*	−0.005 (−0.01, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.03, 0.03)	0.001 (−0.03, 0.03)
Cr	−0.004 (−0.01, 0.01)	−0.01 (−0.02, −0.001)*	−0.01 (−0.02, 0.01)	−0.01 (−0.03, 0.01)	−0.01 (−0.05, 0.04)	−0.01 (−0.06, 0.04)
Cu	−0.01 (−0.03, 0.01)	−0.01 (−0.03, 0.01)	−0.01 (−0.04, 0.01)	−0.01 (−0.04, 0.01)	0.01 (−0.06, 0.09)	0.02 (−0.06, 0.10)
Fe	−0.002 (−0.01, 0.01)	−0.006 (−0.01, −0.001)*	−0.005 (−0.01, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.03, 0.02)	−0.01 (−0.04, 0.02)
Li	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.03, 0.02)	−0.01 (−0.03, 0.02)	0.02 (−0.06, 0.09)	0.01 (−0.07, 0.09)
Mg	0.001 (−0.01, 0.01)	−0.003 (−0.01, 0.01)	−0.004 (−0.01, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.04, 0.02)	−0.01 (−0.04, 0.02)
Mn	0.002 (−0.01, 0.01)	0.001 (−0.01, 0.01)	0.003 (−0.01, 0.02)	0.003 (−0.01, 0.02)	−0.01 (−0.05, 0.03)	−0.01 (−0.05, 0.04)
Mo	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.01)	−0.02 (−0.04, 0.01)	−0.02 (−0.04, 0.01)	0.05 (−0.01, 0.11)	0.05 (−0.01, 0.12)
Pb	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.002)	−0.01 (−0.02, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.05, 0.03)	−0.01 (−0.04, 0.04)
Se	0.02 (0.003, 0.03)*	0.01 (−0.01, 0.02)	0.03 (0.01, 0.05)*	0.03 (0.01, 0.05)*	−0.02 (−0.08, 0.05)	−0.01 (−0.07, 0.06)
Sr	−0.02 (−0.03, 0.01)	−0.02 (−0.03, −0.001)*	−0.03 (−0.05, −0.01)*	−0.03 (−0.05, −0.004)*	0.001 (−0.07, 0.07)	−0.01 (−0.09, 0.06)
Zn	0.002 (−0.01, 0.01)	−0.003 (−0.01, 0.01)	−0.003 (−0.01, 0.01)	−0.01 (−0.02, 0.01)	−0.01 (−0.04, 0.01)	−0.01 (−0.04, 0.02)

*$p < 0.05$.
[a] Urinary metals concentrations were natural-log (ln) transformed. The concentration of Mg was presented as mg/g creatinine and others were presented as ug/g creatinine.
[b] Adjusted for age, gender, body mass index, education, smoking status, current alcohol-drinking, and urinary iodine/creatinine ratio.

phased out, their health effects of long-term exposure in the environment continue to exist. BDE-47 and BDE-153 were reported to have a heavier body burden in various human specimens in Western countries (Bradman et al., 2007; Darrow et al., 2017). However, this burden was different from that of Chinese population, as shown in Supplementary Table S6, possibly caused by different usage patterns of PBDEs congeners. Besides, low-brominated PBDEs may be derived from deca-BDE through debromination (Law et al., 2014). For example, BDE-209, a major deca-BDE, has photochemically unstable feature which can form tetra-to nona-BDE via debromination (Su et al., 2014).Unfortunately, deca-BDE still has a broad application market in China, and those products with low-bromination PBDEs are still being widely used (Ji et al., 2017). Similarly, metals are ubiquitously dispersed in all natural environmental media and metals pollution is gradually becoming a

Table 5
Associations between urinary metals and thyroid hormones levels from all study population in multiple-metal models [β (95% CI)].

Thyroid hormones	Metals[a]	Unadjusted	Adjusted[b]
ln FT3	As	−0.01 (−0.02, −0.002)*	−0.01 (−0.02, −0.001)*
	Cr	−0.003 (−0.02, 0.01)	−0.01 (−0.02, 0.01)
	Fe	0.001 (−0.01, 0.01)	−0.003 (−0.01, 0.004)
	Sr	−0.01 (−0.03, 0.01)	−0.01 (−0.03, 0.01)
ln FT4	Se	0.05 (0.02, 0.07)*	0.04 (0.02, 0.06)*
	Sr	−0.05 (−0.07, −0.02)*	−0.04 (−0.07, −0.02)*

*$p < 0.05$.
[a] Urinary metals concentrations were natural-log (ln) transformed. The concentration of metals was presented as mg/g creatinine.
[b] Adjusted for age, gender, body mass index, education, smoking status, current alcohol-drinking, and urinary iodine/creatinine ratio.

serious health concern. Therefore, these residents living around rivers and lakes should pay more attention to the health effects of exposure to environmental pollutants, including thyroid function.

Since PBDEs congeners structurally resemble T4 and T3, it had been proven that they had thyroid toxicity and neurotoxic effects (Dong et al., 2021; Xiong et al., 2019). Oulhote et al. analyzed the data of 2007–2009 Canadian Health Measures Survey found that the elevated prevalence of hypothyroidism was significantly related to increased concentrations of BDE-47 and BDE-100 (Oulhote et al., 2016). Another study among 308 adult male sport fish consumers suggested that serum PBDEs levels were related with increased T4 (Turyk et al., 2008). In deca-BDE manufacturing workers, it had been reported that serum BDE-209 was positively associated with T4 ($r = 0.270$, $p = 0.029$), and a 10-fold increase in the BDE-209 levels was related to an increase of 8.63 nmol/L (95% CI: 0.93–16.30) in T4 (Chen et al., 2018). In addition, several studies performed in electronic and electric wastes (e-waste) dismantling areas and its surrounding areas also found similar thyroid toxicity, particularly e-waste recyclers (Guo et al., 2018b; Wang et al., 2019; Zhao et al., 2020b). China is a big economic and trade country, simultaneously numerous e-waste were imported or dumped from developed countries. It can be inferred to be the main source of PBDEs pollution in the environment (Sthiannopkao and Wong, 2013). In this study, we did not found BDE-209 was significantly associated with THs, although BDE-209 had the highest concentration in our study population. This phenomenon might be related to the properties of BDE-209, including large molecular size, extreme hydrophobicity and low bioavailability. Previous study had shown that BDE-209 mainly accumulated in adrenals, kidneys, and liver, while lipophilic tissues were the preferred sites for other congeners bioaccumulation (Linares et al., 2015). Furthermore, only a low systemic toxicity of BDE-209 could be detected in the case of long-term chronic exposure, and its NOAEL (No-Observed-Adverse-Effect-Level) values for liver and thyroid gland respectively were 1120 mg/kg b.w. and 2550 mg/kg b.w. (Fromme et al., 2014). Given the pervasive exposure to PBDEs, the persistence and bioaccumulation of it in the environment, more studies are needed to reveal its toxic dose and mechanism of action.

However, the accurate mechanism of effects of PBDEs on thyroid function still remains unclear. The potential mechanism was that PBDEs interfered with the transport and metabolism of THs. First, it has been proven that several hydroxylated PBDEs congeners could be used as THs dependent transcriptional agonists (Ren et al., 2013). In fluorescence competitive binding assay, the different levels of PBDEs congeners and its hydroxylated component (HO-PBDEs) could impede the transcription mediated by THs receptors (TR), but the activities of HO-PBDEs on TR (agonistic or antagonistic) depend on their geometric structure during binding (Li et al., 2010; Ren et al., 2013). Second, PBDEs congeners and its metabolites compete with binding of thyroxine transport proteins (including transthyretin and thyroxine-binding globulin) and directly interfere with the concentrations of FT3 and FT4 (Cao et al., 2010; Guo et al., 2018b). Third, PBDEs congeners and HO-PBDEs may directly deactivate the iodothyronine deiodinases and inhibite the deiodination of T4 (Ren and Guo, 2012; Roberts et al., 2015). In addition, serum PBDEs congeners, including BDE-99, 100, 154, and 209, were reportedly associated with liver impairment (Sun et al., 2020; Zhao et al., 2020b). The potential mechanisms that might be involved in the toxicity of PBDEs potentially could include abnormal metabolism of glycolipids, increased oxidative stress, impairment of mitochondrial function, as well as induction of inflammatory cytokines (Zhao et al., 2020b). Obviously, the liver impairment can easily affect the sulfotransferase bioactivity and deiodination of thyroid hormone deiodinase 1 and 2 in the liver.

The evidence about associations between urinary metals and thyroid function among general population was sparse. Most studies have assessed the concentrations of metals in blood with the focus being on workers in at risk occupations rather than in the general population. Urine samples are a convenient non-invasive biomonitoring media for body's metabolites and used more frequently in epidemiological studies. Urinary excessive metals also reflect the disruption of homeostasis (Wu et al., 2018). In current study, we observed urinary arsenic was inversely associated with FT3. This result was similar to that in Guo et al. study investigated relationship between blood metals and THs among pregnant (Guo et al., 2018a). Arsenic is ubiquitously dispersed in food, soil, water and air, and its pollution sources include natural sources and anthropogenic sources, such as volcanoes, coal power plants, incineration of waste, and pesticide use (Ciarrocca et al., 2012). Contaminated drinking water is the main route of human exposure to excessive arsenic

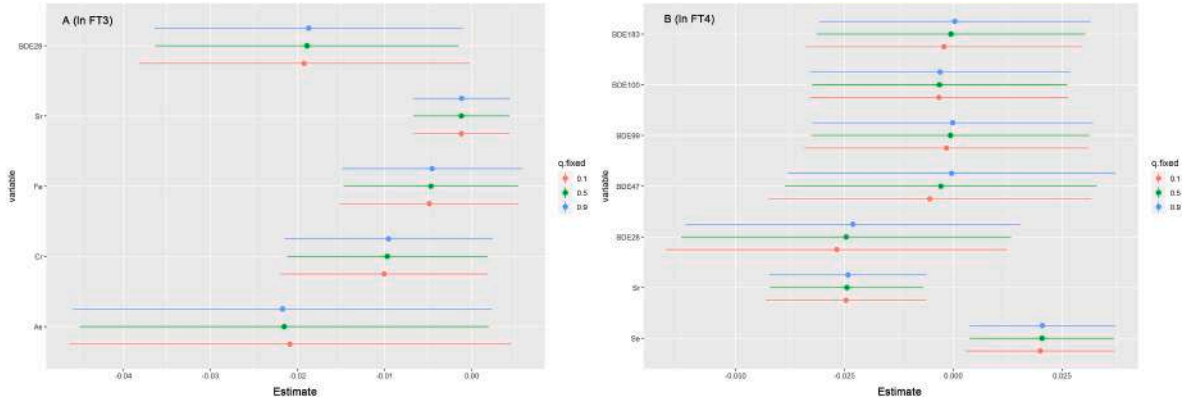

Fig. 2. Effects (estimates and 95% confidence intervals) of each chemical on FT3 (A) and FT4 (B). This figure indicated the relative effects of a single chemical at the 75th versus 25th percentile, while remaining chemicals were set at their 10th, 50th, or 90th percentile levels, respectively. All models were adjusted for age, gender, body mass index, education, smoking status, current alcohol-drinking, and urinary iodine/creatinine ratio.

(Davey et al., 2008). Arsenic was considered an endocrine disruptor and had been reported to alter some hormone receptor-mediated gene regulation, such as glucocorticoid receptor, retinoic acid receptor, and TR (Davey et al., 2008). Specifically, arsenic could disrupt THs homeostasis by acting on the transcription of TR related genes, including type I deiodinase gene (Davey et al., 2008; Sun et al., 2016a). For example, arsenic concentrations at < 150 μg/L disturbed the THs homeostasis of bighead carp larvae by increasing the thyroxine levels and reducing TR mRNA transcriptional levels (Sun et al., 2016b). In addition, the increased arsenic trioxide level was significantly associated with inhibition for vitro thyroid peroxidase activity, and the minimal dose required to inhibit this activity was between 0.1 and 1 ppm (Palazzolo and Jansen, 2008).

As we all know, selenium is an important element for synthesis of THs, and the thyroid gland has the highest selenium content in human organs (Kohrle, 2015). It has been reported that selenium supplementation (80 μg or 200 μg/day as sodium selenite or selenomethionine respectively) was effective against Hashimoto's thyroiditis and reducing thyroid peroxidase autoantibody concentration at 3 months (Toulis et al., 2010). Those pregnant women with thyroid-peroxidase-antibody positive easily suffer from post-partum hypothyroidism, but the risk of post-partum hypothyroidism were notably reduced when those women were treated 200 μg/day selenomethionine (Negro et al., 2007). However, for those with adequate-to-high selenium, excessive intake of selenium supplementation might be adversely affected, which could cause selenosis (Rayman, 2012). In current study, there was a positive association between selenium and FT4 levels. This can involve the contribution of selenium to thyroid function. Glutathione peroxidase rich in selenium has strong antioxidant properties, which is very important for resisting the damage of excess hydrogen peroxide and its reactive oxygen intermediates during THs synthesis. In addition, some selenoenzymes also could regulate the transformation of T4 to T3 (Broberg et al., 2011). Therefore, it can be assumed that selenium should have a negative correlation with T4 (Broberg et al., 2011). At this time, the thyroid gland may be stimulated by TSH to secrete more T4 due to the normal negative feedback regulation of thyroid system. Subsequently, the FT4 concentration is increasing.

For strontium, it is well known as calcium-sensing receptor (CaSR) agonists, Sr^{+2} ions seem to be able to replace Ca^{+2} ions. The experiment study suggested that strontium biased CaSR signaling toward extracellular signal-regulated kinases 1 and 2 (ERK1/2) signaling and the potency of strontium-stimulated calcitonin secretion was higher than calcium (Thomsen et al., 2012). Calcitonin secreted by the thyroid gland can regulate the body calcium balance, and this process is stimulated by the combination of T3, T4 and tyrosine during the secretion calcitonin. However, it is not clear whether strontium in turn affects thyroid function in producing T3 and T4. Although we found urinary strontium level was significantly negatively associated with FT4 in this study, this association and its potential mechanism need to be further confirmed.

To the best of our knowledge, this study is the first to evaluate the simultaneous effects of co-exposure to PBDEs and metals on THs levels in the general population and to investigate the interactions of PBDEs and metals exposure on THs. In order to acquire an exact risk association, we conducted multivariate models and subgroup analyses to adjust some common covariates, such as age, gender, smoking status, and an additional adjustment for menopausal status in the female subgroup. However, this study has several limitations. First, this study is cross-sectional study with a small sample size extracted from community cohort. This study only reveals a phenomenon and does not represent the causality. Second, the single determination of PBDEs and urinary metals may not be accurately exposure status. Multiple determinations will be beneficial to reflect long-term and chronic exposure levels. Third, the urine sample is not the preferred choice for the measurement of certain metal such as iron and lead, although urine metals levels have been used to explore the correlation with various diseases including hypertension, metabolic syndrome, cardiovascular diseases and kidney function (Domingo-Relloso et al., 2019; Wu et al., 2018; Xu et al., 2021; Yang et al., 2019). The interpretation of associations between our urinary metals and THs should be cautious, and these associations warrant further investigation.

5. Conclusions

In current cohort of rural communities along the Yangtze River, we measured the human body burden of PBDEs and metals and their associations with THs. Compared with the general population in other study areas, the burden of PBDEs and some heavy metals (i.e., Cd, As, and Pb) levels seems to be gradually accumulating among the rural residents along the Yangtze River. In addition, FT3 was significantly negatively associated with As and BDE-28; and FT4 was significantly negatively associated with Sr and multiple PBDEs congeners (i.e., BDE-28, 47, 99, 100, 154, and 183), but positively associated with Se levels. Besides, there was no statistically significant association of TSH with plasma PBDEs and urinary metals in current study. Our results indicated that the potential effects of PBDEs and metals exposure on thyroid function should raise concern, and more measures are needed to reduce the release of these pollutants in environment. However, further studies are necessary to investigate these associations and to illuminate the potential mechanisms.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Declaration of competing interest

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

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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.2021.113800.

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Urinary neonicotinoids level among pregnant women in Japan

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ABSTRACT

Neonicotinoids (NEOs) are the most important globally available class of chemical insecticides since the introduction of synthetic pyrethroids. The adverse effects of NEOs for early development have been reported via in vivo and epidemiological studies. Therefore, prenatal NEOs exposure is highly concerning. This study aimed to determine the level of NEOs exposure during daily life among pregnant women in Japan, as well as the sources of exposure. Spot urine samples were collected during the first, second, and third trimesters from 109 pregnant women who delivered their infants at obstetrics and gynecology clinics in Kumamoto city, Japan, between 2014 and 2016. Additional data were obtained from medical records and self-administered questionnaires. thiamethoxam and clothianidin (CLO) were detected in most participants (83.4% and 80.9%, respectively), and at higher concentrations than those in other areas of Japan. Multiple logistic regression analysis showed a statistical significant association of pulses in CLO (1.01 [1.00–1.02]). In conclusion, pregnant women in Japan appear to be exposed to NEOs in their daily lives, and pulses intake may be a source of NEOs exposure. These findings may further the assessment of human NEOs exposure risk.

1. Introduction

Neonicotinoids (NEOs) are the most important chemical class of insecticides introduced to the global market since the introduction of synthetic pyrethroids. NEOs are registered in more than 120 countries and are considered the most effective class of insecticides for controlling sucking insects (Jeschke et al., 2011). NEOs that have been demonstrated to provide excellent biological control while remaining safe for both humans and the environment are used extensively worldwide for crop protection, and account for approximately one-fourth of the global insecticide market (Tomizawa and Casida, 2011). Accordingly, seven NEOs, namely acetamiprid (ACE), imidacloprid (IMI), thiacloprid (THI), clothianidin (CLO), dinotefuran (DIN), thiamethoxam (TMX), and nitenpyram (NTP), are specifically used in many Japanese prefectures (Harada et al., 2016; National Institute for Environmental Studies, 2019). The total shipment amounts of these NEOs have increased by almost 7 times in 2017 relative to the amounts reported in 1996 (National Institute for Environmental Studies, 2019).

The NEOs exposure during early development is an issue of great concern. In vivo studies of mice showed that IMI exposure during the early developmental period (gestational day 4 to postnatal day 21) induces long-lasting changes in behavior and brain function (Buker et al., 2018). Consistent with this observation, studies conducted in the United States have reported associations of residential proximity to NEOs agricultural use with the incidence of tetralogy of Fallot and anencephaly (Carmichael et al., 2014; Yang et al., 2014). Furthermore,

Abbreviations: BMI, body mass index; FFQ, food frequency questionnaire; IS, internal standard; LOD, limit of detection; LOQ, limit of quantitation; SD, standard deviation; SPE, solid-phase extraction; NEOs, Neonicotinoids; ACE, acetamiprid; IMI, imidacloprid; THI, thiacloprid; CLO, clothianidin; DIN, dinotefuran; TMX, thiamethoxam; NTP, nitenpyram; nAChRs, nicotinic acetylcholine receptors; OPs, organophosphates; NACE, acetamiprid-N-desmethyl; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; PesUse, pesticide use; PesNoUse, non-pesticide use.

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proximity to agricultural sources of IMI during pregnancy have been associated with an increased risk of neural tube defects (Rull et al., 2006; Yang et al., 2014) and a reduction in IQ (Gunier et al., 2017). Ling et al. reported that first or second trimester exposure to pesticides, including IMI, or exposure to 2 or more pesticides from three chemical classes (organophosphates [OPs], pyrethroids, and carbamates), was associated with a small increase (3–7%) in the risk of preterm birth (Ling et al., 2018). It is therefore necessary to determine the human exposure levels to NEOs, especially in pregnant women. To the best of our knowledge, only one study has assessed NEOs exposure levels in pregnant women. However, the sample size and population characteristics of that study were very limited (30 pregnant women living in agricultural areas in Spain) (López-García et al., 2017). Moreover, few studies have assessed NEOs exposure levels using biological samples collected in Japan.

Previously, Ueyama et al. developed biomonitoring methods for the assessment of urinary NEOs levels, leading to the determination of these levels in some Japanese studies (Osaka et al., 2016; Ueyama et al., 2014, 2015). One such study analyzed changes in the levels of exposure to NEOs and OPs during daily life by quantifying these chemicals in urine samples collected from 95 adult women between 1994 and 2011 (Ueyama et al., 2015). Other studies also analyzed the levels of NEOs exposure among 703 healthy 3-year-old children (Osaka et al., 2016) and 52 adults (41 males and 11 females) who worked at two companies located in Aichi prefecture (Ueyama et al., 2014). However, no study has assessed NEOs exposure in pregnant women in Japan. Therefore, we aimed to determine the levels of exposure to 6 NEOs (ACE, IMI, THI, TMX, CLO, and DIN) and acetamiprid-N-desmethyl (NACE), a metabolite of ACE, during daily life among pregnant women in Japan, and to examine the sources of exposure.

2. Materials and methods

2.1. Study participants and sampling

Local residents of Kumamoto prefecture in Japan who eventually delivered their infants at obstetrics and gynecology clinics in Kumamoto city between 2014 and 2016 had been asked to donate spot urine specimens during health checkups in the first, second, and third trimesters of pregnancy. A total of 109 participants were enrolled. We could not obtain urine samples from 4 participants during the second trimester or from 9 participants during third trimester. Therefore, we analyzed a total of 314 urine samples (109, 105, and 100 during the first, second, and third trimesters, respectively). Basic maternal information (age, weight, height) was obtained from medical records and used to calculate the maternal body mass index (BMI). Information of pesticide use was obtained via a self-administered questionnaire after delivery. Food intake were assessed using a validated food frequency questionnaire (FFQ) with 138 food items, developed by National Cancer Center, Japan (Sasaki et al., 2003b, 2003c; Tsubono et al., 1996), which was administered during the second trimester of pregnancy. The demographic characteristics of the 109 pregnant women in this study are presented in Table 1.

This study was conducted after receiving approval from the Ethics Committees of Kumamoto University Faculty of Life Sciences on February 5, 2013 (Approval No. 628) and after obtaining written informed consent from all participants.

Table 1
Demographic data of the participants at the first health checkup visit (n = 109).

Variable	Mean	Standard Deviation	Min.	Max.
Age (years)	30.8	4.6	20.0	40.0
Height (cm)	158.5	4.9	148.0	175.2
Weight (kg)	52.4	8.0	39.0	80.0
BMI (kg/m^2)	20.9	3.0	15.9	33.7

BMI; body mass index.

2.2. Analytical method

The urine specimens were stored at −40 °C and later analyzed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). ACE, IMI, THI, TMX, CLO, DIN (purity >99%), formic acid (purity > 99%), acetic acid (purity > 99.5%), and 1 mol/L ammonium acetate (purity >28%) were obtained from Wako Pure Chemical Industries (Osaka, Japan). Acetamiprid-d6 (purity >99.7%) was obtained from Hayashi Pure Chemical Ind. (Osaka, Japan). Imidacloprid-d4 (purity >99.9%) and NACE (purity >99.8%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Phosphoric acid (purity >85%), methanol (purity >99.8%), acetonitrile (purity >99.8%), and ammonium solution (ultrapure reagent) were obtained from Kanto Chemical Co., Inc (Tokyo, Japan). A polymetric strong cation exchange solid-phase extraction (SPE) product, Bond Elut PCX (30 mg) (Agilent Technologies, Inc., Santa Clara, CA, USA), was used to extract NEOs from urine.

The urinary concentrations of NEOs were measured according to previously reported methods (Osaka et al., 2016; Ueyama et al., 2014, 2015). Briefly, LC-MS/MS was performed on an Agilent 1200 Infinity LC coupled with an Agilent 6470 Triple Quadrupole LC/MS System (Agilent Technologies, Inc., Santa Clara, CA, USA). The LC operating conditions were as follows: LC column, ZORBAX Eclipse Plus, C18, 2.1 × 100 mm, 1.8 μm, 600 Bar (Agilent Technologies, Inc., Santa Clara, CA, USA); mobile phase, (A) H_2O containing 17 mmol/L acetic acid and 5 mmol/L ammonium acetate, and (B) acetonitrile containing 17 mmol/L acetic acid and 5 mmol/L ammonium acetate; total flow rate of mobile phase, 0.2 ml/min; total run time including equilibration, 10 min. The initial mobile phase composition was 98% mobile phase A and 2% mobile phase B. The percentage of mobile phase B was changed linearly over 2 min to 5%, and changed linearly to 70% over the next 2 min. This percentage was maintained for 3 min, after which the mobile phase composition was allowed to return to the initial conditions and equilibrate for 3 min. The injection volume was 3 μl.

MS/MS was performed using an Agilent Jet Stream electrospray ionization (AJS ESI, Agilent Technologies, Inc., Santa Clara, CA, USA) source in the positive ion mode with multiple reaction monitoring. The nebulizer gas pressure was set at 35 psi with a source temperature of 325 °C and gas flow of 10 L/min. The capillary voltage was 4000 V (positive mode). The raw chromatograph and mass spectrogram data were processed using MassHunter Workstation software (Agilent Technologies, Inc., Santa Clara, CA, USA). The peak area ratio of each NEOs to the internal standard (IS) was used for the quantitative calculation of the respective NEOs.

The recoveries of ACE, IMI, THI, TMX, CLO, and DIN (6NEOs) standards ranged from 57.3 to 147%. The recoveries of 6NEOs standards were calculated by dividing the sum of the 6NEOs standards immediately before the separation analysis by the sum of the 6NEOs standards immediately after the separation analysis step. For THI, DIN, and NACE, samples measured from days that exceeded 3 standard deviations (SD) on the x-R chart were excluded from the analysis. As a result, 254, 275, and 173 samples were analyzed for THI, DIN, and NACE, respectively. The limit of detection (LOD) were calculated as values resulting in a signal-to-noise ratio of 3, and ranged from 0.26 to 1.59 μg/L (Table 2). Limit of quantitation (LOQ) were calculated as values resulting in a single-to-noise ratio of 10, and ranged from 0.86 to 5.31 μg/L (Table 2). Since prior studies in Japan showed > LOD of urinary NEOs concentration (Osaka et al., 2016; Ueyama et al., 2014, 2015), we have also shown them as comparison. For quality control (QC), urine samples were collected from three healthy volunteers who neither had received medication nor had been occupationally exposed to NEOs. As the concentrations of 6NEOs plus NACE (7NEOs) in the QC urine samples were very low, a standard mixture of the 7NEOs was added to yield a urinary NEOs concentration of 5 μg/L.

Table 2
Limit of quantitation, limit of detection, detection frequencies and percentiles of urinary NEOs concentrations in the study participants (n = 314 total urine samples).

NEOs	LOQ (μg/L)	LOD (μg/L)	>LOD (%)	Selected percentiles				
				Min.	25th	50th	75th	Max.
ACE (ug/g creatinine.)	0.86	0.26	0.6	<LOD	<LOD	<LOD	<LOD	4.79
IMI (ug/g creatinine.)	1.27	0.38	0.6	<LOD	<LOD	<LOD	<LOD	5.44
THI [a] (ug/g creatinine.)	3.85	1.16	0.0	<LOD	<LOD	<LOD	<LOD	<LOD
TMX (ug/g creatinine.)	1.42	0.43	83.4	<LOD	5.06	7.40	9.81	72.0
CLO (ug/g creatinine.)	3.43	1.03	80.9	<LOD	11.3	15.3	19.6	79.9
DIN [b] (ug/g creatinine.)	2.22	0.67	10.9	<LOD	<LOD	<LOD	<LOD	619
NACE [c] (ug/g creatinine.)	5.31	1.59	5.8	<LOD	<LOD	<LOD	<LOD	43.7
ttl4NEOs (mol/g creatinine.)				40.6	76.7	99.9	127	580

LOQ; limit of quantitation, LOD; limit of detection, ACE; acetamiprid, IMI; imidacloprid, THI; thiacloprid, TMX; thiamethoxam, CLO; clothianidin, DIN; dinotefuran, NACE; acetamiprid-N-desmethyl, ttl4NEOs; total 4NEOs (ACE, IMI, TMX and CLO). [a]; n = 254, [b]; n = 275, [c]; n = 173. ttl4NEOs include < LOD sample. For samples with levels < LOD, half the value of the LOD was recorded.

2.3. Data analysis

To determine the sources of NEOs exposure, the potential associations of the urinary NEOs concentration with seasons (n = 312), pesticide use during pregnancy (n = 39) and food intake during pregnancy (n = 93) were considered. For the seasonal analysis of urine samples, March, April, and May were classified as spring; June, July, and August as summer; September, October, and November as autumn; and December, January, and February as winter according to the Japan Meteorological Agency (Japan Meteorological Agency, 2019). To assess pesticide use, the following question was asked: "Have you used pesticides during your pregnancy? Or has anyone used pesticides around you during your pregnancy?" The response options were "yes" or "no." Intake of each food group (g/day) was calculated using the validated FFQ and total energy was adjusted using the method of residuals. Food groups included cereals, potatoes and starches, sugar, confectionaries, fats and oils, nuts and seeds, pulses, fish and shellfish, meats, eggs, milk and daily products, vegetables, pickled vegetables, green and yellow vegetables, other vegetables, fruits, fungi, algae, alcoholic beverages, nonalcoholic beverages, and seasonings and spices. According to Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF), although they were lower than the maximum residue limit, more than one of the NEOs were detected from 14 vegetables such as shungiku, leek and broccoli, and 5 fruits such as mandarins, persimmons and pears in 2014, 2015 and 2016 (Ministry of Agriculture, Forestry and Fisheries, 2016; Ministry of Agriculture, Forestry and Fisheries, 2018). Therefore, in addition to FFQ food groups, we grouped those NEOs detected vegetables and fruits as total leaf vegetables (Leek, shungiku, broccoli, spinach, komatsuna, Chinese cabbage and lettuce), total vegetables (leek, shungiku, broccoli, spinach, komatsuna, Chinese cabbage, lettuce, eggplant, green pepper and green beans), total fruits (mandarins, persimmons, melon, pears) and total vegetables and fruits (total vegetables and total fruits), and association with urinary NEOs concentration was analyzed. The energy intake <500 kcal/day or >5000 kcal/day were defined as outside of energy intake limit and it was referred to previous study which targeted pregnant women in Japan (Takahashi et al., 2016). Subjects with energy intakes outside of the predefined limits was not observed in this study.

The detection frequencies (>LOD) of ACE, IMI, THI, DIN and NACE were very low in this study (Table 2), therefore only TMX, CLO and total of 4 NEOs (ttl4NEOs: ACE, IMI, TMX and CLO) were statistically analyzed. For samples with concentrations below the LOD, the statistical analysis was performed using half the value of the LOD. Friedman test was performed to identify potential associations of seasons, Mann-Whitney *U* test was performed to pesticide use during pregnancy. To identify association of food intake amounts during pregnancy with exposure to NEOs, Mann-Whitney *U* test was performed as first step and multiple logistic regression analysis with stepwise variable selection method was conducted as second step with included variables $p < 0.200$ in Mann-Whitney *U* test, maternal age and maternal BMI before pregnancy. Because tap water could be a potential risk of IMI exposure (Klarich et al., 2017), tap water intake was also included as independent variables for multiple logistic regression analysis. As a result of Friedman test, there was no significant difference observed in any urinary NEOs between first, second and third trimesters. Therefore, average value of urinary NEOs in these 3 trimesters was used for analysis of pesticide use during pregnancy and food intake amount during pregnancy. Statistical analyses were conducted using the IBM® SPSS® statistics 24 (Mac® client version, IBM Co., Armonk, NY, USA). A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Urinary NEOs levels among pregnant women

The urinary NEOs concentrations and distributions in this study are summarized in Table 2. The > LOD of TMX and CLO were high, at 83.4% and 80.9%, respectively. In contrast, ACE, IMI, DIN and NACE were only detected in a few participants (0.6%, 0.6%, 10.9 and 5.8%, respectively). THI was not detected in any analyzed urinary samples. The median and maximum levels of the ttl4NEOs were 40.6 and 580 nmol/g creatinine, respectively.

3.2. Sources of NEOs exposure

The median NEOs concentrations were compared to the seasons and any significant difference was found between four seasons. (spring, summer, autumn, winter; TMX: 7.62, 6.74, 6.69, 7.07 μg/g creatinine, p = 0.186; CLO: 15.6, 16.1, 13.9, 14.3 μg/g creatinine, p = 0.463; ttl4NEOs: 102, 97.8, 97.0, 93.3 nmol/g creatinine, p = 0.481).

To determine the effect of pesticide use during pregnancy on the urinary NEOs concentration, we compared the median concentrations of NEOs between the "pesticide use" (PesUse, n = 20) and "non-pesticide use" (PesNoUse, n = 19) groups. However, no significant associations in the median values were observed (TMX: 7.54 vs 9.20 μg/g creatinine, p = 0.214; CLO: 14.9 vs 17.0 μg/g creatinine, p = 0.322; ttl4NEOs: 97.6 vs 113 nmol/g creatinine, p = 0.258).

To determine the associations between NEOs and food intake, TMX, CLO and tt4NEOs were divided into 2 groups, namely "below median (< median)" and "greater than or equal to median (≥ median)." We put these 2 groups of urinary NEOs as dependent variable and food groups that associated with any NEOs of $p < 0.200$ by Mann-Whitney *U* test (sugar, confectionaries, fats and oils, pulses, fish and shellfish, meats, milk and daily products, fruits, alcoholic beverages), tap water, maternal age and maternal BMI before pregnancy as independent variables to perform multiple logistic regression analysis. Table 3 showed variable $p < 0.05$ in the multiple logistic regression analysis. As a result of the analysis, slightly but significant association of pulses in CLO (1.01 [1.00–1.02]) was observed (Table 3). We also statistically analyzed between NEOs and vegetables and fruits that reported NEOs detection

Table 3
Multiple logistic regression analysis for food intake based on urinary NEOs concentration.

NEOs	Food group	adjusted OR (95%CI)	*P*-value
CLO	pulses	1.01(1.00-1.02)	0.015[a]

[a] ; <0.05, NEOs; neonicotinoids, CLO; clothianidin, OR; odds ratio, CI; confidence interval. n = 93 (n = 46 for < median, n = 47 for ≥ median). Dependent variable: CLO, TMX and ttl4NEOs (AEC, IMI, TMX and CLO). Independent variables: sugar, confectionaries, fats and oils, pulses, fish and shellfish, meats, milk and daily products, fruits, alcoholic beverages, tap water, age and BMI. Reference; <median.

by MAFF. However, statistical significant association were not observed between urinary NEOs and those vegetables and fruits.

4. Discussion

The present study was the first to analyze the urinary concentrations of NEOs in pregnant women in Japan. So far, only three studies have analyzed urinary NEOs concentrations in the Japanese population (Osaka et al., 2016; Ueyama et al., 2014, 2015). These studies included 52 Japanese individuals of both sexes in Aichi prefecture (Ueyama et al., 2014), 95 women in Kyoto prefecture and surrounding areas (Ueyama et al., 2015), and 703 3-year-old children in Aichi prefecture (Osaka et al., 2016). In our study, consistent with the findings of Ueyama and colleagues (Ueyama et al., 2014), high detection frequencies for TMX and CLO in urine samples were observed (83%, and 81% in our study vs 100%, and 96% in the study by Ueyama et al. (2014), respectively). Moreover, the median urinary TMX and CLO concentrations in our study were not only higher than those reported in previous studies, but also higher than the maximum value (TMX: 7.40 μg/g creatinine (median) in our study vs 0.23–0.63 μg/g creatinine (median) and 0.52–7.25 μg/g creatinine (maximum) in the previous studies; CLO: 15.3 μg/g creatinine (median) in our study vs < LOD (median) and 1.67–15.2 μg/g creatinine (maximum) in the previous studies) (Osaka et al., 2016; Ueyama et al., 2015). In this study, LOD of ACE, THI and DIN were not as low as previous studies (ACE: 0.26 μg/L in this study vs 0.01–0.03 μg/L in the previous studies; THI: 1.15 μg/L in this study vs 0.06–0.3 μg/L in the previous studies; DIN: 0.67 μg/L in this study vs 0.1–0.32 μg/L in the previous studies, respectively) (Osaka et al., 2016; Ueyama et al., 2014, 2015) and the detection frequencies of ACE, IMI, THI and DIN were low, therefore we could not compare the urinary concentration level of those NEOs to previous studies.

The greatest concern of NEOs exposure among pregnant women is the effect on fetus. Prior studies suggested the association between maternal IMI exposure during pregnancy and incidence of tetralogy of Fallot, anencephaly (Carmichael et al., 2014; Yang et al., 2014), increase risk of preterm birth (Ling et al., 2018), neural tube defects (Rull et al., 2006; Yang et al., 2014) and reduction in IQ (Gunier et al., 2017) of infant. Furthermore, Ohno et al. suggested CLO through the placental barrier in vivo study (Ohno et al., 2020) and Ichikawa et al. reported DIN and NACE were detected from urine sample of infant within 48 h after birth (Ichikawa et al., 2019). These previous studies suggest that maternal NEOs exposure during pregnancy may result in fetal NEOs exposure and may adversely affect the postnatal health of their offspring. In our study, CLO was detected from most of the subjects (80.9%) and this may imply a number of fetus are exposed to CLO. However, research on the effects of NEOs on the fetus, including in vivo study is limited. Therefore, further in vivo and epidemiological research is needed.

The total shipments of NEOs have increased by almost 7 times in 2017 compared to 1996 (National Institute for Environmental Studies, 2019). The NEOs detection frequencies in urine samples from Japanese women living in Kyoto prefecture and the surrounding area has also increased significantly between 1994 and 2011 (Ueyama et al., 2015). Taken together, these data suggest a potential relationship between the amounts of NEOs shipments and human exposure levels. The total shipment amounts for the 6NEOs analyzed in this study were higher in Kumamoto prefecture, where our subjects resided, than in Kyoto prefecture. In addition, the levels of four NEOs (ACE, THI, DIN, and IMI) were higher than those in Aichi prefecture, the site of previous related studies (Osaka et al., 2016; Ueyama et al., 2014). Therefore, we expected higher urinary concentration levels of these NEOs in our samples, compared to those from other areas of Japan. However, our study did not identify a correlation between the amounts of NEOs shipped to a prefecture and the urinary NEOs concentrations of residents.

The associations of urinary NEOs concentrations with seasons, pesticide use and food intake were analyzed to identify potential sources of NEOs exposure. Osaka et al. reported that urinary NEOs concentrations in 3-year-old children were significantly higher in the summer (August–September) than in the winter (February) (Osaka et al., 2016). Our study also considered urinary NEOs concentrations between four seasons, however no association was observed. The Kumamoto city branch of the Japan Agricultural Cooperatives (JA-Kumamoto) reported 17 fruits and vegetables (rice, soybeans, melon, watermelon, strawberry, eggplant, tomato, green paper, lotus root, cabbage, broccolini, mandarin orange, Japanese pear, Japanese apricot, green onion, onion, and Japanese parsley) that are the major fruits and vegetables consumed in Kumamoto city, where participants in this study resided (Japan Agricultural Cooperatives Kumamotoshi, 2013). Of these major fruits and vegetables (except Japanese parsley, no data), IMI and TMX are used as pesticide for more than half of them (81.3% for IMI and 56.3% for TMX) (Kumamoto prefectural government, 2019). These NEOs were used at various times during the growth cycle (e.g., seeding, planting, before harvesting) and may have varied depending on the type of vegetable (Kumamoto vegetable promotion association, 2019). In addition, many vegetables are cultivated throughout the year. Therefore, these may be a reason that seasonal difference was not observed in our study.

In our study, statistical association between urinary CLO and food intake was observed. The ≥ median CLO group reported slightly but significantly higher pulses intake than the <median group. Soybeans is among the most common ingredients used in foods in Japan, and is a component of many foods such as miso soup, soy sauce, tofu, and natto. Most of the soybeans eaten in Japan are imported product. According to a report by MAFF, domestic production of soybeans from 2014 to 2016, when this study was conducted, was 232,000 to 243,000 tons, and imports were 2,828,000 to 3,243,000 tons (Ministry of Agriculture, Forestry and Fisheries, 2016). In addition, the largest imports are from the United States, with 66.6–72.1% of soybeans imports from 2014 to 2016 coming from United States (Ministry of Finance, 2020). It is reported 34–44% of soybeans hectares in United States were seed-applies by NEOs in 2011 (Douglas and Tooker, 2015). Possibly, soybeans seed-applied by CLO leading to human exposure via processed soybeans food intake.

Drinking water could be a potential risk of NEOs exposure. The distributions and leaching potentials of NEOs were examined in Brazil, where IMI, THI, and CLO were found to potentially leach into groundwater (Miranda et al., 2011). In the United States, IMI was detected in tap water (Klarich et al., 2017). In Japan, NEOs contamination in tap water has not been assessed yet, but the National Institute for Environmental Studies reported that NEOs were detected in waterways throughout Japan (National Institute for Environmental Studies, 2016). Due to this background data, we also included tap water intake amount as an independent variable to multiple logistic regression analysis. However statistical significant association was not observed. Further studies are needed to determine the levels of residue and the assessment of NEOs contamination in tap water in Japan.

There are some limitations in this study. First, the use of spot urine sample to evaluate NEOs exposure level and using the FFQ to evaluate trends in food intake represents have some limitations. Overall, 24-h

urine collection may be preferable because the half-lives of urinary biomarkers are relatively short (Osaka et al., 2016). Moreover, ideally, NEOs with short half-lives would be analyzed using a duplicated method that would better reflect the relationships of these chemicals with the most recently consumed meals. In this study, quantitative data on the amount of the food that was consumed before the sampling was not taken. The FFQ refer to the general dietary habits not directly prior to sampling. Although these limitations, in this study, urine samples were collected during the three pregnancy periods, first, second and third trimester, and there was no statistical significant difference between these three urinary NEOs. Therefore, even though spot urine sample was used, we think it was reflected some daily exposure of NEOs. Furthermore, validity of FFQ is validated using 28 day dietary record and the reproducibility of FFQ is validated at 1 year interval (Sasaki et al., 2003a; 2003c). Although the correlation coefficient is not high in validation, it was reported that it can be used to rank individuals according to intakes for the food groups (Sasaki et al., 2003c). Therefore, we think that the intake amount of each food group reflect higher or lower food intake.

This study had some other limitations. The numbers of participants who answered questions regarding the use of pesticides was very small ($n = 39$), which may have reduced the statistical power. Accordingly, further studies with larger sample sizes are needed. In addition, although statistical significance was observed between urinary CLO and pulses intake in this study, it does not indicate NEOs concentration in food. Therefore, in order to clarify the causal relationship of NEOs human exposure from food intake, further research is necessary including food analysis.

5. Conclusion

In this study we determined the urinary NEOs concentrations in Japanese pregnant women and they were exposed to NEOs in their daily lives. Furthermore, the participants in our study had been exposed to TMX and CLO in levels higher than other populations in Japan. As sources of NEOs exposure, the intake of pulses was suggested as potential source of NEOs exposure. These results may facilitate further assessments of NEOs exposure risk in humans.

Declaration of competing interest

None.

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Urinary parabens and their potential sources of exposure among Korean children and adolescents: Korean National Environmental Health Survey 2015–2017

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ABSTRACT

Parabens are used as a preservative in several consumer products including cosmetics, personal care products, and medicinal products. These chemicals have been suspected for estrogenicity and potential adverse endocrine outcomes in humans. For the first time, exposure profiles and potential sources of major parabens are investigated for a nationally representative population of children and adolescents of Korea. In addition, major determinants of urinary paraben levels were identified. For this purpose, the children, and adolescents (n = 2355, 3–18 years of age) who participated in the Korean National Environmental Health Survey cycle 3 (2015–2017) were studied. Adjusted multiple linear regression models were employed to investigate the relationships of several potential demographic and behavioral determinants of exposure, with the urinary levels of three parabens; methyl, ethyl, and propyl paraben. Methyl and propyl paraben levels of the Korean children and adolescents were comparable to those of the US, but the high exposure group (95th percentile) showed much higher levels of exposure. Moreover, urinary ethyl paraben levels are always higher than those of other countries. The uses of personal care products including liquid soaps, fragrance products, nail polish, or antiseptic products were significantly associated with urinary paraben levels. In addition, dietary sources such as fast food and canned food consumption were identified as major contributors to ethyl paraben levels. For methyl and propyl parabens, the use of fever medications and ointments were identified as major determinants of the exposure, especially among the younger children of 3–5 years of age. These observations are related to the Korean regulations that permit the use of the parabens as preservatives in foods and medications. The findings demonstrate that the exposure profile of parabens among Korean children are unique, and mitigation efforts for some parabens are required in Korea. Further studies are warranted to confirm the exposure sources of parabens and to develop mitigation measures among Korean children and adolescents.

1. Introduction

Parabens are alkyl esters of 4-hydroxybenzoic acid. Since its first use in medicine in the 1920s, primarily to inhibit microbial growth and extend product shelf life, parabens have been extensively used as a preservative in numerous consumer products, including cosmetics, personal care products, and medications (Soni et al., 2005). In cosmetics, parabens are commonly used as preservatives (Rastogi et al., 1995). In personal care products such as moisturizers, deodorants, and shampoo, parabens are added for their antimicrobial functions

Abbreviations: CHMS, Canadian Health Measures Survey; CI, Confidence Intervals; Ctree, Conditional interference trees; GerES, the German Environmental Survey; GM, Geometric mean; KoNEHS, Korean National Environmental Health Survey; LOD, Limit of Detection; MOE, Ministry of Environment; NHANES, National Health and Nutrition Examination Survey; NIER, National Institute of Environmental Research.

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(Gruvberger et al., 1998; Guo et al., 2014).

Parabens are generally considered as safe to humans at the levels permitted by regulatory standards. However, contradictory evidence is increasing in both epidemiologic and experimental studies. Studies have indicated estrogenic properties of parabens with a longer aliphatic chain, although short-chain esters were observed to exert estrogenic effects (Boberg et al., 2010; Sun et al., 2016). In animals, propyl paraben was reported to affect the activity of a sex hormone like testosterone (Oishi, 2002). Parabens have also been associated with breast cancer development (Darbre et al., 2004; Darbre and Harvey, 2014). Parabens have been found to may affect various disorders in the body with endocrine-disrupting chemicals (Boberg et al., 2010; Nishihama et al., 2016; Nowak et al., 2018; Watanabe et al., 2013). Because of their extensive use, exposure to parabens through oral, dermal, and inhalation routes is widespread in daily lives (Błędzka et al., 2014; Dodge et al., 2018). Most of the parabens are excreted through urine as metabolites, e.g., *p*-hydroxyhippuric acid, sulfate-, and glucuronide-conjugated forms (Abbas et al., 2010; Soni et al., 2005).

Because of health concerns, several national scale biomonitoring programs have been conducted for parabens in countries including Canada, France, Germany, and US (CDC, 2021; Fillol et al., 2021; Health Canada, 2019; Murawski et al., 2020). However, among the Korean population, information on the current status of exposure to major parabens, especially in children and adolescents, along with potential sources of exposure, is limited.

This study determined the exposure levels of three most frequently used parabens, methyl, ethyl, and propyl paraben, in the children and adolescents who participated in the Korean National Environmental Health Survey (KoNEHS) Cycle 3 (2015–2017). In addition, potential sources of exposure among Korean children and adolescents were investigated. The results of the study will help understand high priority parabens and subpopulations of high exposure among Korean children and adolescents. Furthermore, this information will aid in developing appropriate mitigation measures for the paraben exposure among the general population of Korea.

2. Methods

2.1. Population and study design

The study population consisted of the children and adolescents (n = 2380; between 3 and 18 years of age) who participated in the KoNEHS Cycle 3 (2015–2017), conducted by the Korean Ministry of Environment. Among them, 25 subjects were excluded from the final analysis because of the insufficient volume of urine samples, and therefore the final number of the participating children and adolescents was 2355.

KoNEHS is a cross-sectional survey designed to monitor the exposure levels of environmental chemicals among representative Korean population using a multiple stage sampling method (Choi et al., 2017; Park et al., 2016). For children and adolescents, random sampling was performed following region, sex, and age stratification based on population data of Korea. The sampling units were the childcare or educational institutions, e.g., kindergarten, schools (Ha et al., 2014).

Written informed consent as well as information regarding the participants were collected through questionnaires from their parents or guardians. The questionnaire included demographic and socioeconomic information, and other factors related to environmental chemical exposure. This study was approved by the Ethical Review Board of the National Institute of Environmental Research (NIER), Korea (IRB No. NIER-2015-BR-006-01, July 20, 2015).

2.2. Measurement of urinary parabens

Once sampled, urine specimens were stored in a refrigerator and transported under cold (0–4 °C) conditions to the laboratory, where the samples were kept at −70 °C until analysis. Urine samples were hydrolyzed using β-glucuronidase/sulfatase, were acidified with formic acid, and preconcentrated using solid-phase extraction. Methyl paraben, ethyl paraben, and propyl paraben in urine were quantified employing an ultra-high performance liquid chromatograph-mass spectrometry (Agilent 6490, Agilent, Santa Clara, CA, USA) (NIER, 2018). Limits of detections (LODs) were as follows: methyl paraben: 0.108 μg/L; ethyl paraben: 0.107 μg/L; and propyl paraben: 0.082 μg/L. An internal quality control of the analysis was ensured by analyzing two quality controls (low and high) in each batch of analyses. External quality assurance including accuracy of the analytical method was conducted by participating in laboratory comparison programs, including the G-EQUAS (German External Quality Assessment Scheme).

Urinary creatinine was measured using the kinetic Jaffe reaction method and was analyzed by the ADVIA 1800 Auto Analyzer (Siemens Medical Solutions Diagnosis, USA). Internal quality control was ensured by analyzing commercial reference materials (Liquichek Urine Chemistry Control, Bio-Rad). External quality and accuracy of the analytical method were assessed by participating in interlaboratory comparison programs (College of American Pathologists).

2.3. Statistical methods

The distribution of urinary parabens was skewed; thus log-transformation was performed before statistical analyses and values below the LODs, LODs were divided by $1/\sqrt{2}$ (Hornung and Reed, 1990). The urinary paraben concentrations were adjusted with the urinary creatinine (μg chemical/g creatinine) for urine dilution (Barr et al., 2005; Uchida and Gotoh, 2002).

For the frequencies of use of personal care products, most responses were grouped into four categories, i.e., 'no use', 'less than once a week', 'once a week and more', 'within two days'; For food consumption questions, most responses were grouped into four categories as well, i.e., 'do not eat', 'eat less than once a week', 'eat once a week or more', 'eat once a day or more.' The covariates such as age, sex, monthly household income, regional area, exercise time, and indoor activity time were included as categorical variables.

The exposure risks of parabens associated with personal care products and food were calculated based on the survey weighted multiple linear regression. In addition, an unbiased recursive partitioning-based permutation test using conditional inference trees (CTree) was conducted to identify and visualize the relationship between critical exposure factors and paraben levels. CTree is a non-parametric class of regression trees embedding tree-structured regression models into a defined theory of conditional inference procedures (Hothorn et al., 2006).

Statistical significance was determined at $p < 0.05$. For statistical analysis of each group, SAS survey procedures ('surveyfreq', 'surveymean', 'surveyreg') were used, reflecting the population weight adjustment as well as the multistage sample design using SAS 9.4 (SAS Institute, Cary, NC, US). For CTree statistical analysis, R version 3.6.3 was used and a *p* value lower than 0.05 indicated statistical significance.

3. Results

3.1. Characteristics of the study population

Demographic characteristics distribution according to age, sex, BMI categories, regional area, monthly household income, exercise time, and indoor activity time are presented in Table 1. The females comprised 50.5% of the population. The median BMIs for subgroups of 3–5, 6–11, and 12–18 years of age were 16.0, 17.6, and 21.4, respectively. Urban and rural areas were divided according to classification of the administrative district (urban: -dong; rural: -eup, -myeon). Then, urbanized rural areas were divided into separate peri-urban areas (Lee and Lee, 2018). Industrial areas were designated as a neighborhood of identifiable industrial facilities. Most subjects lived in the urban area (61.0%),

Table 1
Weighted geometric mean and 95% confidence interval (μg/g creatinine) for parabens according to population group and characteristics of residential environment.

	N	(%)	Weighted N	(%)	Methyl paraben		Ethyl paraben		Propyl paraben	
					GM	95% CI	GM	95% CI	GM	95% CI
Total	2355	100	7337225	100	24.5	(21.6, 27.8)	12.2	(10.3, 14.3)	2.2	(1.9, 2.6)
Age group										
3–5 years	571	24.2	1383291	18.9	***55.1***	***(45.0, 67.5)***	***16.9***	***(12.7, 22.4)***	***5.2***	***(4.0, 6.7)***
6–11 years	884	37.6	2663460	36.3	***26.6***	***(22.8, 31.1)***	***10.5***	***(8.1, 13.7)***	***1.7***	***(1.4, 2.0)***
12–18 years	900	38.2	3290474	44.8	***16.3***	***(13.7, 19.3)***	***11.9***	***(9.1, 15.6)***	***2.0***	***(1.6, 2.5)***
Sex										
Male	1165	49.5	3835294	52.3	***20.0***	***(16.7, 24.1)***	13.1	(10.6, 16.1)	***1.7***	***(1.4, 2.0)***
Female	1190	50.5	3501931	47.7	***30.5***	***(26.8, 34.7)***	11.3	(9.4, 13.5)	***3.0***	***(2.6, 3.6)***
BMI Category [a]										
1st Quartile	590	25.1	1793452	24.4	***24.8***	***(20.1, 30.8)***	12.4	(9.9, 15.5)	***2.5***	***(2.0, 3.1)***
2nd Quartile	592	25.1	1872634	25.5	***26.9***	***(22.4, 32.4)***	12.3	(10.1, 15.1)	***2.4***	***(2.0, 3.0)***
3rd Quartile	587	24.9	1854499	25.3	***25.9***	***(21.5, 31.3)***	11.4	(8.8, 14.8)	***2.3***	***(1.8, 2.8)***
4th Quartile	586	24.9	1816640	24.8	***20.6***	***(17.0, 25.0)***	12.5	(9.8, 16.0)	***1.8***	***(1.5, 2.2)***
Regional area										
Rural area	468	19.9	843273	11.5	28.1	(20.2, 39.1)	***16.8***	***(10.5, 27.0)***	2.3	(1.8, 2.9)
Peri-urban area	124	5.2	490602	6.7	20.8	(13.2, 32.8)	***10.0***	***(5.3, 18.9)***	2.1	(1.5, 2.9)
Urban area	1436	61.0	4916386	67.0	24.6	(21.1, 28.8)	***12.5***	***(10.2, 15.4)***	2.4	(2.0, 2.8)
Industrial area	327	13.9	1086964	14.8	22.9	(16.3, 32.3)	***9.2***	***(6.6, 12.7)***	1.8	(1.2, 2.6)
Monthly household income (US dollars) [b]										
Do not know	107	4.2	329788	4.5	28.8	(18.5, 44.7)	11.5	(7.5, 17.7)	2.9	(1.7, 4.9)
<2350	218	8.9	688044	9.4	22.6	(17.8, 28.6)	12.9	(9.4, 17.8)	2.3	(1.6, 3.2)
2350–3525	341	13.5	998808	13.6	25.2	(20.4, 31.3)	14.3	(10.8, 18.9)	2.3	(1.8, 2.9)
3525–5875	833	35.2	2533437	34.5	25.9	(22.0, 30.5)	12.2	(10.0, 14.9)	2.3	(1.9, 2.8)
>5875	856	38.2	2787148	38.0	23.0	(19.1, 27.7)	11.4	(9.3, 14.0)	2.1	(1.7, 2.6)
Exercise time (min)										
Do not	1001	42.5	2907120	39.6	***29.5***	***(25.0, 34.9)***	11.9	(9.8, 14.4)	***2.6***	***(2.2, 3.2)***
<30	482	20.5	1468494	20.0	***25.8***	***(20.3, 32.7)***	12.8	(10.1, 16.3)	***2.1***	***(1.6, 2.7)***
30–60	543	23.1	1880314	25.6	***20.7***	***(16.9, 25.3)***	12.4	(9.8, 15.7)	***2.0***	***(1.6, 2.5)***
>60	329	13.9	1081297	14.8	***18.4***	***(15.1, 22.5)***	11.5	(8.4, 15.8)	***2.0***	***(1.6, 2.7)***
Indoor activity time (min)										
<600	570	24.2	2058331	28.1	***15.8***	***(13.1, 19.2)***	12.7	(9.2, 17.6)	***1.8***	***(1.4, 2.4)***
600–770	508	21.6	1532847	20.9	***21.2***	***(17.7, 25.5)***	12.6	(10.1, 15.8)	***2.0***	***(1.6, 2.4)***
770–840	686	29.1	1965768	26.8	***28.4***	***(23.6, 34.0)***	11.7	(9.2, 14.8)	***2.5***	***(1.9, 3.1)***
>840	591	25.1	1780279	24.2	***38.8***	***(32.4, 46.5)***	11.7	(9.3, 14.7)	***2.8***	***(2.2, 3.5)***

NOTE: $p < 0.05$.
[a] BMI Category 3–5 years (25%=15.0, 50% =16.0, 75% =17.1), 6–11 years (25%=15.7, 50% =17.6, 75% =20.3), 12–18 years (25%=19.5, 50% =21.4, 75% =23.7).
[b] Currency: 1175 won/dollar.

followed by the rural area (19.9%), industrial area (13.9%), and peri-urban area (5.2%). Age and exercise time were negatively correlated with two or more paraben concentrations in the urine (Table S1).

3.2. Distributions of the concentrations of parabens

Among the young Korean population, the urinary concentrations of all three parabens tended to be higher among the 3–5 years old group, and this trend was more outstanding for methyl and propyl parabens. Methyl parabens were detected at 55.1 μg/g creatinine (GM) among the 3–5 years old children, whereas it was at 26.6 μg/g creatinine among 6–11 years old, and 16.3 μg/g creatinine among the 12–18 years old adolescents. For propyl paraben, GMs of 5.2, 1.7, and 2.0 were detected for the 3–5, 6–11 and 12–18 years old group, respectively. On the other hand, ethyl parabens were detected at 16.9, 10.5, and 11.9 μg/g creatinine (GM) in the 3–5, 6–11 and 12–18 years old groups, respectively. Urinary creatinine concentrations measured for the 3–5, 6–11 and 12–18 years old groups were 0.840, 1.084 and 1.606 g/L (GM), respectively (Table S3). Compared to males, females showed higher methyl, propyl paraben concentrations, and lower ethyl paraben concentrations (Table 1).

3.3. Comparison with other national biomonitoring studies

The urinary parabens for the five countries are shown in Table 2. The national biomonitoring studies except Esteban showed the highest urinary methyl and propyl parabens concentrations in the youngest population and the 95 percentiles of the 3–5 years group had the highest concentration. Among several countries, KoNEHS had the highest concentration of methyl paraben (GM: 55.1 μg/g creatinine), followed by NHANES, CHMS and GerES (GM: 53.5, 17, 15.12 μg/g creatinine). NHANES had the highest concentration of propyl paraben (GM: 7.5 μg/g creatinine), followed by KoNEHS, CHMS and GerES (GM: 5.2, 2.0, 0.695 μg/g creatinine). Interestingly, the KoNEHS had the highest concentration of ethyl paraben (GM: 16.9 μg/g creatinine). In comparison, other countries were very low (GerES 0.930 μg/g creatinine) or not detected.

3.4. Evaluation of the paraben exposure factors

Multiple linear regression analyses showed that the urinary levels of ethyl paraben were positively associated with fast food consumption and canned food consumption, after adjusting for covariates (Table 3). No other dietary factors were associated with urinary levels of methyl and propyl parabens. Among pharmaceuticals and personal care products, several items were identified to be associated with elevated levels of urinary parabens (Tables 4 and 5). Use of liquid soaps including shower gel and shampoo was associated with ethyl paraben levels. For propyl paraben levels, uses of fragrance and antiseptic products were also significantly associated (Table 4). Among pharmaceuticals, the use of medications for fever antipyretics and dermatitis (ointments) were significantly associated with the urinary methyl and propyl parabens (Table 5).

The CTree analysis identified the use of fever medication as an initial discriminator for methyl paraben, followed by age. For children who

Table 2
Urinary concentrations of three parabens (μg/g creatinine) among Korean children and adolescents, in comparison with those reported in national biomonitoring programs.

	KoNEHS 2015–2017					NHANES 2015–2016[a]					CHMS 2016–2017[b]					GerES 2014–2017[c]				Esteban 2014–2016[d]			
	N	DR	GM	75th	95th	N	DR	GM	75th	95th	N	DR	GM	75th	95th	N	DR	GM	95th	N	DR	GM	95th
Methyl paraben																							
3–5 years	571	100	55.1	178	5365	140	97.9	53.5	198	2210	542	88.9	17	44[e]	-[f]	93	96	15.12	1130	398	94.2	5.3	311.4
6–11 years	884	100	26.6	89.8	754	415	96.1	22.2	60.2	684	531	88.4	8.7	19	-[f]	155	95	7.255	715				
12–18 years	900	100	16.3	58.7	309	405	98.3	18.8	65.4	467	531	87.5	7.2	30	190[e]	145	99	5.208	261				
Ethyl paraben																							
3–5 years	571	99.0	16.9	81.4	456	140	38.6	NC	5.00	83.0	542	35.8	NC	2.5[e]	-[f]	99	58	0.930	3.53	398	29.4	NC	17.5
6–11 years	884	98.9	10.5	56.8	440	415	35.9	NC	2.54	16.0	531	26.3	NC	NC	-[f]	166	71	0.921	6.54				
12–18 years	900	99.6	11.9	38.3	177	405	39.3	NC	1.64	12.7	531	28.2	NC	1.0	27[e]	149	76	0.912	10.1				
Propyl paraben																							
3–5 years	571	99.3	5.2	21.3	824	140	100	7.5	16.3	724	542	70.7	2.0[e]	5.8[e]	53[e]	99	27	0.695	101	398	30.9	NC	45.1
6–11 years	884	94.6	1.7	5.7	99.4	415	98.3	2.7	6.21	69.5	531	70.3	-[f]	-[f]	-[f]	166	33	0.551	40.7				
12–18 years	900	97.9	2.0	5.8	94.9	405	98.8	2.4	10.1	103	531	70.1	-[f]	3.8[e]	-[f]	149	36	0.469	10.0				

Abbreviations: KoNEHS, Korean National Environmental Health Survey; NHANES, National Health and Nutrition Examination Survey; CHMS, Canadian Health Measures Survey; GerES, the German Environmental Survey; GM, Geometric mean; DR, Detection rate; LOD, Limit of Detection; LOQ, Limit of Quantification, NC, Detection rate <60% the analysis was performed but the geometric mean was not calculated.

[a] US national biomonitoring 3–5 years, 6–11 years, 12–19 years, U.S. (CDC, 2021).

[b] Canada national biomonitoring 3–5 years, 6–11 years, 12–19 years (Health Canada, 2019).

[c] Germany national biomonitoring 3–5 years, 6–10 years, 14–17 years (Murawski et al., 2020).

[d] France national biomonitoring 6–17 years (Fillol et al., 2021).

[e] Use data with caution (CV between 16.6% and 33.3%).

[f] Data is too unreliable to be published (CV > 33.3%).

Table 3
Multiple linear regression analysis and 95% confidence intervals for parabens in dietary factors.

	N (%)	Weighted N (%)	Methyl paraben			Ethyl paraben			Propyl paraben		
			β	95% CI		β	95% CI		β	95% CI	
Consumption frequency											
Fast food[a]											
Do not eat	578 (24.5)	1666968 (22.7)	ref.			ref.			ref.		
Less than once a week	1456 (61.8)	4591778 (62.6)	−0.199	−0.448	0.049	0.076	−0.272	0.424	−0.060	−0.357	0.237
Once a week or more	316 (13.5)	1067780 (14.6)	0.157	−0.285	0.600	***0.538***	***0.047***	***1.030***	0.008	−0.439	0.456
Once a day or more	5 (0.2)	10699 (0.1)	−0.687	−1.818	0.444	***2.385***	***1.349***	***3.421***	0.321	−1.097	1.739
Frozen food											
Do not eat	1000 (42.5)	3064147 (41.8)	ref.			ref.			ref.		
Less than once a week	1120 (47.6)	3478993 (47.4)	−0.068	−0.280	0.144	0.022	−0.278	0.321	−0.104	−0.323	0.115
Once a week or more	222 (9.4)	760698 (10.3)	−0.078	−0.555	0.399	−0.008	−0.527	0.511	−0.149	−0.740	0.441
Once a day or more	13 (0.5)	33386 (0.5)	1.369	−0.449	3.187	0.542	−2.584	3.668	1.568	−1.609	4.745
Beverages											
Do not eat	1114 (47.3)	3194723 (43.5)	ref.			ref.			ref.		
Less than once a week	536 (22.7)	1759684 (24.0)	−0.156	−0.519	0.208	−0.245	−0.654	0.163	−0.084	−0.461	0.292
Once a week or more	597 (25.4)	2073712 (28.3)	0.083	−0.270	0.435	***−0.398***	***−0.757***	***−0.038***	0.144	−0.270	0.558
Once a day or more	108 (4.6)	309107 (4.2)	−0.025	−0.520	0.471	0.095	−0.381	0.572	−0.056	−0.646	0.533
Canned food											
Do not eat	879 (37.3)	3028724 (41.3)	ref.			ref.			ref.		
Less than once a week	850 (36.1)	2408615 (32.8)	−0.206	−0.515	0.103	***0.464***	***0.043***	***0.884***	−0.124	−0.515	0.268
Once a week or more	571 (24.3)	1698995 (23.2)	0.006	−0.360	0.372	***0.609***	***0.192***	***1.026***	0.042	−0.401	0.485
Once a day or more	55 (2.3)	200891 (2.7)	−0.188	−1.196	0.820	0.596	−0.834	2.026	0.297	−0.752	1.345

NOTE: ***p < 0.05.***
[a] Pre-cooked meals, mass-produced ingredients.

Table 4
Multiple linear regression analysis and 95% confidence intervals for parabens in personal care products.

	N (%)	Weighted N (%)	Methyl paraben			Ethyl paraben			Propyl paraben		
			β	95% CI		β	95% CI		β	95% CI	
Product use frequency											
Liquid soap [a]											
No use	444 (18.9)	1410477 (19.2)	ref.			ref.			ref.		
Less than once a week	71 (3.0)	232412 (3.2)	***−0.595***	***−1.165***	***−0.025***	0.111	−0.614	0.836	−0.490	−1.078	0.097
Once a week and more	897 (38.1)	2709395 (36.9)	−0.277	−0.593	0.039	***0.311***	***0.013***	***0.609***	−0.198	−0.537	0.141
Within 2 days	943 (40.0)	2984941 (40.7)	0.032	−0.281	0.346	***0.386***	***0.052***	***0.720***	0.118	−0.236	0.473
Color cosmetics											
No use	1730 (73.5)	5384730 (73.4)	ref.			ref.			ref.		
Less than once a week	43 (1.8)	140440 (1.9)	0.135	−0.809	1.078	−0.168	−1.348	1.012	0.065	−0.937	1.066
Once a week and more	130 (5.5)	386090 (5.3)	0.148	−0.355	0.652	0.244	−0.327	0.815	0.370	−0.293	1.033
Within 2 days	452 (19.2)	1425965 (19.4)	−0.07	−0.422	0.283	0.297	−0.063	0.657	0.109	−0.423	0.642
Fragrance products											
No use	2142 (91.0)	6633288 (90.4)	ref.			ref.			ref.		
Less than once a week	45 (1.9)	148257 (2.1)	0.137	−0.998	1.271	−0.072	−1.427	1.283	−0.002	−1.375	1.372
Once a week and more	76 (3.2)	267292 (3.6)	1.451	−0.311	3.214	1.034	−1.335	3.403	1.685	−0.922	4.293
Within 2 days	92 (3.9)	288388 (3.9)	−0.447	−1.998	1.103	0.14	−0.651	0.93	***2.373***	***1.291***	***3.455***
Nail polish											
No use	2221 (94.3)	6949431 (94.7)	ref.			ref.			ref.		
Less than once a week	63 (2.7)	200905 (2.7)	0.202	−0.430	0.834	***0.976***	***0.284***	***1.668***	−0.085	−0.814	0.644
Once a week and more	13 (0.5)	33644 (0.5)	−1.008	−2.180	0.163	−0.771	−3.105	1.564	−0.746	−2.531	1.039
Within 2 days	58 (2.5)	153245 (2.1)	0.468	−0.191	1.127	0.285	−0.524	1.094	0.177	−0.641	0.995
Antiseptic products [b]											
No use	1724 (73.2)	5411351 (73.8)	ref.			ref.			ref.		
Less than once a week	262 (11.1)	801408 (10.9)	0.268	−0.043	0.580	0.045	−0.344	0.435	0.201	−0.180	0.582
Once a week and more	266 (11.3)	806904 (11.0)	0.254	−0.252	0.760	0.440	−0.158	1.038	0.244	−0.281	0.770
Within 2 days	103 (4.4)	317561 (4.3)	0.220	−0.229	0.669	0.325	−0.157	0.807	***0.414***	***−0.039***	***0.866***
Hair care products [c]											
No use	2189 (93.0)	6777098 (92.4)	ref.			ref.			ref.		
Less than once a week	27 (1.1)	99463 (1.4)	0.326	−0.941	1.594	−0.223	−1.714	1.267	0.686	−0.510	1.883
Once a week and more	84 (3.6)	282739 (3.9)	−0.265	−0.929	0.398	0.082	−0.756	0.920	−0.539	−1.216	0.138
Within 2 days	55 (2.3)	177925 (2.3)	0.065	−0.878	1.009	−0.548	−1.824	0.727	0.317	−0.634	1.268

NOTE: Adjusted for age, sex, regional area, monthly household income, exercise time and indoor activity time. ***p < 0.05***.
[a] Liquid soap includes shower gel and shampoo.

[b] Antibacterial products include those containing antibacterial (sterilizing) ingredients such as mouthwash, acne treatment, deodorant, and hand sanitizer.

[c] Hair products include hair gel, mousse, spray, wax, essence, and dye.

Table 5
Medication intake due to disease, and multiple linear regression and 95% confidence.

	N (%)	Weighted N (%)	Methyl paraben			Ethyl paraben			Propyl paraben		
			β	95% CI		β	95% CI		β	95% CI	
Take a medication											
Due to fever											
No	2185 (92.8)	6839186 (93.2)	ref.			ref.			ref.		
Yes	170 (7.2)	498039 (6.8)	***2.139***	***1.698***	***2.581***	***−0.542***	***−0.918***	***−0.167***	***2.019***	***1.495***	***2.544***
Due to dermatitis [a]											
No	2232 (94.8)	6951773 (94.7)	ref.			ref.			ref.		
Yes	123 (5.2)	385452 (5.3)	***0.887***	***0.409***	***1.365***	0.349	−0.167	0.864	***1.162***	***0.570***	***1.755***
Due to gastroenteritis											
No	2346 (99.6)	7316474 (99.7)	ref.			ref.			ref.		
Yes	9 (0.4)	20751 (0.3)	0.004	−2.261	2.269	***−0.695***	***−1.287***	***−0.104***	0.287	−2.452	3.027
Other reasons											
No	2323 (98.6)	7242916 (98.7)	ref.			ref.			ref.		
Yes	32 (1.4)	94308 (1.3)	0.395	−0.504	1.293	0.415	−0.160	0.990	0.387	−0.817	1.591

NOTE: Adjusted for age, sex, regional area, monthly household income, exercise time and indoor activity time. ***p < 0.05***.
[a] Both oral and dermal drugs included.

administered fever medication and are 7 years and younger, GM of methyl parabens was 502 μg/g creatinine; and for children who are older than 7 years of age who spent more than 770 min/day indoors, GM of methyl parabens was 129 μg/g creatinine. For children who did not administer fever medication, GM of methyl parabens was 106 μg/g creatinine in the group who are 8 years and younger and used dermatitis medication. For ethyl paraben, indoor activity time was an initial discriminator and fast-food consumption was next. For children who consumed fast food once a week or more showed 22.4 μg/g creatinine (GM). Lastly for propyl paraben, it showed a similar pattern as methyl paraben as the use of fever medication was the initial discriminator, followed by age. For children who administered fever medication and are 7 years and younger, GM of propyl parabens was 31.7 μg/g creatinine (Fig. 1).

4. Discussion

4.1. Concentration of parabens in Korean young population

The parabens were detected in more than 94% of the present children and adolescents, suggesting their widespread exposure among the young Korean population. The present observations showed that younger children generally exhibited greater levels of exposure to the parabens. This observation may be due to the fact that younger children tend to show greater intake amount and skin surface area per body weight (Miller et al., 2002), and is not different from those reported from other national biomonitoring programs (Table 2) (CDC, 2021; Health Canada, 2019; Murawski et al., 2020).

Urinary paraben concentrations are presented as unadjusted, i.e., μg paraben/L urine, and creatinine-adjusted urinary levels, i.e., μg paraben/g creatinine. Our results showed that urinary creatinine levels increased with age in children, similar to the observations of other reports (Aylward et al., 2011; Remer et al., 2002). Regardless of creatinine adjustment urinary methyl and propyl paraben concentrations were the highest in the 3–5 years old group. This observation suggests the presence of age specific sources of exposure among this group of young children. For ethyl paraben (unadjusted only), the highest levels were observed in the 12–18 years old group (Table S2).

Compared by sex, the concentrations of methyl and propyl parabens were higher in females. This finding is similar to those made on adults. Urinary levels of methyl, ethyl, and propyl parabens were reported to be higher in females (Engel et al., 2014; Genuis et al., 2013; Honda et al., 2018; Wang et al., 2013). In contrast, the concentrations of ethyl paraben were higher in male children and adolescents.

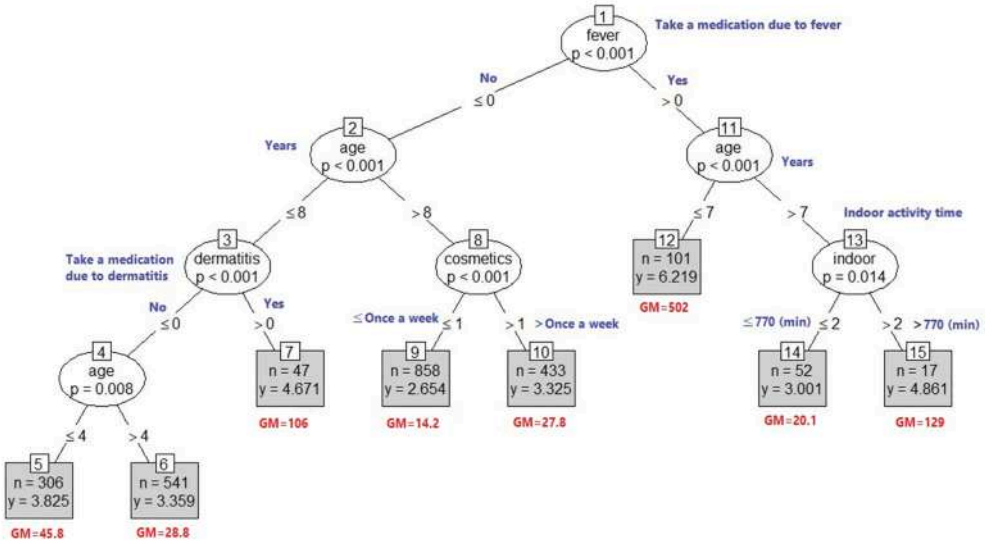
4.2. Comparison with other countries

The levels of methyl and propyl parabens among the Korean population were generally comparable to those of the US. Among the high exposure groups, e.g., 95th percentile, Korean children of 3–5 years of age showed more than two folds of methyl paraben levels than that of the US children of the matching age (5365 vs 2210 μg/g creatinine). For propyl parabens, a similar pattern, e.g., higher 95th percentile level than that of the US (824 vs 724 μg/g creatinine), was observed, while the GM was lower than that of the US (5.2 vs 7.5 μg/g creatinine). Compared to the Canadian and German children, Korean subjects showed up to 3 times higher levels of exposure on average for methyl parabens, and 2 times higher levels for propyl parabens. Another interesting finding was the frequent detection of ethyl parabens with consistently higher levels of exposure. Other biomonitoring programs generally showed very low or negligible levels of ethyl paraben in urine (CDC, 2021; Fillol et al., 2021; Health Canada, 2019; Murawski et al., 2020). Available literature indicates that the urinary ethyl paraben levels of the Korean population were always higher, i.e., up to 4 folds higher than those of the US children. In addition, ethyl paraben levels were more than 10-times higher than the levels reported from several Asian and Middle East countries (China, Japan, India, Saudi Arabia, Kuwait, and Vietnam: median concentration range: 0.19–2.74 ng/mL) (Honda et al., 2018). These observations suggest the presence of specific exposure sources and pathways of ethyl parabens among the Korean population.

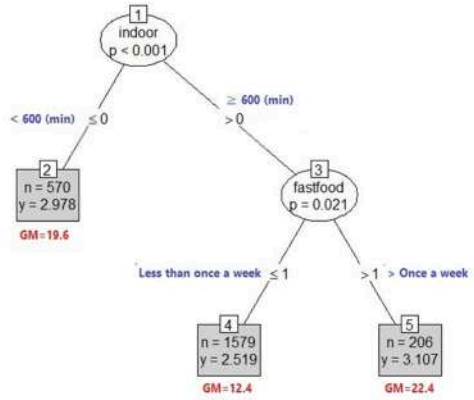
4.3. Exposure to ethyl parabens through food

Our findings suggest the presence of specific sources of exposure among Korean children and adolescents to the measured parabens. For ethyl parabens, fast foods and canned foods consumption were identified as major contributors of the exposure among the children and adolescents, in addition to the use of liquid soap and nail polish. While contribution of personal care products has frequently been reported for ethyl parabens (Fisher et al., 2017; Guo and Kannan, 2013; Li et al., 2020), dietary sources have seldom been investigated. In Korea, ethyl parabens are commonly used as a food preservative, and have been used in diverse condiments (Jeong et al., 2020). In particular, studies have reported detection of methyl and ethyl parabens in soy sauce and condiments (vinegar and sauces) (National Institute of Food and Drug Safety Evaluation, 2020), with the highest levels found in the fermented condiments (red pepper paste and soy paste) (Jo et al., 2020). A recent report on 'temple stay participants' of Korea found that urinary ethyl paraben levels increased after the 5-day temple stay and suggested that the increased consumption of traditional Korean condiments that are frequently used in a vegetarian style temple diet might explain this

(A) Methyl paraben



(B) Ethyl paraben



(C) Propyl paraben

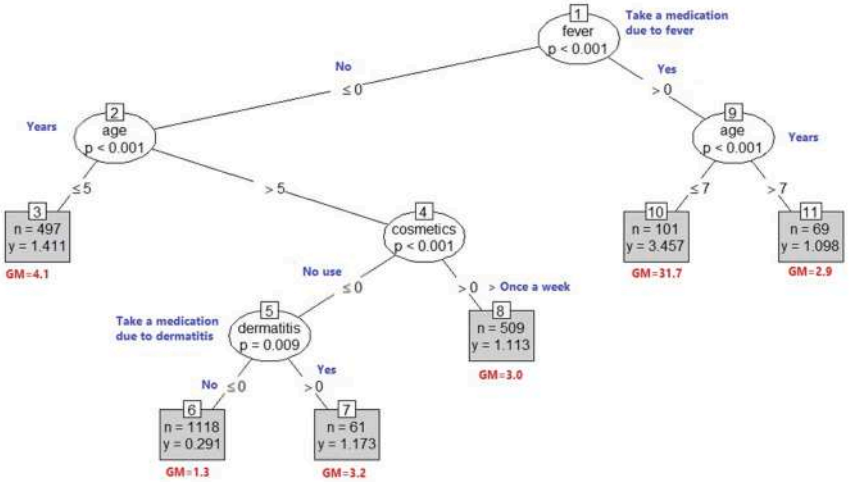


Fig. 1. Decision tree to identify sources of exposure to (A) methyl (B) ethyl and (C) propyl paraben. ***p<0.05.***

increase (Jo et al., 2020). Contribution of the dietary sources among Korean population to the ethyl parabens exposure should be subject to more refined exposure assessment in the future.

4.4. Exposure to parabens in personal care products

For methyl and propyl parabens, use of several personal care products such as nail polish, antiseptic products, hair care products, or color cosmetics were identified as potential sources. Both methyl and propyl parabens are predominantly used as preservatives in personal care products (Karthikraj and Kannan, 2018). Use of cosmetics and personal care products are among the well-known sources of paraben exposure in the general population (Wang et al., 2013). For example, urinary methyl paraben concentration is related to perfume and liquid soap among adults (Braun et al., 2014; Soni et al., 2002). For children and adolescents, the use of nail products and other cosmetics are also among the important sources of exposure to parabens, similar to other countries (Manová et al., 2013). The relationships of the use of color cosmetics, nail polish, and hair products with urinary paraben levels observed in the current study are in line with previous studies (Guo et al., 2014). Several studies have reported positive correlations between urinary biomarkers of parabens and personal care products(Guo et al., 2014; Kim et al., 2018; Kizhedath et al., 2019). A study conducted in Slovenia showed higher urinary methyl paraben levels in children who had used children cosmetic items such as lipstick and perfume (Tkalec et al., 2020). A positive association between methyl and propyl parabens and body lotion usage for a mother-children study has also been previously reported in Swedish literature (Larsson et al., 2014). This study results support previous international findings of association between parabens and personal care products.

4.5. Exposure to parabens in medications

Statistically significant associations were observed medications for between fever and atopic dermatitis medications, and the urinary levels of methyl and propyl parabens (Table 5), shed light on potential sources of these parabens among Korean children and deserve further discussions. Methyl parabens are widely used as a preservative in medicinal products, and it is frequently used in combination with propyl paraben (Brand et al., 2018). Paraben concentration was the highest in liquid medicines followed by soft gels, while medicines in solid form contained the least paraben content (Moreta et al., 2015). Among tablet, particle, oral liquid of pharmaceuticals products, the estimated daily intake (EDI) of parabens via liquid medications was the highest (Ma et al., 2016). While the levels of parabens used in the medicinal products of Korea are not readily available, the levels of parabens in medications varies by country (Moreta et al., 2015).

The importance of medication as a source of exposure to methyl and propyl parabens is also supported by the results of CTree analysis (Fig. 1). The results showed that a recent medication followed by the age of the medicated, were the most important discriminators that influence the urinary levels of both parabens. Paraben containing medications may be a source of high urinary methyl and propyl paraben concentrations reported previously (Dodge et al., 2015). In addition, a previous report showed that the neonatal who took liquid medications were found to be exposed to methyl and propyl parabens which might be used as their preservatives (Mulla et al., 2015). For Chinese children, the GM and median values of EDI of parabens via pharmaceutical ingestion were approximately three times higher than those of adults (Ma et al., 2016). Therefore, for young children, the use of medications containing parabens, e.g., antipyretic medicine in liquid formulation, warrants concern and further efforts to find safer alternatives. In line with this concern, more medications are sold in preservative-free or paraben-free formulations (Cutaia et al., 2018). In the present population who did not take the fever medicine, the use of ointment was again identified as the most important discriminator for urinary methyl paraben (Fig. 1A), outlining the importance of medication as sources of methyl paraben exposure among the children and adolescents of Korea. In addition, the CTree results suggested that the indoor activity time was another important discriminator among the Korean population. This discriminator implies that indoor pollution such as house dust and indoor air may contribute to the body burden of the parabens for the preschoolers and young children, which is comparable to several reports that were made elsewhere (Chen et al., 2018; Hartmann et al., 2016; Zhu et al., 2020).

5. Conclusions

National representative children and adolescents of Korea who participated in KoNEHS Cycle 3 showed that younger children tended to exhibit higher urinary paraben concentrations. Our observations demonstrate that urinary ethyl paraben levels are generally higher than those of other countries including the US and Canada. In addition, among the high exposure group, e.g., 95th percentile, levels of methyl and propyl parabens are higher in the young Korean population. For ethyl paraben, dietary sources such as fast food and canned food consumption were identified as major contributors. For methyl and propyl parabens, in addition to use of personal care and cosmetic products, use of fever medications and ointments was identified as major determinants of exposure, especially among the younger children. Further confirmation of the contribution of these exposure sources is needed. Considering that the young children are more likely to exhibit higher level of exposure and at the same time are expected to possess greater vulnerability to paraben exposure, identification of major exposure pathways for parabens and developing their mitigation measures are important. The present observations will help design further exposure assessments that would confirm major sources of paraben exposure among the children and adolescents of Korea and develop their management policies.

Disclaimer

The results and conclusions in this article are those of the authors and do not necessarily represent the views of the Ministry of Environment and the National Institute of Environmental Research of Korea.

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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.2021.113781.

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Web-based behavioral intervention to reduce exposure to phthalate metabolites, bisphenol A, triclosan, and parabens in mothers with young children: A randomized controlled trial

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ABSTRACT

In this study, a web-based behavioral intervention was designed, which aimed to reduce exposure to phthalate metabolites, bisphenol A, triclosan, and parabens in mothers with young children. A randomized controlled design with two groups was used to verify the effects of the intervention pre- and post-test. In total, 51 mothers participated in the study, categorizing 26 and 25 in the intervention and control groups, respectively. The web-based behavioral intervention focused on changes in diet, personal care products, and health behavior and reinforced behavior through encouragement. This program included an educational video, a game for locating endocrine disruptors at home, a method for locating facilities potentially emitting endocrine disruptors, resources, and a questions and answers mode. Data were collected from May 18 to June 30, 2020. Participants allocated to the intervention group were provided access to the behavioral intervention website via a computer or smartphone. Participants allocated to the control group were sent written information about endocrine disruptors via mail. For both the intervention and control groups, questionnaire results and maternal urine samples were assessed at baseline, during the intervention, and after one month. After the intervention, the urinary concentrations of mono (2-ethylhexyl) phthalate (MEHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), bisphenol A (BPA), methylparaben (MP), ethylparaben (EP), and propylparaben (PP) were found to be significantly decreased in the intervention group. Compared with the control group, the intervention group showed significantly decreased urinary geometric mean values of MEHP, MEOHP, BPA, MP, and PP after one month compared with those during the intervention (3.8%, 16.3%, 28.4%, 9.2%, and 24.4%, respectively). Hence, the web-based behavioral intervention was effective at reducing the exposure to endocrine disruptors in mothers with young children.

1. Introduction

Human health is regulated by the endocrine system, which releases and regulates certain hormones essential for metabolism, growth and development, sleep, and reproduction. Endocrine disrupting chemicals (EDCs), such as phthalates, bisphenol A (BPA), triclosan (TCS), and parabens, adversely affect health by altering or disrupting the functioning of hormonal systems (World Health Organization, 1996). Humans are exposed to EDCs through the ingestion of food and water and exposure to dust, inhalation of airborne gases and particles, and skin contact, which are closely related to the components of daily life, such as diet, food additives, personal care products (PCPs), and cosmetics (Kavlock et al., 2006). Several studies have associated phthalate metabolites, BPA, TCS, and parabens with disorders in the human reproductive system and nervous system development, and attention deficit and hyperactivity disorder, thyroid cancer, and breast cancer (Bai et al., 2015; CDC, 2019; Cho, 2012; Cullen et al., 2017; Fisher et al., 2017; Giulivo et al., 2016; Kim et al., 2018; Kim et al., 2020b; Larsson et al.,

Abbreviations: mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP); mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), methylparaben (MP); ethylparaben (EP), propylparaben (PP); bisphenol A (BPA), endocrine disrupting chemical (EDC); triclosan (TCS), personal care product (PCP); bisphenol S (BPS), di(2-ethylhexyl) phthalate (DEHP); short message service (SMS), limit of detection (LOD); Korean National Environmental Health Survey (KoNEHS), geometric mean (GM); monobutyl phthalate (MBP), monomethyl phthalate (MMP); 2-C-methyl-d-erythritol-2,4-cyclopyrophosphate (MECPP), monoethyl phthalate (MEP); mono-n-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP).

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2014; Ley et al., 2017; World Health Organization, 1996).

Interestingly, these EDCs have a half-life of 6–29 h and most are excreted through the urine (Haines et al., 2017; Koch et al., 2004; LaKind et al., 2019; Larsson et al., 2014; Moos et al., 2015; Völkel et al., 2002). Owing to the short half-life of EDCs, dietary and behavioral interventions, even for a short period, can reduce the exposure to these chemicals (Ackerman et al., 2014; Chen et al., 2015; Harley et al., 2016; Jo et al., 2020; Kim et al., 2020; Rudel et al., 2011; Sears et al., 2020). Chen et al. (2015) reported that handwashing and the reduced use of plastic cups are the most effective interventions among seven one-week strategies for reducing urinary phthalate levels in 4–13-year-old girls. Kim et al. (2020) performed a 3-day dietary intervention for mothers and infants from 37 families (93 people in total). Consequently, BPA levels decreased by 53.1% and bisphenol S (BPS) levels reduced by 63.9% in the maternal urine, and BPA levels decreased by 47.5% in the urine of infants. These findings suggest that dietary or behavioral strategies can effectively minimize exposure to EDCs on an individual level. From the perspective of the social cognition theory, behavioral change begins with the individual's perception of the effect of their behavior on health, and is reinforced by a sense of self-efficacy, such that they are able to perform a specific behavior that will produce desirable results (Middleton et al., 2013). Theory of Planned Behavior is widely used in the public health field as a conceptual framework for achieving human health-promoting behavior, which has been reported the most important variable in the process of intentional human behavioral change (Ajzen, 1985, 1991). These human intentions involve the attitude towards the behavior, subjective norm, and perceived behavioral control (Ajzen and Madden, 1986). Therefore, the intervention strategy should include not only educating subjects with information on exposure to EDCs but also recognizing the risk of exposure to EDCs and enhancing self-efficacy. In addition, mother–infant paired studies on urine concentrations of phthalate metabolites, BPA, TCS, and parabens have been conducted in many countries, and the results have indicated that the concentrations of toxic chemicals in mothers are highly correlated with those in their children (Ait Bamai et al., 2015; Covaci et al., 2015; Cullen et al., 2017; Hlisníková et al., 2019; Kim et al., 2020b; Tratnik et al., 2019). Particularly, infants and toddlers are highly dependent on their mothers, who are usually the primary caregivers (Dennedy and Dunne, 2010); therefore, the behavior of the mother greatly impacts the health of the child.

Previous studies on dietary and behavioral interventions have demonstrated that offline intervention for 3–7 days is effective in reducing EDC levels (Ackerman et al., 2014; Barrett et al., 2015; Chen et al., 2015; Harley et al., 2016; Jo et al., 2020; Kim et al., 2020; Rudel et al., 2011; Sathyanarayana et al., 2013. However, it is effective only for a short time. Behavioral change takes a long time, and long-term offline intervention is expensive and time-consuming. Thus, more effective alternate strategies are needed. A recent meta-analysis reported that web-based online intervention is more effective than offline intervention for changing lifestyle habits (Beleigoli et al., 2019). This web-based intervention has already been proven effective in many public health fields, such as prenatal and postnatal education, and education of cancer survivors and patients with chronic diseases (Jiao et al., 2019; Lee et al., 2014; Wantland et al., 2004). The advantages of web-based programs include their accessibility, user anonymity, and flexibility. In particular, as the participants in these previous studies were mothers who spent most of their time caring for their young children, web-based programs that can be remotely accessed have been shown to be suitable (Lee et al., 2014; Wantland et al., 2004). In addition, some questions are sensitive or personal, which may be difficult to answer in person, and hence, collection of honest and accurate answers is possible in web-based programs owing to user anonymity (Jiao et al., 2019; Wantland et al., 2004). Mothers can freely access the intervention program whenever possible and view or experience the content multiple times (Jiao et al., 2019). Moreover, owing to the restrictions associated with the COVID-19 pandemic, web-based programs are preferred by participants. In Korea, 99.8 and 96% of 20–40-year-old women are computer and smartphone users, respectively (Gallup Korea, 2020). This suggests that this technology is sufficient for a web-based behavioral intervention program that can be easily operated at home, and mothers being familiar with smartphone and internet usage further bolsters the application of these types of programs. Thus, we aimed to develop a web-based behavioral intervention to reduce the exposure of mothers with young infants to EDCs and confirm the effects of the intervention over a period of one month.

2. Materials and methods

2.1. Study design

This study involved the development of a web-based behavioral intervention to reduce exposure to phthalate metabolites, BPA, TCS, and parabens in mothers with young children. A randomized controlled design with two groups was used to evaluate the effects of the intervention pre- and post-test (Fig. 2).

2.2. Participants and randomization

Participants were recruited from 221 individuals who participated in the Endocrine Disruptors Project for Mothers, which prospectively studied the association between 15 toxic chemicals in the breast milk and urine, and the lifestyle of Korean women postpartum (Kim et al., 2020a, 2020b). The participants in this study were mothers with young children. The inclusion criteria were as follows: 1) mothers who stayed with the infant for the majority of the time during the day, and 2) mothers who understood the study purpose and provided informed consent. Mothers with metabolic disturbances or abnormal urine excretion were excluded. Among the 221 participants, 101 had a job and spent most of their time away from their children; six were receiving urinary system treatment, and 52 refused to participate. Subsequently, 62 mothers were randomized to either an experimental or control group by an independent statistician, using a random number function in Microsoft Excel. From five and six subjects in the experimental and control group, respectively, urine samples could not be collected and incomplete questionnaires were received. Finally, 51 mothers participated in this study (Fig. 1). This study was approved by the Institutional Review Board at Kyung Hee University, Seoul, Korea (KHSIRB-20-166).

2.3. Development of a web-based behavioral intervention

This program focused on the individuals' behavior (dietary habits, PCPs, and health) to reduce exposure to phthalate, BPA, TCS, and parabens, which was developed using previous evidence-based research. A literature review on endocrine disruptors (phthalate metabolites, BPA, TCS, and parabens) was conducted and the need for education was investigated to develop the intervention. Basic data and policies for these chemicals were retrieved using a search engine, including PubMed, as well as information provided by the World Health Organization (WHO), US Environmental Protection Agency (US EPA), US Food and Drug Administration (US FDA), and European Environment Agency (EEA). To develop an intervention program, we reviewed 12 studies published in the past decade. Among these, seven involved dietary interventions, two involved interventions against PCPs, two involved interventions against residential environments, and one involved both dietary and PCP interventions (Table 1). We encouraged the participants to eat fresh organic foods when possible, avoid foods containing high levels of fat and dairy products such as cheese and ice cream (Barrett et al., 2015; Dong et al., 2017; Harley et al., 2016; Hlisníková et al., 2019; Jo et al., 2016, 2020; Kim et al., 2020, 2020a; Larsson et al., 2014), and use stained glass or glassware instead of plastic products for cooking. Some chemicals such as phthalate are not chemically bound to the polymer; hence, they can be easily outgassed and inhaled into the human body (Koch and Calafat, 2009). Therefore, we requested mothers

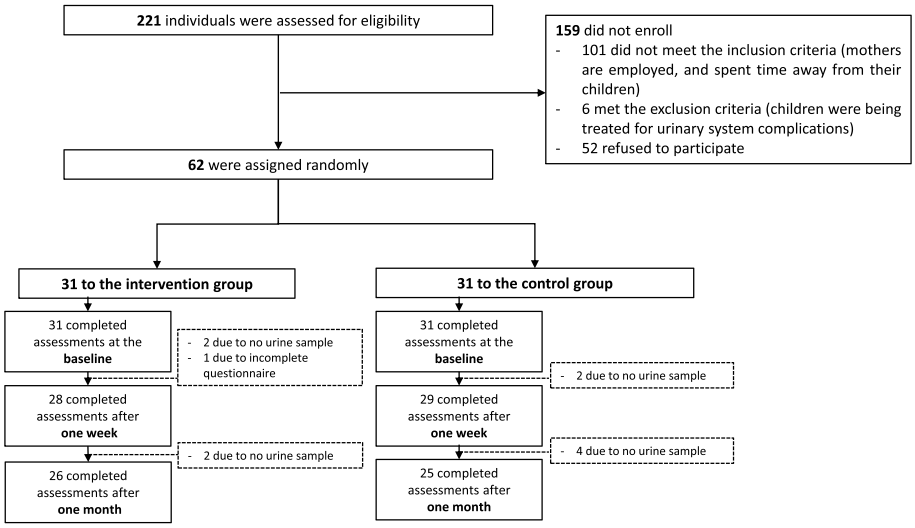

Fig. 1. Participant flow diagram.

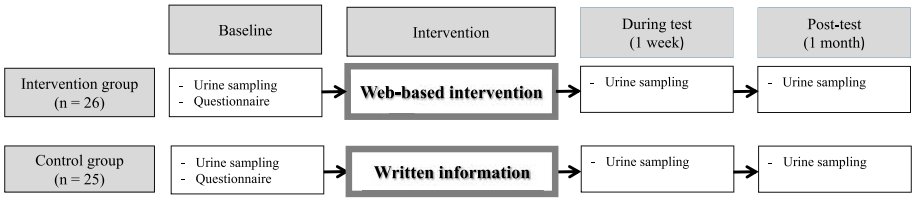

Fig. 2. Study design.

to avoid using new furniture and cars during the intervention period and refrain from using PCPs and cosmetics with strong fragrances and colors, as reported previously (Berger et al., 2019; Chen et al., 2015; Dodson et al., 2020; Kim et al., 2020a; Larsson et al., 2014; Nassan et al., 2017). In our intervention, we also encouraged participants to sweat for at least 30 min as a daily health activity, and frequently wash their hands and drink water to induce the release of endocrine disruptors, as reported previously (Kim et al., 2020a; Dong et al., Larsson et al., 2014). Based on previous research (Biedermann et al., 2010; Ferguson et al., 2017; Geens et al., 2012; Li et al., 2021; Liao and Kannan, 2014; Martínez et al., 2018; Mielke et al., 2011; Serrano et al., 2014), an intervention was developed that involved the following criteria: 1) intake of organic foods, 2) reduction in fish intake, 3) reduction in animal fat intake, 4) reduction in dairy products (cheese and ice cream) intake, 5) avoiding the use of new cars and new furniture, 6) using stainless steel and glassware for cooking, 7) avoiding the use of strong scented PCPs and cosmetics, 8) reduction in the use of color makeup, 9) exercising/sweating for more than 30 min daily, and 10) washing hands frequently.

To implement the behavioral intervention more effectively, we developed a web-based intervention program, which consisted of the following five components: an educational video explaining the health effects of endocrine disruptors as well as steps to reduce exposure to them; a game to find items containing endocrine disruptors at home; a search for facilities that release endocrine disruptors; resources; and a questions and answers mode (Q&A). The educational materials were validated by five experts. Moreover, the developed web-based program was shown to five mothers and their feedback on understanding the content, image speed, text, and effect processing was collected. The contents of the final web-based program are described in Supplementary Fig. S1.

2.4. Data collection

Data were collected from May 18 to June 30, 2020, to study the effects of the web-based behavioral intervention program on the participants' exposure to EDCs. Participants allocated to the intervention group were provided access to a behavioral intervention website via a computer or smartphone. Participants increased their EDC risk awareness by exploring facilities and materials, studying the videos, and asking questions via the Q&A mode. We encouraged the participants to access the web-based program at least three times a week. Furthermore, the researcher reinforced their behavior via weekly short message service (SMS) and phone calls for a month. We sent a message to the participants three times a week on the guidelines for preventing exposure to endocrine disruptors and encouraged them through phone calls once a week. In addition, we frequently communicated with the participants about precautions to be taken when collecting urine samples through SMS, allowing immediate feedback. Participants allocated to the control group were sent written information about endocrine disruptors by mail. The written information included the definition, forms, health effects, and methods of prevention of exposure to endocrine disruptors (Supplementary Fig. S2). They were provided access to a web-based intervention program upon completion of data collection for this study. Participants in both the intervention and control groups completed the questionnaire and provided urine samples for measurements at baseline (T1) and during the intervention at the end of week 1 (T2). Subsequently, urine samples were collected at the end of 1 month of the intervention and reinforcement (T3). The questionnaires included general characteristics, such as age, education level, household income, residential area, infant sex, and infant age. Samples (20 mL) were collected from the first morning urine in a container without exposure to endocrine disruptors and refrigerated immediately.

Table 1
Interventional studies on endocrine disruptors.

	Author (year)	Research design	Participants	Intervention period	Intervention	Analyte(s)
1	**This study (2021)**	Pre- and post-intervention, randomized controlled trial	51 mothers with infants (26 experimental/25 control)	4 weeks	**Web-based behavioral intervention** - dietary habits, personal care products, and health behavior	MEHP, MEOHP, MEHHP, BPA, TCS, parabens
2	Sears et al. (2020)	Pre- and post-intervention, randomized controlled trial	288 infants	NA	**Residential intervention** - removal of lead hazards in paint, dust, water, and soil in and around the home, extensive cleaning, and removal of dust - paint stabilization, involving repairing any deteriorating or water-damaged wall material, removing loose or peeling paint, reapplying paint, and thoroughly cleaning the area using wet methods	MEHHP, MEOHP, MEHP, MECPP, MEP, BzP, MCOP, MCNP, MiBP, MnBP
3	Kim et al. (2020)	Pre-, mid-, and post-intervention, one group	93 participants (37 mothers and their 56 infants)	3 days	**Dietary intervention** - refrain from canned foods and drinks - refrain from foods in plastic packaging, including takeaway and instant foods - refrain from bottled water -consume fresh home-cooked foods	BPA, BPS
4	Jo et al. (2020)	Pre- and post-intervention, one group	25 temple-stay participants	5 days	**Dietary intervention** - strict dietary replacement during temple stay, with Buddhist vegetarian diet excluding meat, eggs, dairy, and fish products.	Parabens
5	Rutkowska et al. (2020)	Pre- and post-intervention, one group	26 participants from 9 households	6 months	**Residential intervention** - introduction of recommended lifestyle changes to lower exposure to selected endocrine disruptors in the indoor home environment	BPA, BPS, 4-NP, DEP, DiBP, DEHP
6	Ley et al. (2017)	Cohort design, randomized trial	154 pregnant females (78/76)	NA	**Personal care products intervention** - participants provided commercially available wash products (liquid and bar soap, toothpaste, dishwashing liquid), which did or did not contain TCS	Triclosan, triclocarban
7	Galloway et al. (2018)	Pre- and post-intervention, one group	94 students	7 days	**Dietary intervention** - consuming a diet designed to reduce the consumption of BPA by avoiding processed foods and foods packaged in known sources of BPA - minimized intake of known sources of BPA according to a set of guidelines	BPA
8	Harley et al. (2016)	Pre- and post-intervention, one group	100 girls	3 days	**Personal care products intervention** - small polyethylene containers of shampoo, conditioner, body wash, and moisturizing lotion, a bar of hand soap, a container of liquid soap, and roll-on deodorant - four items from among liquid or powder foundation, mascara, eyeliner, lipstick/lip gloss/lip balm, and sunscreen	Phthalate, parabens, TCS, BP-3
9	Chen et al. (2015)	Pre- and post-intervention, one group	30 girls	7 days	**Intervention strategy** - nutrition supplements and medication, cosmetic and personal care products, plastic containers, microwaved food, food contained in a plastic bag or plastic wrapping, building material and handwashing, time-activity pattern	MMP, MEP, MBP, MBzP, MEHP, MEHHP, MEOHP, MECPP
10	Barrett et al. (2015)	Pre-, mid-, and post-intervention, one group	10 pregnant females	3 days	**Dietary intervention** - balanced diet intended to minimize dietary phthalate exposure	MEHHP, MEOHP, MEHP, MECCP, MBP, MEP, MiBP, MBzP, MCPP, MCOP, MCNP
11	Ackerman et al. (2014)	Pre- and post-intervention, one group	5 families, 20 participants	3 days	**Dietary intervention** - foods provided by a caterer, prepared from fresh ingredients (no canned or frozen foods), and packaged almost exclusively without contact with plastic	MEHHP, MEOHP, MEHP
12	Sathyanarayana et al. (2013)	Pre- and post-intervention,	10 families, 40 participants	5 days	**Dietary intervention**	Phthalate, BPA

(*continued on next page*)

Table 1 (*continued*)

	Author (year)	Research design	Participants	Intervention period	Intervention	Analyte(s)
		randomized, two arms			- complete dietary replacement with fresh and organic catered foods prepared without plastic	
13	Rudel et al. (2011)	Pre- and post-intervention, one group	20 participants	3 days	**Dietary intervention** - foods provided by a caterer, prepared from fresh ingredients (no canned or frozen foods), and packaged almost exclusively without contact with plastic	BPA, phthalate

2.5. Laboratory analysis of chemicals

To determine the extent of EDC exposure in this study, the concentrations of mono (2-ethylhexyl) phthalate (MEHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), BPA, TCS, MP, PP, and EP were quantitatively analyzed in the urine samples using previously reported procedures (Alves et al., 2016a, 2016b; Atkinson and Roy, 1995; Brotons et al., 1995; Colborn, 1995; Colerangle and Roy, 1997; Yamamoto and Yasuhara, 1999). All chemicals, reagents, and solvents were of analytical grade or higher quality. MEHP, MEHHP, and MEOHP were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA) and BPA, MP, EP, PP, and TCS were purchased from Sigma-Aldrich (St Louis, MO, USA). Individual isotope-labeled internal standards, $^{13}C_2$-MEHP, $^{13}C_4$-MEOHP, and $^{13}C_2$-MEHHP were purchased from Cambridge Isotope Laboratories, Inc., and $^{13}C_{12}$-BPA, $^{13}C_6$-MP, $^{13}C_6$-EP, and $^{13}C_6$-PP were purchased from Wellington Laboratories (Ontario, Canada). The limits of detection (LODs) were as follows: MEHP, 0.21 μg/g; MEOHP, 0.09 μg/g; MEHHP, 0.12 μg/g; BPA, 0.02 μg/g; MP, 0.09 μg/g; EP, 0.16 μg/g; PP, 0.10 μg/g; and TCS, 0.04 μg/g. The enzyme solution for hydrolysis was prepared by dissolving β-glucuronidase mixture from *Helix pomatia* (Sigma-Aldrich). β-Glucuronidase (*Escherichia coli* K12) was obtained from Roche Diagnostics GmbH (Mannheim, Germany). HPLC-grade water and acetonitrile were obtained from Merck. Hydrogen chloride, methyl tertiary butyl ether, ammonium acetate, and acetic acid were purchased from Sigma-Aldrich. To prepare samples, 1 mL of each sample (or blank, calibration curve level, and quality control) was added to 500 μL of ammonium acetate buffer (1 M, pH 6.5) and 5 μL of the internal standard working solution. Next, 30 μL of β-glucuronidase from *E. coli* K12 was added, and the solution was vortexed and incubated at 37 °C for 4 h. After incubation, 100 μL of 2 N HCl was added and the mixture was extracted using 3 mL of ethyl acetate. Further, 2 mL of supernatant was transferred to a new glass tube; the solution was evaporated; and the residue was dissolved in 300 μL of 60% acetonitrile. We used 5 μL of the resultant mixture for high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) analysis. All chemicals were analyzed using HPLC. For phthalate metabolites, the chromatographic separation was performed using an ACE Excel 2 C18-AR column (150 × 2.1 mm; particle size 2 μm; Advanced Chromatography Technologies, Ltd.). The CTC-PAL Leap cooling unit was set at 4 °C, and the sample injection volume was 10 μL. The mobile phase included 0.012% acetic acid in water (solvent A) and 0.012% acetic acid in acetonitrile (solvent B) passed through the column at a flow rate of 4 mL/min. The mobile phase gradient was as follows: 2% solvent B for 0.0 min; increased linearly to 80% solvent B from 0.1 min; maintained at 98% solvent B from 0.1 to 12.0 min; returned to 2% solvent B from 12.1 to 15.5 min; and finally, the gradient was maintained from 15.6 to 18.0 min. For phenols, chromatographic separation was performed on an ACE Excel 2 C18-AR column (150 × 2.1 mm; particle size 2 μm, Advanced Chromatography Technologies, Ltd.). The CTC-PAL Leap cooling unit was set at 4 °C and the sample injection volume was 3 μL. The mobile phase included 0.012% acetic acid in water (solvent A) and 0.012% acetic acid in acetonitrile (solvent B) passed through the column at a flow rate of 4 mL/min. The mobile phase gradient was as follows: 2% solvent B for 0.0 min; increased linearly to 80% solvent B from 0.1 min; maintained at 98% solvent B from 0.1 to 12.0 min; returned to 2% solvent B from 12.1 to 15.5 min; and lastly, the gradient was maintained from 15.6 to 18.0 min.

2.6. Statistical analysis

Concentrations below the LOD were assigned a proxy value as the LOD divided by the square root of two (Hornung and Reed, 1990). In accordance with the WHO criteria, spot urine samples with creatinine concentration as very dilute (<0.3 g/L) or concentrated (>3.0 g/L) were excluded from the analysis (World Health Organization, 1996). General characteristics of the subjects are presented using descriptive statistics, including frequency, percentage, average, geometric mean (GM), median, and standard deviation. The normality of distribution of variables (maternal age, education level, household income, resident area, infant sex, and infant age) was tested using the Shapiro–Wilk test. For verification of the homogeneity in baseline characteristics of the intervention and control groups, we performed a parametric test (Student's t-test, chi-square test) when variables followed a normal distribution through the normality test (maternal age, infant age, and household income), or a non-parametric test (Mann–Whitney *U* test) when variables did not follow a normal distribution (education level, residential area, and infant sex). We performed the Wilcoxon matched-pairs signed-rank test to assess if there was a difference in urinary concentration of chemicals before and after the intervention between the intervention and control groups. We used a mixed-effect regression model to determine if there was a difference in the urinary concentration of chemicals between the two groups with time after adjustment for age, education level, and household income. Statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Homogeneity of the baseline characteristics

We randomized 26 and 25 participants to the intervention and control groups, respectively, meeting the inclusion and exclusion criteria from among 221 subjects who participated in the Endocrine Disruptors Project for Mothers. We collected data on age, educational level, monthly income, residential area, infant sex, and infant age from both these groups. We found no significant differences in the baseline characteristics, including age, educational level, monthly income, residential area, infant sex, and infant age between the intervention and control groups (Table 2).

3.2. Chemical concentrations

We analyzed the concentrations of EDCs in the urine of participants in the intervention and control groups. Urinary creatinine-adjusted concentrations of chemicals measured pre-intervention are displayed in Table 3. MEHP, MEOHP, MEOHP, BPA, TCS, and parabens were detected in 80–100% of samples from the 51 subjects, with median concentrations of 0.14–41.86 μg/g and GM concentrations of

Table 2
Baseline characteristics of participants in the intervention and control groups.

Characteristic	Category	Intervention group (n = 26) n (%)/mean ± SD	Control group (n = 25) n (%)/mean ± SD	t/ x^2/ U	*p*-value
Age (years)		35.8 ± 3.9	35.1 ± 2.9	0.06	0.951
Education level	<College	4 (15.4)	5 (20.0)	0.97	0.156
	≥College	22 (84.6)	20 (80.0)		
Household income (USD/month)	<5000	13 (50.0)	14 (56.0)	2.43	0.407
	≥5000	13 (50.0)	11 (44.0)		
Residential area	Metropolitan	15 (57.7)	13 (52.0)	0.31	0.659
	Non-metropolitan	11 (42.3)	12 (48.0)		
Infant sex	Male	11 (42.3)	12 (48.0)	1.78	0.890
	Female	15 (57.7)	13 (52.0)		
Infant age (months)		22.3 ± 1.7	22.1 ± 1.8	0.38	0.705

SD, standard deviation; the Student's t-test, chi-square test, and Mann–Whitney *U* test were performed for testing homogeneity of baseline characteristics.

0.12–50.27 μg/g. EP concentration was detected at the highest level (GM: 50.27 μg/g), followed by MP (GM: 17.25 μg/g), MEHHP (8.44 μg/g), and MEOHP (GM: 4.24 μg/g). The concentrations of MEOHP, MEHHP, BPA, MP, EP, and PP were lower than those reported in the third Korean National Environmental Health Survey (KoNEHS) conducted in 2017 (12.1 μg/g, 16.1 μg/g, 1.18 μg/g, 41.7 μg/g, 39.3 μg/g, 3.9 μg/g, respectively, in median value) (Table 3).

3.3. Change in urinary concentrations of chemicals in the intervention and control groups

Table 4 shows the changes in the geometric mean (GM) of chemical concentrations before (T1) and after (T3) the intervention in the intervention and control groups. After the intervention, the urinary concentrations of MEHP, MEOHP, BPA, MP, EP, and PP decreased significantly in the intervention group ($p = 0.011$, $p = 0.036$, $p = 0.039$, $p = 0.013$, $p < 0.001$, and $p = 0.044$, respectively), whereas only the concentration of EP decreased significantly ($p = 0.044$) and that of MP increased significantly ($p = 0.036$) in the control group (Table 4).

Table 5 shows the changes in the GM of chemical concentrations before the intervention (T1) and during the intervention (T2) and the changes during and after the intervention (T3) in the intervention and control groups. The percentage change in the GM BPA concentration was only statistically significant in the intervention group between T1 and T2 (−35.5%, $p = 0.033$). Additionally, the urinary GM values for MEHP, MEOHP, BPA, MP, and PP were significantly lower (MEHP: −3.8%, MEOHP: −16.3%, BPA: −28.4%, MP: −9.2%, and PP: −24.4%) at T3 than at T2 in the intervention group compared with the control group. In the control group, urinary GM values of most chemicals, excluding BPA, MEOHP, and MEHHP, were increased from T2 to T3 (Table 5).

4. Discussion

In this study, we found that the urinary concentrations of six EDCs (MEHP, MEOHP, BPA, MP, EP, and PP) were significantly decreased in the intervention group after a month of intervention compared with those in the control group. Comparing the effects of the intervention between the two groups is difficult because most previous interventional studies have been performed using a single group. In this study, the urinary concentrations of phthalate metabolites (MEHP and MEOHP)

Table 3
Urinary creatinine-adjusted concentrations of chemicals measured pre-intervention (n = 51) in comparison with KoNEHS.

Analyte (μg/g creatinine)	LOD	% >LOD	GM	Percentile (μg/g)						KoNEHS*
				Min	25th	50th	75th	95th	Max	Median
MEHP	0.21	98	2.71	<LOD	1.28	2.42	3.82	6.67	14.51	–
MEOHP	0.09	93	4.24	<LOD	1.74	4.01	4.39	10.83	33.40	12.1
MEHHP	0.12	100	8.44	1.83	4.21	8.57	9.96	16.49	58.59	16.1
BPA	0.02	81	0.84	<LOD	0.3	0.71	1.37	2.78	8.92	1.18
TCS	0.04	80	0.12	<LOD	0.06	0.14	0.32	1.08	6.44	–
MP	0.09	84	17.25	<LOD	4.79	12.53	50.26	568.31	953.06	41.7
EP	0.16	100	50.27	1.17	12.59	41.86	154.24	868.29	2742.91	39.3
PP	0.1	92	0.63	<LOD	0.12	0.42	2.13	31.58	110.23	3.9

KoNEHS, third Korean National Environmental Health Survey (2017); LOD, limit of detection; GM, geometric mean; MEHP, mono (2-ethylhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl); BPA, bisphenol A; TCS, triclosan; MP, methylparaben; EP, ethylparaben; PP, propylparaben.

Table 4
Urinary concentration changes in chemicals pre- (T1) and post-intervention (T3) in the intervention (n = 26) and control groups (n = 25).

Analyte (μg/g creatinine)	Intervention group, GM (95% CI)			Control group, GM (95% CI)		
	Pre-intervention (T1)	Post-intervention (T3)	*p*-value	Pre-intervention (T1)	Post-intervention (T3)	*p*-value
MEHP	2.65 (1.07–13.09)	2.20 (1.02–7.31)	0.011	2.76 (1.26–14.51)	2.67 (1.87–13.84)	0.318
MEOHP	4.28 (1.70–30.92)	3.33 (1.07–13.96)	0.036	4.20 (1.67–33.40)	3.99 (1.01–33.61)	0.412
MEHHP	8.60 (2.87–43.10)	7.63 (3.14–51.02)	0.051	8.28 (1.83–58.59)	8.22 (1.13–68.88)	0.827
BPA	0.87 (0.53–8.92)	0.40 (0.23–4.11)	0.039	0.80 (0.07–5.90)	0.64 (0.07–5.12)	0.055
TCS	0.13 (0.01–6.39)	0.12 (0.06–10.33)	0.494	0.12 (0.04–6.44)	0.15 (0.03–10.20)	0.262
MP	16.76 (3.86–639.94)	11.0 (3.55–111.62)	0.013	17.76 (3.64–953.06)	20.09 (3.98–771.12)	0.044
EP	51.38 (1.17–2742.91)	33.0 (14.67–566.31)	<0.001	49.16 (2.07–2401.07)	42.00 (11.07–813.91)	0.036
PP	0.70 (0.02–87.19)	0.31 (0.17–43.15)	0.044	0.60 (0.08–110.23)	0.64 (0.04–113.62)	0.437

Pre-intervention, baseline measurement before the intervention (T1); post-intervention, measurement at the end of 1 month of the intervention (T3); CI, confidence interval; GM, geometric mean; MEHP, mono (2-ethylhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl); BPA, bisphenol A; TCS, triclosan; MP, methylparaben; EP, ethylparaben; PP, propylparaben.

Table 5
Change in geometric mean concentrations of phthalate metabolites, BPA, TCS, and parabens in the intervention and control groups over time.

	% change from T1 to T2 (T1 GM–T2 GM)			% change from T2 to T3 (T2 GM–T3 GM)		
Analyte (μg/g creatinine)	Intervention group	Control group	*p*-value	Intervention group	Control group	*p*-value
MEHP	−13.6 (2.65–2.29)	−5.4 (2.76–2.61)	0.491	−3.8 (2.29–2.20)	2.4 (2.61–2.67)	0.013
MEOHP	−7.0 (4.28–3.98)	−4.5 (4.20–4.01)	0.677	−16.3 (3.98–3.33)	−0.5 (4.01–3.99)	0.046
MEHHP	- 7.1 (8.60–7.99)	−0.4 (8.28–8.25)	0.092	−4.5 (7.99–7.63)	−0.4 (8.25–8.22)	0.299
BPA	−35.5 (0.87–0.56)	−11.3 (0.80–0.71)	0.033	−28.4 (0.56–0.40)	−9.9 (0.71–0.64)	0.038
TCS	2.4 (0.13–0.14)	17.2 (0.12–0.14)	0.721	−12.6 (0.14–0.12)	6.7 (0.14–0.15)	0.127
MP	−27.7 (16.76–12.11)	−15.3 (17.76–15.04)	0.091	−9.2 (12.11–11.00)	33.6 (15.04–20.09)	<0.001
EP	−38.5 (51.38–31.59)	−22.9 (49.16–37.88)	0.051	4.5 (31.59–33.00)	10.9 (37.88–42.00)	0.067
PP	−41.3 (0.70–0.41)	−34.1 (0.60–0.39)	0.088	−24.4 (0.41–0.31)	62.2 (0.39–0.64)	<0.001

Mixed-effect regression model examining the changes in geometric mean concentrations between two time points in the two groups after adjusting for age, education level, and monthly income. *P*-value for the interaction term group*time; T1, measurement at baseline before intervention; T2, measurement at 1 week during the intervention; T3, measurement at the end of 1 month of the intervention; MEHP, mono (2-ethylhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl); BPA, bisphenol A; TCS, triclosan; MP, methylparaben; EP, ethylparaben; PP, propylparaben.

were significantly decreased in the intervention group compared with the control group, which is comparable to the findings of previous studies (Ackerman et al., 2014; Chen et al., 2015; Harley et al., 2016; Rudel et al., 2011; Sears et al., 2020). Harley et al. (2016) found that the concentration of monoethyl phthalate (MEP) decreases significantly by 27.4% after a 3-day PCP intervention, while there are no significant changes in the concentrations of mono-n-butyl phthalate (MnBP) and mono-isobutyl phthalate (MiBP). Chen et al. (2015) proposed seven intervention strategies that combine diet and PCPs, and showed that hand washing, consuming fewer beverages in plastic cups, and using lower amounts of shampoo and shower gel reduces the urinary concentrations of monobutyl phthalate (MBP), monomethyl phthalate (MMP), MEHHP, 2-C-methyl-d-erythritol-2,4-cyclopyrophosphate (MECPP), and MEP. Two studies involving dietary interventions of fresh organic food and the avoidance of plastic packaging also reported a significant decrease of over 50% in the DEHP concentration in urine after the intervention (Ackerman et al., 2014; Rudel et al., 2011). However, several studies have reported conflicting results (Barrett et al., 2015; Sathyanarayana et al., 2013). Notably, Sathyanarayana et al. (2013) reported that the urinary DEHP concentration increased unexpectedly from a median of 283.7–7027.5 nmol/g after dietary intervention. The authors suspected that food contamination or ineffective self-guided intervention could account for these results. Therefore, a tailored and detailed system, such as a web-based program, is necessary to provide self-guided intervention and allow users to access and obtain information from anywhere at any time.

The urinary concentration of BPA was found to be significantly decreased in the intervention group compared with that in the control group, which was consistent with the results of previous studies (Kim et al., 2020; Rudel et al., 2011; Rutkowska et al., 2020). Kim et al. (2020) showed that the urinary BPA concentration decreases in both mothers (53.1%) and children (47.5%) after a 3-day dietary intervention. In addition, the frequent consumption of canned food, take-out drinks, and fast food has been associated with an increase in BPA concentration. In the studies of Rutkowska et al. (2020) and Rudel et al. (2011), the urinary concentration of BPA has been shown to be significantly decreased through dietary interventions and lifestyle changes, respectively. However, Sathyanarayana et al. (2013) reported that the urinary BPA concentration increases significantly after a 7-day dietary intervention, whereas Galloway et al. (2018) reported that dietary intervention has no effect on the BPA concentration. These discrepancies could be explained by either a non-stringent intervention or contamination of the food used.

The concentration of TCS in urine did not significantly change in the experimental and control groups before and after the intervention. This was inconsistent with the results of previous studies (Harley et al., 2016; Ley et al., 2017). Harley et al. (2016) confirmed that TCS concentration decreases significantly before and after intervention in a group using toothpaste containing TCS and reported that toothpaste is a major source of TCS exposure. Ley et al. (2017) also reported that the decrease in TCS concentration after intervention in the group using PCPs containing TCS is 7–10-fold higher than that in the group using PCPs without TCS. Although the US FDA banned the use of TCS in wash products in 2016 and in hospital products in 2018, owing to its toxicity, this compound is still used in some toothpastes, mouthwashes, and deodorants (Berger et al., 2019). This may explain the contradiction in the results obtained in the present and previous studies, as the daily use of toothpaste and mouthwash was not prohibited in the present study. However, TCS concentrations from T2 to T3 in the intervention group decreased by 12.6% and that in the control group increased in this study. TCS is used as a preservative in many PCPs, such as makeup products, soaps, and deodorants (Bever et al., 2018; Toms et al., 2011). Our intervention included avoiding the use of PCPs with strong scents and colors, but it would not be easy for subjects to replace their products with alternatives in a short period of time because most PCPs, including TCS-containing products, are a daily necessity. We believe this is the reason behind the urinary TCS concentration after the intervention not decreasing significantly. As the use of these products for personal hygiene is steadily rising, further research is needed to determine whether they are the main sources of TCS exposure.

In this study, the urine concentrations of MP, EP, and PP decreased significantly in the intervention group, which was consistent with the findings of Harley et al. (2016), who conducted an interventional study on the use of PCPs for 3 days in 100 girls. Interestingly, the concentrations of MP and PP decreased continuously from T2 to T3, whereas the concentration of EP increased from T2 to T3. This may be because MP and PP are the most frequently used materials in PCPs such as sunscreens, hand/body lotions, and shampoos, whereas EP is mainly used as a food preservative (Harley et al., 2016; Nassan et al., 2017). Koreans have unique cultural eating habits, including the use of traditional fermented cabbage with seasoning (*Kimchi*) and traditional pepper/-bean/soy seasoning (*Gochujang*, *Doenjang*, and *Ganjang*) (Jo et al., 2020). The *Gochujang* and soy sauce sold in Korea are reported to contain up to 29.7 mg/kg of EP (Choi et al., 2008; Jo et al., 2020). For this reason, many studies have reported that the urinary concentration of EP is higher in Koreans than in individuals from other countries (Honda et al., 2018; Kang et al., 2016; Kim et al., 2018, 2020b). Therefore, studies should be undertaken to investigate additional causes resulting in high levels of EP in traditional Korean foods.

Our study has a few limitations. Firstly, although we demonstrated that web-based behavioral programs were effective at reducing exposure to EDCs, the sample size used in this study was relatively small to allow for generalizations. However, when assuming a significance level of 0.05, a power of 0.85, a medium effect size of 0.2, two groups, three measurement time points (baseline, 1 week, 1 month), and a 0.5 correlation between points using the G-power 3.1.9.4 program, the target sample size for the experiment was 48 subjects. Hence, in this study, the sample size was sufficient to determine the effects of the intervention on reducing the exposure of mothers with young infants to phthalate

metabolites, BPA, TCS, and parabens. Secondly, we compared the concentration of EDCs in morning spot urine samples before and after the intervention based on a previous study, which showed the first morning urine to have a higher reproducibility than the lunch-time and bed-time urine (Kim et al., 2020b). However, the levels of chemicals with a short half-life may also show inter- and intra-day variations (Kim et al., 2020b; Koch et al., 2004; LaKind et al., 2019). Hence, additional studies are needed to measure the effectiveness of web-based interventions, not only using morning urine, but also using urine samples obtained at lunch or before bedtime. Lastly, we confirmed the effect of the web-based behavioral intervention program only by noting changes in the concentration of EDCs in the urine. In the future, it is necessary to investigate the changes in subjects' health behavior during the intervention period and the barriers and facilitators for the behavior change.

5. Conclusions

This study was conducted to develop a web-based behavioral intervention and confirm the effectiveness in reducing EDC exposure by evaluating the urinary concentration of MEHP, MEOHP, MEHHP, BPA, TCS, and parabens after a month. The web-based intervention was developed based on evidence from previous studies (Berger et al., 2019; Chen et al., 2015; Dodson et al., 2020; Kim et al., 2020a; Larsson et al., 2014; Nassan et al., 2017), including dietary, PCP, and health-promoting interventions, and reinforced through SMS and phone calls. After the intervention, the urinary concentrations of MEHP, MEOHP, BPA, MP, EP, and PP decreased significantly in the intervention group. These findings suggested that web-based behavioral intervention was effective for reducing the exposure to EDCs among mothers with young children. In the future, the web-based intervention program would be broadened and customized to each life cycle. Additionally, it will be necessary to develop an integrated program to reduce the exposure to environmentally harmful factors through the development of programs for EDCs as well as other environmental hazards, including air pollution.

Author contributions

Conceptualization and methodology, JHK; investigation, JHK, JMK, and HK; formal analysis, JHK and JMK; visualization, resources, and data curation, JMK and HK; validation, JHK and JMK; writing—original draft, JHK; writing—review and editing, JHK, JMK, and HK; supervision, JHK; project administration, JHK; funding acquisition, JHK. All authors have read and agreed to the published version of the manuscript.

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Declaration of competing interest

The authors declare no conflict of interest.

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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.2021.113798.

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