Association of neighborhood greenness exposure with cardiovascular diseases and biomarkers

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ABSTRACT

Aim: Living in areas with neighborhood greenness may be associated with the incidence of cardiovascular diseases (CVDs). However, little evidence in this regard has emerged from developing countries. In the present study, we examined neighborhood greenness associated with CVDs and the lipid accumulation product (LAP) and pulse pressure (PP) in China.

Methods: We undertook our analysis using a community cross-sectional survey conducted in Longzihu District of Bengbu from July to August 2015. We measured triglyceride levels, waist circumference, and blood pressure. To assess exposure to neighborhood greenness, we used the average normalized difference vegetation index (NDVI) at 1,000-, 1,500-, and 2,000-m buffers in the participant community. We employed generalized mixed models to determine the association among neighborhood greenness, CVDs, LAP, and PP. We conducted stratified analysis by age, gender, income, and education. We assessed the potential mediating effects of road proximity and physical activity on greenness and CVDs, PP, and LAP.

Results: The highest tertiles of $NDVI_{1500-m}$ were steadily and significantly associated with lower odds of CVDs prevalence: the adjusted OR of such prevalence was 0.612 (95% CI, 0.462–0.811); higher NDVI was significantly associated with lower PP levels. The NDVI was strongly associated with CVDs prevalence among participants who were male and had high income. Ambient road proximity significantly mediated 9.7% of the estimated association between greenness and PP, there was no evidence of mediation effects for physical activity.

Conclusions: Higher neighborhood greenness could have a beneficial effect on CVDs and biomarkers. There were higher associations between residential greenness and CVDs among male and higher-income individuals; road proximity partially mediated the observed association between greenness and PP.

1. Introduction

The World Health Organization (WHO) reports that cardiovascular diseases (CVDs), including coronary heart disease (heart attacks), cerebrovascular disease (stroke), and raised blood pressure (hypertension), are globally the biggest cause of death (Joseph et al., 2017). In China, the prevalence and mortality of CVDs are continuously rising. The number of CVD patients in China in 2016 amounted to 290 million; the number of CVDs deaths was 17.9 million (Zhou et al., 2019). The occurrence and development of CVDs are related to the interaction between individual lifestyles and their environment; the ecological environment plays a crucial role with respect to the above risk factors. From a public health perspective, identifying the relationship between CVDs and environmental factors is particularly important; that relationship can be modified by changes in individual lifestyles or population-level government policies.

In recent decades, a population's exposure to the natural environment has been found to have a possible causal relationship with health outcomes; that has become a focus in the growing field of environmental epidemiology (Markevych et al., 2017). That relationship includes the results of cardiovascular health, such as the following: prevalence of CVDs (Astell-Burt and Feng, 2020; Chum and O'Campo, 2015; Massa et al., 2016; Ngom et al., 2016; Picavet et al., 2016; Yang et al., 2020b; Yitshak-Sade et al., 2017); CVDs and mortality (Lachowycz and Jones, 2014; Mitchell and Popham, 2008; Schanzer et al., 2015; Silveira and Junger, 2018; Vienneau et al., 2017; Wang et al., 2017; Wilker et al., 2014); and CVDs risk factors (Seo et al., 2019; Wang et al., 2019). Many

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Abbreviations

CVDs	cardiovascular diseases
CHD	coronary heart disease
LAP	lipid accumulation product
WC	waist circumference
TG	triglyceride
SBP	systolic blood pressure
DBP	diastolic blood pressure
BP	blood pressure
PP	pulse pressure
NDVI	normalized difference vegetation index
OR	odds ratio
CI	confidence interval
WHO	World Health Organization

studies have examined the CVDs associations of greenness. However, the results have been somewhat inconsistent. Studies have mainly focused on the relationship among neighborhood greenness, CVDs mortality, and CVDs morbidity; research has been conducted in Western developed countries, rarely in developing countries.

In environmental epidemiological studies, CVDs markers are often considered as intervention variables in the association between environmental exposure and CVDs (Yang et al., 2020a). The lipid accumulation product (LAP) and pulse pressure (PP) have gained attention as predictors of cardiovascular biomarkers. In 2005, Kahn proposed a relatively new CVD risk factor index—LAP—as a simple indicator of excessive fat accumulation in adults. LAP is an indicator based on a combination of waist circumference (WC) and fasting triglyceride levels (Kahn, 2005). Studies have shown that LAP is better than other indicators in predicting the risk of CVDs (Etyang et al., 2016; Hosseinpanah et al., 2016; Kyrou et al., 2018; Wiltgen et al., 2009). PP is the difference between systolic blood pressure (SBP) and diastolic blood pressure (DBP), and it is an easily measurable parameter in basic health evaluation. Both SBP and DBP increase with age up to the 6th decade of life. Thereafter, SBP continuously increases; DBP remains constant or decreases, resulting in a rise in PP (Etyang et al., 2016). PP is a key variable associated with blood pressure; it is an important risk factor for CVDs, including hypertension, stroke, and myocardial infarction death (Glasser et al., 2014; Said et al., 2018). Previous studies have focused only on the relationship between the composition of LAP, PP, and greenness. For example, Yang et al. examined the relationship between greenness and triglyceride (TG) levels (Yang et al., 2019a); higher neighborhood greenness was found to be associated with lower SBP levels and decreased prevalence of hypertension (Yang et al., 2019). Few studies have directly investigated the relationship among neighborhood greenness, LAP, and PP.

Over recent decades, China has undergone rapid urbanization (Guan et al., 2018) that has presented a huge challenge for urban residents in accessing green space (Nieuwenhuijsen et al., 2017). Greenness is spatially correlated with road proximity and traffic-related air pollution; it may also have some impacts on health outcomes (Gascon et al., 2016; Wilker et al., 2014; Yuchi et al., 2020). In addition, lifestyle changes, such as the use of motorized transport to commute to work and reduced leisure time, have caused an epidemic of physical inactivity in China (Kohl et al., 2012). The present study investigated whether living in areas with higher neighborhood greenness was associated with lower CVDs prevalence. It also examined the relationship among LAP, PP, and neighborhood greenness, which are critical to CVDs development. At the same time, we explored further whether road proximity and physical activity could mediate the association between greenness and prevalence of CVDs.

2. Methods

2.1. Study participants

The analysis was based on a community cross-sectional survey conducted in Longzihu District, Bengbu from July to August 2015. Bengbu is a city located in northern Anhui Province, China. It covers an area of 5,952 km^2 and had a population density of 592 inhabitants/km^2 in 2015; 56.2% of the population lived in urban areas. There were seven subdistricts (*jiedao*) and 32 communities in Longzihu (Fig. 1). Using a random number generator, we applied stratified sampling to select potential participants. From each random sample of 125 residents who had lived in the community for over 1 year, we selected 4,000 participants from the 32 communities (Fig. 2). Researchers visited each community and organized a free health checkup site for consenting participants. We excluded pregnant women, residents who had lived in the area for less than 1 year, and people with severe preexisting conditions. We recruited 3,652 participants, aged 18–91 years. Among them, 3,354 returned completed questionnaires (response rate, 91.80%). We excluded 1,254 individuals who refused to provide a venous blood sample. Thus, we conducted our analysis on 2,100 participants. The sociodemographic characteristics of the included and excluded groups were similar (Table S1). This study was approved by the Ethics Committee of Bengbu Medical College.

2.2. Outcome assessment

We measured blood pressure (BP) using American Heart Association guidelines (Pickering et al., 2005). The study participants were asked not to smoke, drink coffee or tea, or exercise for over 30 min before BP measurement. Participants had to rest for 5–10 min before determining BP. The BP of each participant was measured three times, and the average was calculated. We obtained the PP by subtracting the average diastolic pressure from the average systolic value (Said et al., 2018).

Using a tape measure with an insert buckle at one end, we determined the WC to the nearest 0.5 cm (Goh et al., 2014).

At the same time, participants' blood samples were taken after they had fasted overnight for more than 8 h. We calculated LAP with reference to TG (Wiltgen et al., 2009) as follows: [WC (cm) – 65] × [TG (mmol/L)] for men; [WC (cm) – 58] × [TG (mmol/L)] for women (Hosseinpanah et al., 2016).

CVDs mainly involves hypertension, coronary heart disease (CHD), and stroke (Barnett et al., 2017). We considered participants hypertensive if they had SBP ≥140 mmHg, DBP ≥90 mmHg, if they had ever been told by a doctor or health-care professional that they had hypertension, or had taken prescribed antihypertensive medication (Annor et al., 2015). We determined CHD by means of a coronary computed tomography enhanced scan or coronary angiography; we defined it as having at least one major epicardial coronary artery with a lumen stenosis of ≥50%. We based the diagnosis of stroke on the criteria presented at the 4th China Conference on Cerebrovascular Diseases, which were revised according to the diagnostic criteria of stroke of the WHO (Cai et al., 2018). In this study, all subjects' health outcomes were based on the same criteria.

2.3. Neighborhood greenness

We used the latitude and longitude coordinates of a residential area to define the neighborhood greenness exposure. We employed the normalized difference vegetation index (NDVI) to evaluate neighborhood greenness. We measured NDVI by satellite using the Moderate Resolution Imaging Spectroradiometer (MODIS) instrument at a 250-m × 250-m resolution every 16 days. We calculated the NDVI as the ratio of visible red and near-infrared reflectance; land surfaces with green vegetation reflect more near-infrared and absorb more visible red wavelengths (Rhew et al., 2011). We normalized the NDVI values from
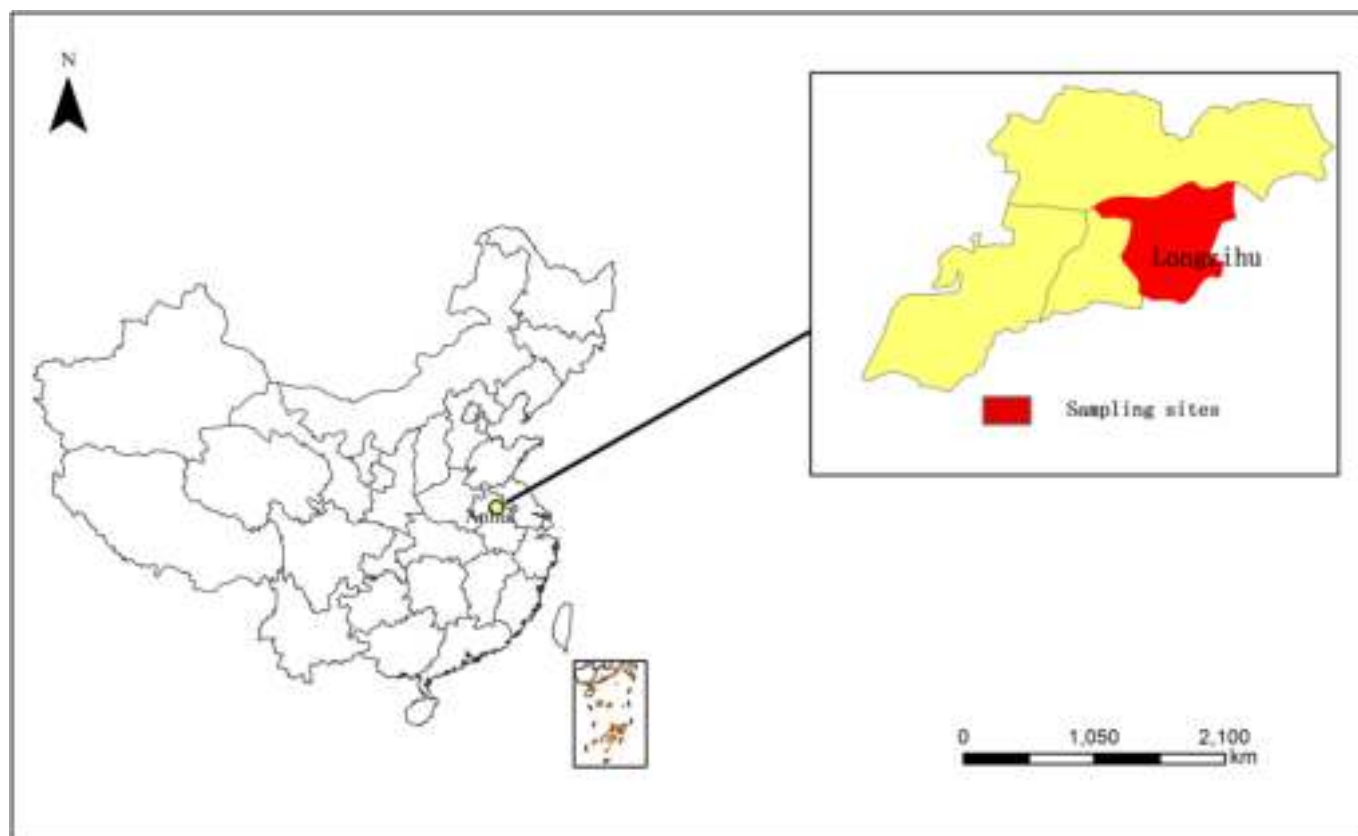
Fig. 1. Locations of study areas in Longzihu District,Bengbu, Anhui Province.

−1 to +1 to reflect the range of vegetation from very sparse to dense. We downloaded NDVI data from the Global Agricultural Monitoring Project (Younan et al., 2016) for August 2015: August is the greenest month in Bengbu, and 2015 was the closest year to the study period. We defined greenness as the average NDVI with 1,000-m, 1,500-m, and 2,000-m buffers around the corresponding residential address. We selected 1, 500 m for the main buffer analysis; we used the lower and higher buffers for the sensitivity analysis. We also report results using other buffers. We extracted NDVI values using ArcGIS 10.2 (ESRI, Redlands, CA, USA) and R version 3.3.2 (University of Auckland, Oakland, New Zealand).

2.4. Covariates and mediators

To collect information on demographic characteristics, we used a self-administered questionnaire delivered using a face-to-face interview. Among those characteristics, cigarette smoking was defined as the status of pre-smoking or current smoking. We evaluated alcohol consumption by asking whether in the previous month, the subject had drunk alcohol; if the answer was no, we regarded the status as non-drinking. We categorized educational level as follows: up to primary school and below; up to secondary school; and up to junior college and above. We classified monthly income as follows: 0–1,000 RMB; 1,000–3,000 RMB; over 3,000 RMB. A family history of CVDs referred to individuals with at least one parent or sibling who had suffered from such diseases. We assessed physical activity with the question, "Do you engage in any kind of physical exercise on a regular basis?" The answers were either yes or no. We employed a directed acyclic graph (DAG Fig. S1), developed using DAGitty v2.3 software (Canon Montanez and Rodriguez Acelas, 2019), to choose a minimum sufficient set of covariates to adjust for confounding and also to identify intervening variables between exposure and outcome based on the literature. From the DAG (Fig. S1), we adjusted for age, gender, educational level, and income level. Greenness is spatially correlated with road proximity and traffic-related air

pollution; it may also have some impacts on health outcomes (Gascon et al., 2016; Wilker et al., 2014; Yuchi et al., 2020). Evidence suggests that greenness can encourage physical activity (Twohig-Bennett and Jones, 2018), which is correlated with CVDs prevalence. Thus, road proximity and physical activity appear to be related to greenness exposure and prevalence of CVDs, and we considered them as potential mediators. We geocoded residential addresses using ArcGIS 10.2, and we calculated the distance to the nearest major roadway, defined as a freeway, freeway ramp, or prime arterial road. We categorized proximity to the road as ≤ 100 m, 100–200 m, 200–1000 m (reference category).We collected information about physical activity using a self-reported questionnaire (Jia et al., 2018).

2.5. Statistical analysis

We calculated descriptive statistics of the population characteristics and exposure variables. Continuous variables were expressed as means ± standard deviations, medians, and the 25th to 75th percentiles; categorical variables were expressed as frequencies and percentages. We compared differences among the groups using the *t*-test for continuous variables; we employed the Pearson χ^2 and Kruskal-Wallis *H* tests for categorical variables. We used a generalized linear mixed model to evaluate the relationship among neighborhood greenness, CVDs prevalence, LAP, and PP. In our model, we examined the role of the community as a random effect; we evaluated residential greenness and covariables as fixed effects. We estimated the effect of the continuous variables using the regression coefficient (β) and corresponding 95% confidence interval (CI); we evaluated the effect of the categorical variables using the odds ratio (OR) and corresponding 95%CI. We used the covariates to modify the unadjusted and adjusted models according to age, gender, education, and income (we selected those covariates using the DAG).

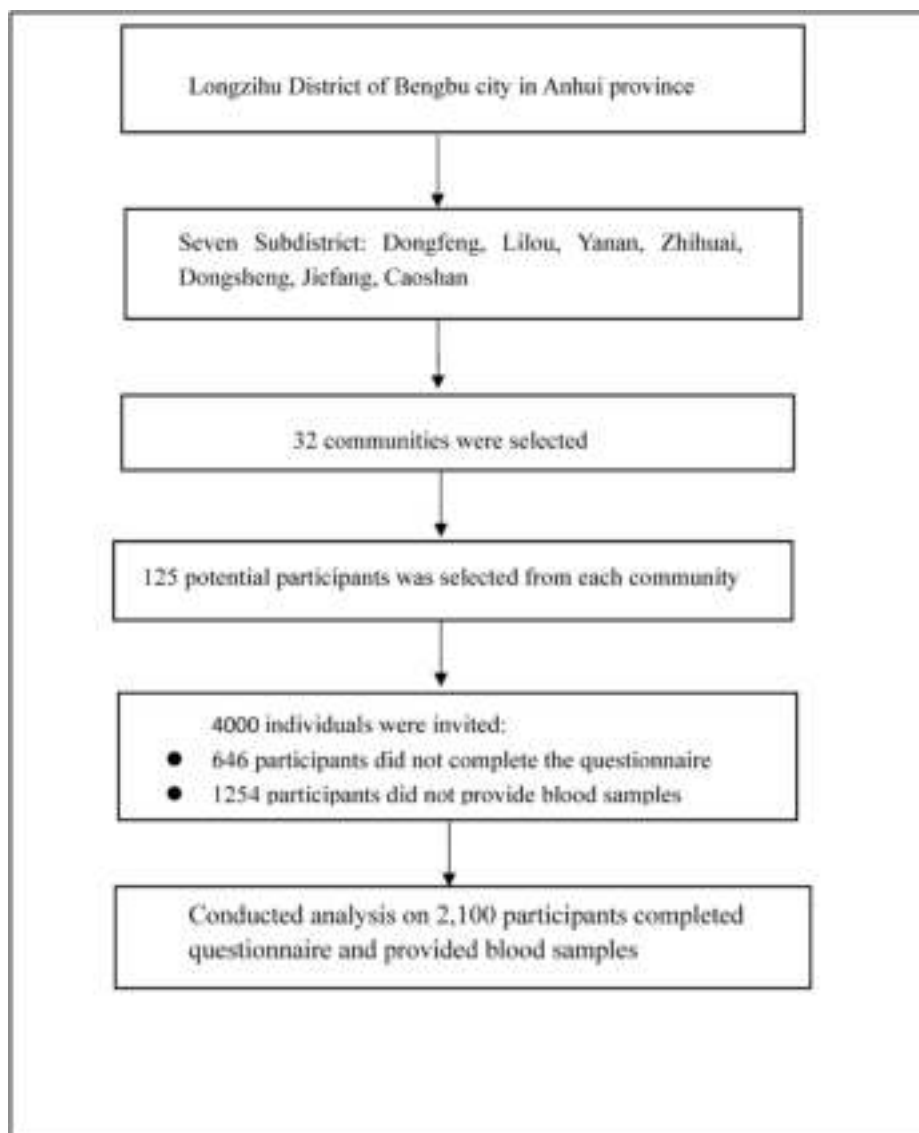We categorized the NDVI values into three groups based on the

Fig. 2. Study sampling process.

tertiles in our models. To determine the change in CVDs probability, LAP, and PP for every 0.1-unit increase in NDVI value, we conducted stratified analyses according to gender, age, income, and education. We tested effect modification in subgroup analyses and considered the effect to be present if a regression cross-product term (e.g., greenness × age) was statistically significant.

Finally, we used mediation analysis to examine whether road proximity and physical activity could be the mechanisms by which greenness affected CVDs and the biomarkers. We estimated the proportions of the mediated effect using the package mediation in the R (Imai et al., 2010). That method compared the exposure effect determined from the full model, which included the greenness, mediator, and all covariates (multivariate model), with the exposure effect estimates obtained from a univariate model, where exposure was regressed on the mediator (mediation model). We tested the mediators individually. Standard errors were generated by bootstrapping (5000 simulations).

All the data in this study were analyzed using R version 3.3.2 (University of Auckland, Oakland, New Zealand). We set statistical significance as $P < 0.05$.

3. Results

3.1. Participant characteristics

The final analysis covered 2,100 participants; their basic characteristics appear in Table 1. The prevalence of CVDs was 37.14%. Participants with CVDs differed from those without CVDs in all sociodemographic variables. Individuals diagnosed with CVDs were more likely to be aged ≥65 years and in a middle-to high-income group (80% had a monthly family income of >1,000 RMB) compared with participants without CVDs. Participants with CVDs had higher levels of LAP and PP than those without. Participants without CVDs had higher NDVI values in the 1,000-m, 1,500-m, and 2,000-m buffer

3.2. Neighborhood greenness, CVDs, and biomarkers

Table 2 shows the association between NDVI values and CVDs. In the crude models, the highest tertiles of $NDVI_{1500-m}$ were steadily and significantly associated with lower odds of CVDs prevalence. That association was notable for the highest tertiles of $NDVI_{1500-m}$ after adjusting for age, gender, education, and income. In the fully adjusted model, being in the highest-tertile $NDVI_{1500-m}$ had a significant protective effect

Table 1

Participant characteristics.

Variables	Total	CVDs	Non-CVDs	$\chi 2/t/H$	P
Overall	2100	780	1320		
Age (n, %)				258.3	<0.001
<65years	678	69 (9.6)	609 (44.1)		
≥65years	1422	651 (90.4)	771 (55.9)		
Gender (n, %)				6.187	0.013
Male	889	335 (46.5)	564 (40.9)		
Female	1201	385 (53.5)	816 (59.1)		
Education (n, %)				19.894	<0.001
Primary schools and below	604	251 (34.9)	353 (25.6)		
Secondary school	1283	402 (55.8)	881 (63.8)		
Junior College and above	213	67 (9.3)	146 (10.6)		
Income per month (n, %)				8.27	0.016
<1000,RMB	471	145 (20.1)	326 (23.6)		
1000–3000,RMB	1288	472 (65.6)	816 (59.1)		
≥3000,RMB	341	103 (14.3)	238 (17.2)		
Regular exercise (n, %)					
Yes	834	251 (34.9)	583 (42.2)	10.77	0.001
No	1266	469 (65.1)	797 (57.8)		
Road proximity (n, %)					
≤100 m	433	129 (17.9)	304 (22.0)		
100–200m	307	106 (14.7)	201 (14.6)	5.03	0.08
200–1000 m	1360	485 (67.4)	875 (63.4)		
Triglyceride, mmol/l[a]	1.64 ± 1.15	1.85 ± 1.27	1.53 ± 1.06	6.11	<0.001
Waist circumference,cm[a]	85.47 ± 9.82	88.54 ± 9.68	83.87 ± 9.51	−10.59	<0.001
SBP, mmHg[a]	124.55 ± 15.05	132.12 ± 15.61	120.60 ± 13.12	17.87	<0.001
DBP, mmHg[a]	79.69 ± 10.06	82.76 ± 10.44	78.12 ± 9.47	10.31	<0.001
LAP, mmol.cm/l[a]	42.34 ± 38.17	52.49 ± 45.50	37.04 ± 32.51	−8.967	<0.001
PP,mmHg[a]	44.85 ± 11.50	49.36 ± 12.93	42.49 ± 9.90	−13.53	<0.001
$NDVI_{1000-m}$[b]	0.294 (0.263,0.332)	0.288 (0.261,0.301)	0.294 (0.263,0.345)	21.915	<0.001
$NDVI_{1500-m}$[b]	0.293 (0.264,0.343)	0.288 (0.259,0.313)	0.297 (0.264,0.383)	21.579	<0.001
$NDVI_{2000-m}$[b]	0.301 (0.279,0.338)	0.296 (0.276,0.319)	0.305 (0.279,0.384)	22.54	<0.001

[a] Mean ± standard deviation.

[b] Median (1st to 3rd quartile).

Table 2

Associations between NDVI tertiles and CVDs.

NDVI	OR (95% CI)	
	Non-adjusted	Adjusted[a]
1000-m		
Tertile 1	1 (ref)	1 (ref)
Tertile 2	1.045 (0.751–1.429)	0.998 (0.758–1.313)
Tertile 3	0.562 (0.411–0.767)	0.641 (0.482–0.851)
1500-m		
Tertile 1	1 (ref)	1 (ref)
Tertile 2	0.843 (0.620–1.147)	0.860 (0.661–1.120)
Tertile 3	0.526 (0.356–0.776)	0.618 (0.434–0.879)
2000-m		
Tertile 1	1 (ref)	1 (ref)
Tertile 2	0,936 (0.684–1.280)	0.936 (0.715–1.225)
Tertile 3	0.531 (0.388–0.727)	0.612 (0.462–0.811)

[a] Adjusted by age, gender, income, and education.

on CVDs (OR, 0.618; 95% CI, 0.434–0.879) compared with participants in the lowest-tertile $NDVI_{1500\text{-m}}$. The associations among NDVI, LAP, and PP appear in Table 3. In the unadjusted model, the second tertile of $NDVI_{1500\text{-m}}$ showed a clear relationship with lower LAP. The highest tertiles of $NDVI_{1000\text{-m}}$, $NDVI_{1500\text{-m}}$, and $NDVI_{2000\text{-m}}$ were consistently and conspicuously related to lower PP levels. After adjustment for age, gender, education, and income, a tertile increase in $NDVI_{1500\text{ -m}}$ was significantly associated with a 1.12% lower PP. There was a statistically significant relationship between neighborhood greenness and LAP.

3.3. Subgroup analysis

We conducted subgroup analyses stratified by age, gender, education, and income. Among the participants, we examined stratified models of change in CVDs, LAP, and PP for a 0.1-unit increase in average

Table 3

Associations between NDVI tertiles and cardiovascular biomarkers.

NDVI	β (95% CI)			
	LAP		PP	
	Non-adjusted	Adjusted[a]	Non-adjusted	Adjusted[a]
1000-m				
Tertile 1	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Tertile 2	−5.89 (-13.18,1.40)	−6.23 (-13.46,1.00)	−1.19 (-3.54,1.16)	−1.35 (-3.64,0.94)
Tertile 3	−4.11 (-11.49,3.27)	−3.47 (-10.88,3.94)	−3.52 (-5.89,-1.15)	−2.90 (-5.23, −0.57)
1500-m				
Tertile 1	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Tertile 2	−10.02 (-16.08,-3.96)	−9.90 (-15.90,-3.90)	−2.29 (-4.43,-0.15)	−2.17 (-4.23,-0.11)
Tertile 3	−3.72 (−10.85, 3.41)	−2.86 (-10.07, 4.35)	−4.00 (-6.53,-1.47)	−3.29 (-5.78,-0.80)
2000-m				
Tertile 1	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Tertile 2	−9.99 (-16.56,-3.42)	−10 (-16.51, −3.49)	−2.06 (-4.31,0.19)	−2.03 (-4.21,0.15)
Tertile 3	−5.27 (-11.95,1.41)	−4.54 (-11.24,2.16)	−3.81 (-6.10,-1.52)	−3.14 (-5.39,-0.89)

[a] Adjusted by age, gender, income, and education.

$NDVI_{1500\text{-m}}$ neighborhood greenness (Table 4). We determined the estimated OR for a 0.1-unit increase in average $NDVI_{1500\text{-m}}$ to be 0.768 (95% CI, 0.663–0.890) and 0.906 (95% CI, 0.805–1.020) in male and female participants, respectively; the interaction was statistically significant ($P = 0.017$). We also observed a significant interaction between $NDVI_{1500\text{-m}}$ and income: there was a greater association with higher-income participants. No significant modification effects were evident

Table 4

Associations between an 0.1 unit increase in $NDVI_{1500-m}$ and LAP, PP and CVD prevalence.

	β (95% CI)[a]			OR (95% CI)[a]		
	LAP	P for interaction	PP	P for interaction	CVD	P for interaction
Age						
<65	0.289 (-1.440,2.018)	0.097	−1.199 (-1.969,-0.429)	0.899	0.805 (0.669,0.969)	0.981
≥65	−1.984 (-5.298,1.330)		−0.858 (-1.907,0.191)		0.836 (0.752,0.930)	
Gender						
Male	−0.803 (-3.535,1.929)	0.634	−0.933 (-1.772,-0.094)	0.327	0.768 (0.663,0.890)	0.017
Female	−0.206 (-2.827,2.415)		−1.216 (-1.982,-0.450)		0.906 (0.805,1.020)	
Education						
Primary schools and below	−1.927 (-5.171,1.317)	0.154	−1.005 (-1.752,-0.258)	0.934	0.832 (0.741,0.934)	0.197
Secondary school	1.080 (-1.674,3.834)		−1.101 (-1.940,-0.262)		0.842 (0.727,0.975)	
Junior College and above	−6.186 (-11.684,-0.688)		−1.043 (-3.219,1.133)		0.346 (0.123,0.968)	
Income per month						
<1000 yuan	−1.865 (-4.658,0.928)	0.005	−0.957 (-1.529,-0.385)	0.926	0.845 (0.739,0.966)	0.016
1000–3000yuan	−0.150 (-3.143,2.843)		−1.218 (-2.010,-0.426)		0.934 (0.824,1.058)	
≥3000yuan	1.811 (-1.060,4.682)		−0.845 (-1.996,0.306)		0.269 (0.138,0.526)	

[a] Adjusted by age, gender, income, and education.

for the remaining subcategories. The associations were generally consistent in sensitivity analyses using 1000-m and 2000-m NDVI buffers (Table S2. S3).

3.4. Mediation analyses

Table 5 shows the results of our mediation analysis to quantify the proportion of the $NDVI_{1500-m}$ associations attributable to road proximity and physical activity. We found that ambient road proximity significantly mediated only 9.7% of the estimated association between greenness and PP. No significant mediating effects were evident for physical activity. The associations were generally consistent in sensitivity analyses using 1000-m and 2000-m NDVI buffers (Table S6. S7).

4. Discussion

4.1. Key findings

This cross-sectional analysis of 2,100 participants showed that higher neighborhood greenness could be beneficial with respect to the prevalence of CVDs and their biomarkers. Those associations were partially mediated by road proximity but were entirely independent of physical activity. We found that the associations were still significant when using different buffers for greenness or with a selected adjustment set. These associations were stronger in male and higher-income participants. To our knowledge, this is one of only a few studies to investigate the relationship among neighborhood greenness, LAP, and PP in a developing country. Our findings contribute to the discussion about the importance of neighborhood greenness for health promotion; they underline the importance of considering that factor in urban planning.

4.2. Comparison with previous studies and interpretations

Many studies have focused on greenness and CVDs. Consistent with our findings, investigations in Spain (Plans et al., 2019), China (Jia et al.,

Table 5

Mediation of association between greenness ($NDVI_{1500m}$) and CVDs by road proximity and physical activity.

Outcome	Potential mediators	Proportion mediated (95% CI)[a]	P value
CVD	Road proximity	0.059 (-0.009, 0.190)	0.096
	Physical activity	−0.057 (-0.214,0.06)	0.32
LAP	Road proximity	0.067 (-1.314,1.370)	0.83
	Physical activity	−0.877 (-6.759, 7.970)	0.424
PP	Road proximity	0.097 (0.039,0.180)	<0.001
	Physical activity	−0.026 (-0.124,0.070)	0.57

[a] Adjusted by age, gender, income, and education.

2018), and Brazil (Massa et al., 2016) observed that exposure to higher neighborhood greenness was significantly related to lower CVD occurrence. Similarly, greater tree canopy—but not green space—was found to be associated with lower odds of developing CVDs (Astell-Burt and Feng, 2020). In Korea, a study involving 73 districts found that a high level of greenness was associated with decreased risk of CVD-related mortality (Kim et al., 2019). However, results have been mixed. In a cross-sectional study of 100,000 adults from the United Kingdom, no significant association was observed between the proportion of green space and CVDs mortality (Bixby et al., 2015). In addition, proximity to greenness was not associated with CVDs prevalence in two cross-sectional studies of 12,546 adults in the Netherlands (Picavet et al., 2016) and among 2,411 adults in Canada (Chum and O'Campo, 2015). Discrepancies in the results may be attributable in part to differences in the study populations (e.g., race, gender proportion, age, and lifestyle factors), various exposure assessment strategies (e.g., NDVI, distance to green space, and percentage of green space), and the presence or absence of other factors (e.g., air pollutants, noise, and psychosocial stress). For example, one study used the distance from a neighborhood area to a green space as an indicator of neighborhood greenness exposure (Paquet et al., 2013). In the present study, we employed the average of $NDVI_{1000-m}$, $_{1500-m}$, and $_{2000-m}$ as an indicator of neighborhood greenness exposure. Our research is based on old communities in Bengbu, which are densely inhabited. 2000 m is a good buffer size in our community, since it is suitable distance that people often choose to walk on their way to the bus stop or supermarket or other activities, and previous studies have shown significant associations between greenness and health outcomes with the buffer sizes we adopted.

LAP and PP have previously been used as biomarkers of CVDs (Kyrou et al., 2018; Said et al., 2018). In the present study, we found that participants with higher neighborhood exposure to greenness had significantly lower LAP and PP. To our knowledge, no study has previously evaluated the association among neighborhood greenness, LAP, and PP. Thus, it is not possible to compare our findings with those of other reports. However, LAP is a combined index of TG and WC; PP is a combined index of SBP and DBP (Hosseinpanah et al., 2016; Said et al., 2018). Our findings are in contrast with those of several related studies. Higher greenness has been found to be associated with lower TG levels (Tamosiunas et al., 2014; Yang et al., 2019a). A cohort study of 3,205 adults in Australia reported no significant relationship between neighborhood greenness and WC (Huang et al., 2020). Some studies have identified an association among greenness, SBP, DBP, and hypertension (Brown et al., 2016; Dzhambov et al., 2018). One investigation observed no direct correlation among greenness, SBP, and hypertension (Morita et al., 2011); that result is inconsistent with our own. A possible reason for the discrepancy is differences in study populations, exposure

assessment methods, and co-exposures (Paul et al., 2020). No previous research has investigated the potential link among greenness, LAP, and PP. Thus, future studies are needed to confirm our findings.

We observed a stronger protective effect of greenness for CVDs among participants who were male and with high incomes. There are inconsistent results about the association between greenness reported in age subgroups and health benefits. Studies have examined the influence of gender on the association between greenness and health outcomes (Markevych et al., 2017). One prospective cohort study determined that exposure to green space could be protective against CVDs in female, which is contrary to our findings (Ji et al., 2019). It could be that male people are more likely to use green spaces and thereby derive benefit from them. In addition, gender is strongly associated with CVDs, which could have masked any moderate beneficial effects of greenness. We observed a notable association between greenness and CVDs among participants with high incomes. In line with our results, other studies have reported that the beneficial effects of neighborhood greenness on health outcomes are greatest among high-income individuals (Silveira and Junger, 2018); such income levels are often closely related to education (Dadvand et al., 2014). One explanation for that effect could be that lower-income residents tend to be in poor health and live in areas with greater pollution and less greenness than people with higher incomes (Yeager et al., 2020). However, in the present study, detailed data about pollution were unavailable, which limited our ability to directly assess such factors. Future studies with more detailed green space information are needed to validate our results.

4.3. Potential underlying mechanisms

In this study, we observed that higher neighborhood greenness could be beneficial regarding the prevalence and biomarkers of CVDs. We believe this association demands further investigation: several pathways could underlie it (James et al., 2015). First, residents who live in proximity to greenness, which offers attractive zones for physical activity, are more likely to participate in such activity (Orban et al., 2017; Sadeh et al., 2019); physical activity helps protect against the development of CVDs (Tielemans et al., 2013). In the mediation analyses, we did not detect evidence of mediating effects of physical activity. Our findings are in agreement with those of (Thiering et al., 2016; Yang et al., 2019b). Second, the following ecosystem factors are most related to public health: clean air; noise reduction; heat effect reduction; and biodiversity conservation (Takebayashi, 2017; Zhang et al., 2020). We did not have access to data about pollution; however, we were able to include a proxy estimate: road proximity. One study has shown that greenness is spatially correlated with road proximity and traffic-related air pollution; it also found that greenness can have some impact on health outcomes (Jendrossek et al., 2017). In the mediation analyses, road proximity significantly mediated the association between greenness and PP. That relationship may be explained by road proximity involving long-term exposure to traffic-related air pollution (Gaskins et al., 2018).Third, it has been found that exposure to greenness can have a positive impact on mental health (Orban et al., 2017; Wood et al., 2017). In turn, better mental health, reduced stress, and improved social relationships may impede the development of CVDs. However, in the present study, we lacked data about those factors; thus, we were unable to use them as potential mediators regarding the relationship among greenness, CVD prevalence, and biomarkers.

4.4. Limitations and strengths

This study has some important limitations. First, our results were based on a cross-sectional survey, which could not establish a causal relationship: it identified only a link between greenness and CVDs. Second, we lacked data about potential mediators, such as air pollution. Thus, we were unable to examine them as potential variables in the association among greenness, CVDs prevalence, and biomarkers. Third,

the NDVI provides reliable information about the general level of vegetation, but it does not include data about types of greenness or their quality and structure. Fourth, we excluded some subjects who were unable to complete the survey: that may have led to underestimating the prevalence of CVDs and produced selection bias. Fifth, physical activity, the potential mediator, was assessed by questionnaire, and more detailed information like exercise kind, time and location were not available.

This investigation has some notable strengths. First, it is one of few studies to evaluate the association between greenness and CVDs biomarkers in China. We observed the protective effect of greenness with respect to LAP and PP. Second, we were able to test different buffer sizes, thereby providing greater insight into the connections between greenness and health. We were also able to produce a simple, comprehensive set of covariates (including age, gender, education, and income) and test interaction between NDVI and the putative modifiers.

5. Conclusions

The present study found that higher neighborhood greenness may have a beneficial effect on CVDs and biomarkers. Those associations were stronger in male and higher-income participants; road proximity significantly mediated the association between greenness and PP. The results of this study may offer an important insight to inform future studies about the relationship between greenness and CVDs; that could provide support for developing greenness-based public health policy.

Authors' contributions

The authors' responsibilities were as follows: LT: designed the study, analyzed the data and drafted the manuscript; CB: managed and undertook data collection; PWJ: established the mathematical model; XLP: undertook data collection; SHY: co-drafted the manuscript; GHQ: supervised establishing the mathematical model; WXS: supervised the data analysis and co-drafted the paper; JXJ: had primary responsibility for final content. All authors read and approved the final manuscript.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113738.

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A time series analysis of the short-term association between climatic variables and acute respiratory infections in Singapore

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ABSTRACT

Background: Acute respiratory infections (ARIs) are among the most common human illnesses globally. Previous studies that examined the associations between climate variability and ARIs or ARI pathogens have reported inconsistent findings. Few studies have been conducted in Southeast Asia to date, and the impact of climatic factors are not well-understood. This study aimed to investigate the short-term associations between climate variability and ARIs in Singapore.

Methods: We obtained reports of ARIs from all government primary healthcare services from 2005 to 2019 and analysed their dependence on mean ambient temperature, minimum temperature and maximum temperature using the distributed lag non-linear framework. Separate negative binomial regression models were used to estimate the association between each temperature (mean, minimum, maximum temperature) and ARIs, adjusted for seasonality and long-term trend, rainfall, relative humidity, public holidays and autocorrelations. For temperature variables and relative humidity we reported cumulative relative risks (RRs) at 10th and 90th percentiles compared to the reference value (centered at their medians) with corresponding 95% confidence intervals (CIs). For rainfall we reported RRs at 50th and 90th percentiles compared to 0 mm with corresponding 95% CIs.

Results: Statistically significant inverse S-curve shaped associations were observed between all three temperature variables (mean, minimum, maximum) and ARIs. A decrease of 1.1 °C from the median value of 27.8 °C to 26.7 °C (10th percentile) in the mean temperature was associated with a 6% increase (RR: 1.06, 95% CI: 1.03 to 1.09) in ARIs. ARIs also increased at 23.9 °C (10th percentile) compared to 24.9 °C of minimum temperature (RR: 1.11, 95% CI: 1.07 to 1.16). The effect of maximum temperature for the same comparison (30.5 °C vs 31.7 °C) was non-significant (RR: 1.02, 95% CI: 0.99 to 1.05). An increase in ambient temperature to 28.9 °C (90th percentile) was associated with an 18% decrease (RR: 0.82, 95% CI: 0.80 to 0.83) in ARIs. Similarly, ARIs decreased with the same increase to 90th percentile in minimum (RR: 0.84, 95% CI: 0.80 to 0.87) and maximum (RR: 0.89, 95% CI: 0.86 to 0.93) temperatures. Rainfall was inversely associated with ARIs and displayed similar shape in all three temperature models. Relative humidity, on the other hand, exhibited a U-shaped relationship with ARIs.

Conclusion: Our findings suggest that lower temperatures increase the risk of ARIs. Anticipated extreme weather events that reduce ambient temperature can be used to inform increased healthcare resource allocation for ARIs.

1. Introduction

Acute respiratory infections (ARIs) are among the most common of illnesses among humans across the globe (Monto, 2002). ARIs are primarily caused by respiratory syncytial virus (RSV), coronavirus, rhinovirus, influenza viruses, parainfluenza viruses, adenovirus, and human metapneumovirus (Hayden, 2006). They are estimated to cause 4.2 million deaths annually and contribute to a loss of 97 million

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disability adjusted life years (Mathers, 2008). Due to the high prevalence of ARIs, the cost of respiratory illnesses in United Kingdom alone was estimated to be £11.1 billion in 2014 (Burki, 2017). The elderly and children are particularly susceptible to ARIs, experiencing higher mortality and morbidity (Masse et al., 2017; Monto, 2002). It was estimated that 1.3 million children below the age of 5 die from ARIs annually (World Health Organization, 2013).

In Singapore, ARIs are the most common illnesses reported in primary care settings (Ministry of Health (Singapore), 2014). Previous studies investigating the association between weather parameters and ARI pathogens (Chew et al., 1998; Tang et al., 2010b) or all-cause ARIs (Loh et al., 2011) in Singapore conducted on children led to somewhat unexpected results. Chew et al. reported that the occurrence of RSV, the main etiological agent of ARI in children, was positively associated with daily maximal temperature difference and negatively associated with relative humidity, while influenza B was positively associated with rainfall (Chew et al., 1998). Tang et al. reported similar inverse association between relative humidity and influenza (Tang et al., 2010b). Loh et al. found inverse association between relative humidity and respiratory tract infections (Loh et al., 2011).

In all three studies conducted in Singapore the effect of relative humidity on ARIs or ARI pathogens was the most consistent finding. This finding corroborates with the finding in Malaysia but is inconsistent with sub-tropical Hong Kong, which found a positive association between and RSV and influenza A cases with relative humidity (Chan et al., 2002; Tang et al., 2010a). In addition, studies in neighboring Malaysia found inverse associations between RSV infections and mean temperature, and positive association with number of rain days (Chan et al., 2002; Khor et al., 2012). While no inverse association between temperature and ARIs or ARI pathogens was reported in studies in Singapore, inverse associations between temperature variables and ARIs or ARI pathogens was consistently reported in studies conducted in Finland (Mäkinen et al., 2009), United Kingdom (Hajat et al., 2004), Greece (Falagas et al., 2008), Mexico (Costilla-Esquivel et al., 2014) and Germany (du Prel et al., 2009). The absence of the associations between temperature and ARIs in studies in Singapore warrants further investigations.

In this study, we investigated the short-term associations between climate variability and reported ARI cases in Singapore using a time-series analytical study design with the most recent data. Understanding how weather influences the number of ARIs can inform resource planning in primary care settings.

2. Methodology

Acute respiratory infections (ARIs) are diagnosed based on the presence of any key respiratory symptoms including cough, runny nose, sore throat or shortness of breath (Jiang et al., 2017). Weekly ARI cases (onwards just ARIs) collected from 2005 to 2019 were obtained from the Infectious Diseases Bulletin published by Ministry of Health, Singapore (https://www.moh.gov.sg/resources-statistics). These cases, which were reported by epidemiological week (which starts on Sunday and ends on Saturday), were collected from all 18 polyclinics in Singapore. Singapore polyclinics are government institutions that together with private clinics provide primary healthcare services at subsidized and affordable rates to Singapore residents. Polyclinics serve approximately 20% of all primary care patients in Singapore (Ministry of Health (Singapore), 2014). The ARIs data was reported as a cumulative number of ARIs a week until the end of 2011, and average daily ARIs from the beginning of 2012 (cumulative number per week averaged over 5.5 working days). Data from the beginning of 2012 was normalized to a weekly scale by multiplying it by 5.5.

2.1. Climatic variables

Daily climatic data matching the time frame of ARIs (January 2,

2005 to December 28, 2019) were obtained from 23 meteorological stations located throughout the island of Singapore. Due to Singapore's small geographical size, the data from all stations were averaged to produce national-level mean measurements. Mean weekly values for ambient temperature (°C), minimum temperature (°C), maximum temperature (°C), and relative humidity (%) were then calculated for each corresponding week, while the values for total rainfall (millimeter (mm)) were summed up into weekly values.

2.2. Statistical analysis

To account for overdispersion, we assumed a negative binomial distribution to investigate the short-term associations between climate variables and weekly ARIs. To avoid collinearity, 3 separate models for mean ambient temperature, minimum temperature, and maximum temperature were built. We accounted for the potential confounding effects of seasonality and long-term trends by using b-cubic splines. The number of splines was determined using visual analysis and Akaike's Information Criterion (AIC) (Yamaoka et al., 1978).

Cross basis terms of all climatic variables using *crossbasis()* function of distributed lag non-liner models (DLNM) package in R were constructed. DLNM is a framework that allows flexible descriptions of potentially non-linear exposure-response dependencies and delayed effects simultaneously. Cross basis terms carry two dimensions in their structure, one that specifies conventional association along the predictor and outcome space and an additional dimension that specifies the lag-response relationship (Gasparrini, 2011). Previous studies have shown that the effect of the climatic variables on ARIs lasts up to 3 weeks (Hajat et al., 2004; Vandini et al., 2013). We therefore specified lags of up to 4 weeks in order to capture the delayed effects of climate variables. We explored the linearity of the association between ARIs and climatic variables using natural cubic splines with 3 degrees of freedom (*df*). We accounted for the effect of public holidays by including a categorical variable that took on the number of statutory public holidays in the week. The final temperature models are described below (before accounting for residual autocorrelation):

1. $\log E(Y_t) = \beta_0 + \beta_1 \text{TempMean}_{t,l=4} + \beta_2 \text{RF}_{t,l=4} + \beta_3 \text{RH}_{t,l=4} + \beta_4 \text{Holiday} + bs(t, df = 13/\text{year} \times \text{no. of years})$
2. $\log E(Y_t) = \beta_0 + \beta_1 \text{TempMin}_{t,l=4} + \beta_2 \text{RF}_{t,l=4} + \beta_3 \text{RH}_{t,l=4} + \beta_4 \text{Holiday} + bs(t, df = 13/\text{year} \times \text{no. of years})$
3. $\log E(Y_t) = \beta_0 + \beta_1 \text{TempMax}_{t,l=4} + \beta_2 \text{RF}_{t,l=4} + \beta_3 \text{RH}_{t,l=4} + \beta_4 \text{Holiday} + bs(t, df = 13/\text{year} \times \text{no. of years})$

Here, $E(Y_t)$ is the expected number of weekly ARIs at week t; β_0 is the intercept; β_1 to β_4 are the coefficients for the respective independent variables; $\text{TempMean}_{t,l=4}$, $\text{TempMin}_{t,l=4}$, $\text{TempMax}_{t,l=4}$, $\text{RF}_{t,l=4}$, $\text{RH}_{t,l=4}$ are the matrices obtained by applying DLNM to weekly climatic variables: mean ambient temperature, minimum temperature, maximum temperatures, rainfall and relative humidity respectively, l corresponds to number of lags; bs(.) is b-cubic spline function; *df*, degrees of freedom.

Partial autocorrelation function plots (PACF) were used to assess the degree of residual autocorrelation. Any remaining autocorrelation was then accounted for by adding lags of the model's deviance residuals into the final model. Sensitivity analysis was conducted by adopting different *dfs* for the long term and seasonality control. All plots can be found in the Supplementary data (Appendix B, Figures B.1-B.3).

For all models we examined the lag structure of the association and the cumulative effect of the weather variables on ARIs. We reported cumulative relative risks (RRs) of ARIs for 10th and 90th percentiles of temperature variables and relative humidity compared to their reference values (centered at their medians) with corresponding 95% confidence intervals (95% CI). For rainfall we reported RRs for 50th and 90th percentiles compared to the reference value (centered at 0 mm). All statistical tests were two-tailed, and P values < 0.05 were considered statistically significant. All statistical analyses were performed using R

software Version 3.6.3.

3. Results

3.1. Descriptive statistics

There was a total of 11, 236, 597 ARIs over the study duration. ARIs averaged 14,369 (SD: 2411) per week (Table 1). There were low to moderate variations in climatic variables, typical of tropical settings.

Mean and minimum temperatures had a similar weekly pattern with both peaking from April to June every year (Appendix A, Figure A.1). The weekly number of ARIs displayed some seasonal fluctuations. No distinct temporal pattern was observed for relative humidity and cumulative rainfall.

3.2. Multivariable regression modeling

After adjusting for the potential confounding effects of rainfall, relative humidity, public holidays, seasonality and long-term trends, and residual autocorrelation, we observed an inverse relationship between temperature and reported ARIs characterized by a reverse 'S-shaped curve' (Figure 1). A decrease of 1.1 °C from the median value of 27.8 °C to 26.7 °C (10th percentile) in the mean temperature model was associated with a 6% increase (RR: 1.06, 95% CI: 1.03 to 1.09) in ARIs. ARIs also increased at 23.9 °C (10th percentile) compared to 24.9 °C of minimum temperature (RR: 1.11, 95% CI: 1.07 to 1.16). The effect of maximum temperature for the same comparison in percentiles (30.5 °C vs 31.7 °C) was non-significant (RR: 1.02, 95% CI: 0.99 to 1.05). An increase in ambient temperature to 28.9 °C (90th percentile) was associated with 18% decrease (RR: 0.82, 95% CI: 0.80 to 0.83) in ARIs. Similarly, ARIs decreased with the same increase to 90th percentile in minimum (RR: 0.84, 95% CI: 0.80 to 0.87) and maximum (RR: 0.89, 95% CI: 0.86 to 0.93) temperatures. The lag structure of the association between all three types of temperatures and ARIs was similar (Appendix A, Figures A.2 to A.4), where at temperature values below the median, the effect was mainly contributed by lags 0, 1, and 2 and at temperatures above the median the effect was statistically significant at all lags. Similar to temperature, we observed an inverse relationship between rainfall and the number of weekly ARIs in all three temperature models (Figure 2). An increase in the amount of rainfall from 0 mm to 41.2 mm, was associated with 13% decrease (RR: 0.87, 95% CI: 0.86 to 0.89) in ARIs in the mean ambient temperature model. The same increase in rainfall was associated with a decrease in ARIs in both the minimum (RR: 0.87, 95% CI: 0.85 to 0.88) and the maximum (RR: 0.94, 95% CI: 0.92 to 0.95) temperature models. An increase in rainfall from 0 mm to 96.1 mm (90th percentile) was associated with 23% decrease (RR: 0.77, 95% CI: 0.76 to 0.79) in ARIs in the mean ambient temperature model. ARIs also decreased with the same increase in rainfall in the minimum (RR: 0.74, 95% CI: 0.72 to 0.75) and the maximum (RR: 0.82 95% CI: 0.81 to 0.84) temperature models. The lag structure of the association between rainfall and ARIs was consistent in all three temperature models (Appendix A, Figures A.5 to A.7). The inverse shape of the relationship was observed to be statistically significant for all lags, with lag 4 having slower decline in RRs for higher values of rainfall compared to other lags.

We observed a U-shaped association between relative humidity and the ARIs in all three temperature models (Figure 3). At 74.5% (10th percentile) relative humidity compared to 80.1% there was a 7% increase in the risk (RR: 1.07, 95% CI: 1.06 to 1.09) of ARIs in the mean ambient temperature model. Although the shape of the relationship was similar in all three models, the RR values for the same comparison in the minimum and to a lesser degree in the maximum temperature models were lower than the null. Thus the change in relative humidity from 80.1% to 74.5% was associated with 4% decrease (RR: 0.96 95% CI: 0.95 to 0.97) in the minimum, and was non-significant in the maximum (RR: 0.99, 95% CI: 0.98 to 1.00) temperature models. An increase in relative humidity from 80.1% to 84.9% (90th percentile) was associated with a 10% increase (RR: 1.10, 95% CI: 1.09 to 1.12) in ARIs in the mean ambient temperature model. Similarly, ARIs increased in the minimum (RR: 1.16, 95% CI: 1.15 to 1.17) and the maximum (RR: 1.32, 95% CI: 1.27 to 1.37) temperature models. The shape of the lag structure of the association between relative humidity and ARIs was similar in all temperature models (Appendix A, Figures A.8 to A.10). In all three models the shape of the association was consistent for lags 0–3. Lag 4 had lower RRs for values above the median in mean and minimum temperature models and for values below the median in maximum temperature model.

In the mean temperature model, public holidays were associated with a reduction in ARIs (single day RR: 0.930, 95% CI: 0.926–0.933 and dual day RR: 0.788, 95% CI: 0.779–0.798). The public holiday findings in the minimum and the maximum temperature models were similar: RR for single day: 0.927 (95% CI: 0.920–0.933), RR for dual day: 0.782 (95% CI: 0.766–0.800) and RR for single day: 0.925 (95% CI: 0.918–0.932), RR for dual day: 0.790 (95% CI: 0.773–0.808) respectively.

3.3. Seasonality and long-term trend adjustment

We chose 13 *df* per year for b-cubic splines to control for seasonality and long-term trend. AIC values from the negative binomial models with ARIs as the outcome and b-cubic splines as a smooth function of time are highlighted in Appendix A, Table A.1. The reduction in AIC value was marginal beyond 13 *df* per year. Visual fit analysis from the model with 13 *df* per year appeared to yield a satisfactory result, while at 19 *df* (at lowest AIC value) there was some evidence of overfitting (Appendix A, Figure A.11 – B, C). Predicted vs actual ARI plot from this model indicated good predictive ability of the model (correlation coefficient, ρ = 0.91) (Fig. 1- D). Plots with fitted ARIs from models with 7 df and 19 df were visually under fitted and overfitted respectively (Fig. 1- A and C). Final plots with predicted fit over raw data are displayed in Appendix A, Figure A.12.

4. Discussion

In this study, we found significant inverse associations between temperature and rainfall with the number of ARIs. We also found a significant U-shaped association between relative humidity and ARIs. For all the weather parameters tested, the effect on and the shape of the association with ARIs was the most consistent for up to 3 lags, corroborating the findings in the previous studies (Hajat et al., 2004; Vandini
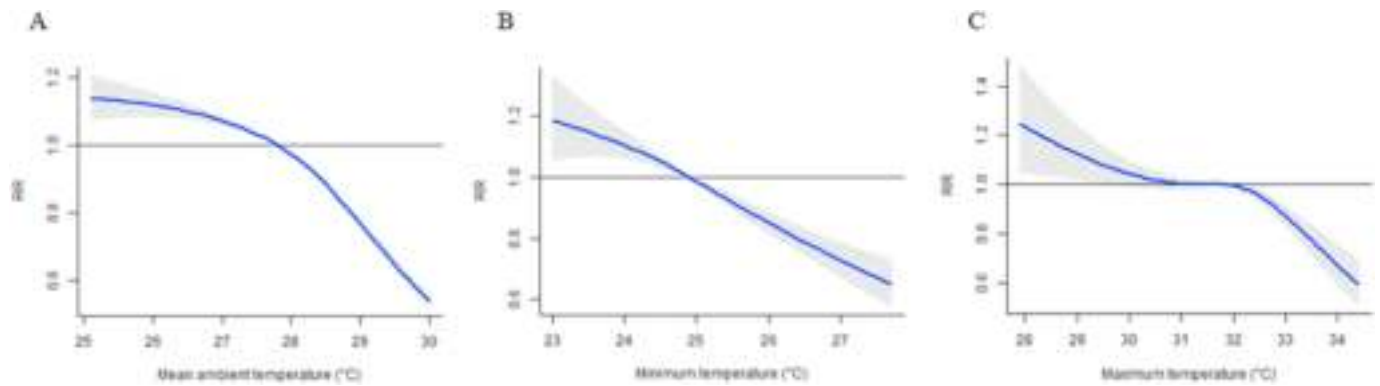
Table 1
Descriptive summary of ARIs and climatic variables.

Variable	Mean	SD	Median	Interquartile range	Minimum value	Maximum value
Number of weekly ARI cases	14,369	2411	14,235	12,902–15,845	7684	24,477
The daily mean ambient temperature averaged over a week (°C)	27.8	0.9	27.8	27.3–28.4	25.1	30.1
Daily minimum ambient air temperature averaged over a week (°C)	25.0	1.0	24.9	24.4–25.5	22.9	27.7
Daily maximum ambient air temperature averaged over a week (°C)	31.7	0.9	31.7	31.2–32.3	27.8	34.4
Mean daily relative humidity averaged over a week (%)	79.9	4.0	80.1	77.0–82.7	63.5	91.4
Weekly cumulative rainfall (mm)	48.1	38.8	41.2	20.1–66.6	0	440.0

Fig. 1. The cumulative effect of mean (A) minimum (B) and maximum (C) temperature on ARIs. The RRs are derived from comparing each temperature on x-axis against the median of that temperature variable. Shaded grey area denotes 95% CI.
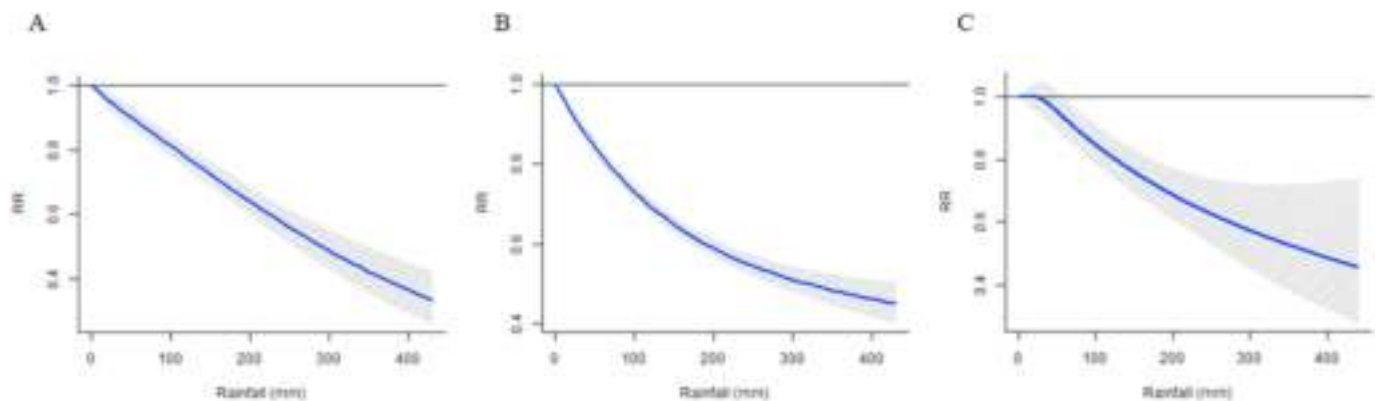

Fig. 2. The cumulative effect of rainfall on ARIs from the mean (A), minimum (B) and maximum (C) temperature models. The RRs are derived from comparing each rainfall value on x-axis against 0 mm. Shaded grey area denotes 95% CI.
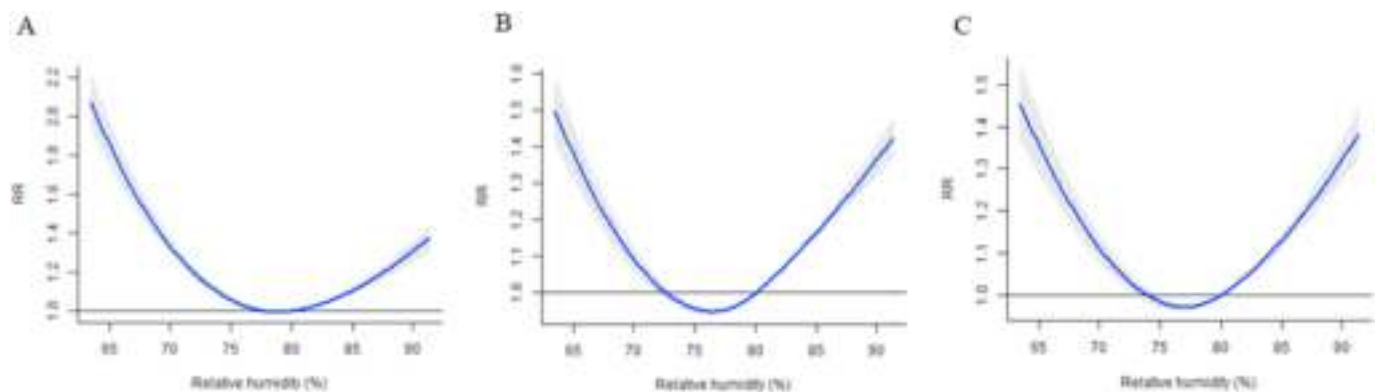

Fig. 3. The cumulative effect of relative humidity on ARIs from the mean (A), minimum (B) and maximum (C) temperature models. The RRs are derived from comparing each relative humidity value on x-axis against its median. Shaded grey area denotes 95% CI.

et al., 2013).

The inverse association between temperature and the number of ARIs found in this study corroborated by numerous previous studies across the globe, including those in Germany (du Prel et al., 2009), Greece (Falagas et al., 2008), UK (Hajat et al., 2004), Finland (Mäkinen et al., 2009), and Mexico (Costilla-Esquivel et al., 2014). Contrary to our findings, previous studies in Singapore found a positive association between maximal temperature difference and ARI pathogens, and a negative association between relative humidity and ARIs or ARI pathogens (RSV, Influenza A) (Chew et al., 1998; Loh et al., 2011; Tang et al., 2010b). The differences between these studies and ours may be due to

the different ARI data and statistical methods used. All three previous studies in Singapore used children hospitalization records, while in our study we used all ARIs reported in government public healthcare institutions. In addition, these previous studies included diagnosis of RSV, Influenza A and B as independent variables in the models (Loh et al., 2011; Tang et al., 2010b). This could have potentially diminished the effect of temperature, rainfall, and relative humidity if those are associated with the included pathogens. Furthermore, the relationship between climatic variables and ARIs was allowed to be non-linear in our study, whereas all the previous studies conducted in Singapore used linear regression modeling approaches.

Possible biological mechanisms of the inverse relationship between temperature variables and ARIs are well described in the literature. Lower temperatures can facilitate the infection with ARI pathogens through several pathways. Viruses that cause ARIs were shown to be more stable in the environment at lower temperatures (Hambling, 1964; Marr et al., 2019). Lower temperatures can also trigger changes in human physiology, specifically decreased mucosal secretion rate, decreased ciliary beat frequency and decreased activity of immune cells, which may leads to higher ARI susceptibility (Eccles, 2002; Proctor, 1977).

In our study, rainfall was inversely associated with ARIs. There are two possible mechanisms by which rainfall could hinder virus transmission. One possible mechanism is by washing away the viruses on exposed surfaces in the environment. In addition, people may be more prone to stay home instead of going out to public places on rainy days, therefore effectively preventing ARI pathogens transmission. Interestingly, this argument was previously used to explain higher transmission rates due to indoor crowding in temperate countries (Dowell, 2012). This, however, would only apply to leisure activities and not mandatory presence at workplaces or educational institutions. Previous studies in Malaysia found that RSV infections were positively associated with the number of rain days in a month but not rainfall in general (Chan et al., 2002; Khor et al., 2012). The differences in human behavior and how much time people spend indoors in different countries can potentially explain inconsistencies in the findings between the countries.

The association between humidity and ARIs was found to be U-shaped. Although the RRs were lower than the null in minimum and maximum temperature for values between 10th and 50th percentile, the general direction of increasing RRs with lower and higher relative humidity values was observed for all models. A few possibilities may explain these associations; however, these are speculative in nature. From previous studies, it is evident that various viruses associate differently with relative humidity. Rhinoviruses survive better at higher levels of relative humidity (du Prel et al., 2009; Karim et al., 1985), while influenza viruses are thought survive longer in a drier environment or display a V-shape relationship with relative humidity (Tang, 2009). It could be that the cumulative risk for all-cause ARIs is contributed by different causative pathogens of ARI, that were favored differently when relative humidity deviates from the optimal values, which may increase the transmission of those pathogens. Lower relative humidity can also impair the defense mechanisms in the mucosal layers of respiratory tract, leading to higher susceptibility to ARIs (Kudo et al., 2019).

In this study, we have employed b-cubic splines with 13 *df* per year to control for seasonality and long-term trend. Typically, 7 *df* per year is chosen in air pollution studies, based on extensive research in that field (Bhaskaran et al., 2013). For ARI, there are no widely accepted guidelines on seasonality and long-term trend control. Our sensitivity analyses using 7, 13 or 19 *df* per year produced similar exposure-outcome relationship curves with narrower CIs in models with 7 *df* and wider CIs in models with 19 *df*. Therefore, the outcome-exposure relationship produced in all models with 13 *df* to control seasonality and long-term trends were robust and consistent.

There are a few limitations to this study. Firstly, the island-wide weather data in our study does not reflect individual-level exposures, a common limitation of ecological studies. Moreover, the etiology of ARIs in primary care settings is not investigated. Viruses may be affected differently by the changes in climatic variables leading to more complex associations. In this study, the ARI data is collected weekly, although daily data would have been advantageous in countries with smaller fluctuations in climatic variables across the week. The data from polyclinics represents approximately 20% of all ARI cases in Singapore (Ministry of Health (Singapore), 2014). However, the effect of climatic variability on ARIs should be similar to the remaining 80% who visit private primary care institutions. Individual demographic data was not available and therefore, it was not possible to perform further analysis based on age, gender or race and stratify by risk groups. In addition, we did not have information on the time spent indoors in air-conditioned spaces and was unable to assess how this would affect the associations. Another potential limitation is that people may self-treat themselves or do not show any symptoms despite being infected, and therefore they would not be included in this study. However, there are several strengths to this study. The study utilized long time series data spanning 14 years from 2005 until the end of 2019. In this study, we used distributed lag non-linear modeling framework in a time-series regression analysis. This allowed us to investigate the associations between climatic variables and ARIs in a non-linear manner.

In conclusion, we found significant inverse association between temperature, rainfall and ARIs in Singapore. The findings on inverse association between temperature and ARIs could be used in environments where the temperature can be controlled. For instance, increasing temperature indoors could potentially reduce the number of ARI cases in Singapore. Future studies on the effects of individual level exposure to indoor and outdoor temperatures are warranted.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113748.

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Burden of osteoporosis and costs associated with human biomonitored cadmium exposure in three European countries: France, Spain and Belgium

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ABSTRACT

Cadmium (Cd) is a toxic heavy metal widespread in the environment leading to human exposure in particular through diet (when smoking is excluded), as documented by recent human biomonitoring (HBM) surveys. Exposure to Cd at environmental low-exposure levels has been associated with adverse effects such as renal toxicity and more recently bone effects. The implication, even if limited, of Cd in the etiology of osteoporosis can be of high importance at the population level given the significant prevalence of osteoporosis and the ubiquitous and life-long exposure to Cd. Therefore, the osteoporosis cases attributable to Cd exposure was estimated in three European countries (Belgium, France and Spain), based on measured urinary Cd levels from HBM studies conducted in these countries. The targeted population was women over 55 years old, for which risk levels associated with environmental Cd exposure were available. Around 23% of the cases were attributed to Cd exposure. Moreover, in a prospective simulation approach of lifelong urinary Cd concentrations assuming different intakes scenarios, future osteoporosis attributable cases were calculated, based on urinary Cd levels measured in women aged under 55. Between 6 and 34% of the considered populations under 55 years were at risk for osteoporosis. Finally, the costs associated to the burden of osteoporosis-related fractures attributable to Cd for each country targeted in this paper were assessed, standing for a major contributing role of Cd exposure in the overall social costs related to osteoporosis. Absolute costs ranged between 0.12 (low estimate in Belgium) and 2.6 billion Euros (high estimate in France) in women currently over 55 years old and at risk for fractures. Our results support the importance of reducing exposure of the general population to Cd.

1. Introduction

Cadmium (Cd) is a non-essential toxic metal widely distributed in the environment. It is naturally abundant but enriched through e.g. industrial and agricultural activities. Measured levels of Cd in agricultural products vary widely, depending on soil type, plant varieties, growing conditions, climate and agricultural methods. Anthropogenic sources, including smelter emissions and the application of fertilizers and sewage sludge to land, may contribute to the contamination of both soils and crops. Previous studies indicated that Cd-bearing fertilizers, especially manures, are an important source of Cd entering into the soil (Bergkvist et al., 2003; Grant and Sheppard, 2008; IPCS 1992). Cadmium occurs in

the environment as a divalent cation and exhibits higher rates of soil-to-plant transfer than other toxic heavy metals (e.g. lead or mercury). Isotope dilution analysis shows that for most soils, indigenous Cd is equally available to plants as freshly added Cd. This makes Cd a food-chain contaminant of great concern. Next to foodstuff also cigarette smoke is a major source of Cd exposure for the general population (Satarug and Moore 2004). Cadmium is classified by the International Agency on Cancer (IARC) as carcinogenic to humans (Group 1) based on sufficient evidence that long-term occupational exposure to Cd contributes to the development of lung cancer (IARC 1993).

Safety limits of Cd in the environment and foodstuffs were established to safeguard population health. Concerning foodstuffs, Regulation

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No. 1881/2006 of the European Commission (EC) sets Cd maximum levels ranging from 0.05 mg/kg (some meat products and vegetables) to 1.0 mg/kg (kidney from some animals, bivalve molluscs and cephalopods) (EC, 2006). A safety limit of 3 mg Cd/kg dry matter is proposed since 2016 by the EC for Cd in inorganic fertilisers with a total phosphorus content of less than 5% phosphorus pentoxide (P_2O_5) equivalent by mass (EU 2016), while a limit of 5.0 µg/L is applied to drinking water (Council Directive 98/83/EC 1998).

In 2009, the European Food Safety Authority (EFSA) performed a risk assessment for dietary Cd exposure by comparing the calculated dietary Cd exposures of the EU general population with the recommended TWI (Tolerable Weekly Intake) of 2.5 µg/kg bw based on kidney function (EFSA 2009). Conclusions were that the mean Cd exposure for adults across Europe was close to, or slightly exceeding the TWI. The exposure of some subgroups of the population, such as vegetarians, children, smokers and people living in highly contaminated areas revealed the exceedance of the TWI by about 2-fold. As the TWI is based on an early indicator of changes in kidney function and not on actual kidney damage, the risk for adverse effects on kidney function at dietary exposure across Europe was considered low at the individual level. Nevertheless, EFSA concluded that the current human exposure to Cd at the population level should be reduced (EFSA 2009; EFSA 2012).

According to the scientific literature, exposure to Cd may not only cause toxic effects to kidneys but also to bones, which became evident with the outbreak of the Itai-Itai disease in a highly Cd-polluted area of Japan, after World War II (Buha et al., 2019; James and Meliker 2013; Nordberg 2004; Nordberg et al., 2018; Staessen et al., 1999). The Itai-Itai disease is characterised by osteomalacia, osteoporosis and multiple bone fractures besides renal dysfunction (WHO 1992). Since then, increasing evidence for the effect of Cd on bones at low-doses brought the scientific community to consider this effect as possibly occurring below the threshold value of urinary Cd (U–Cd) set by EFSA at 1.0 µg/g creatinine (crea) based on kidney effects (Alfven et al., 2000; Nordberg et al., 2018; Wallin et al., 2016).

Cadmium is a cumulative toxicant, of which the body burden increases with age because of the slow elimination rate (ATSDR 2012). While levels of Cd measured in blood are generally associated with Cd short-term exposure over the past 3–4 months (even though blood Cd can also partially reflect accumulated or long-term exposure as shown by e.g. Adams and Newcomb (2014) and Hecht et al. (2016)), U-Cd is considered the best biomarker to assess long-term Cd exposure (Akesson et al., 2014; Lauwerys et al., 1994). The high degree of temporal stability of the biomarker, regardless of spot samples or first morning voids, suggests that short-term variability in dietary Cd exposures does not contribute significantly to U–Cd levels (Vacchi-Suzzi et al., 2016).

The present paper focuses further on this effect on bones and intends to estimate the burden of osteoporosis attributable to Cd exposure among women from EU countries and its related costs. Therefore, the following stages were applied:

1) **Hazard characterisation**: Selection of an exposure-response relationship for the selected health event, i.e. osteoporosis, determined at environmental (low-level) exposure to Cd.
2) **Exposure assessment**: Selection of human biomonitoring (HBM) datasets reflecting the Cd aggregated chronic exposure of population subgroups having similar characteristics to the one for which an exposure-response relationship for osteoporosis was identified. As Cd can be taken up by the oral and inhalation routes of exposure, using HBM data is particularly valuable here, as it reflects the aggregate exposure (i.e. exposure coming from multiple sources) and thus the actual body burden of individuals.
3) **Estimation of the fraction of the population at risk for osteoporosis**: Estimation of the fractions of the HBM study populations at risk for osteoporosis (as a representative sample of the target population at the national level), considering the U–Cd levels at their age.

4) **Estimation of the Cd attributable burden of osteoporosis**: Estimation of the number of osteoporosis cases attributable to Cd exposure, among the population of each selected country.
5) **Costs assessment**: Evaluation of the costs associated to the burden of osteoporosis-related fractures attributable to Cd for each country targeted in this paper.

The present study was performed within the HBM4EU (Human Biomonitoring for Europe) project. This is a Horizon 2020 Framework Project for the development of a sustainable European wide HBM network (2017–2021). HBM4EU will provide better evidence of the actual exposure of citizens to chemicals and the possible health effects to support policy making (https://www.hbm4eu.eu/about-hbm4eu/).

2. Methods

2.1. Hazard characterisation

As part of the work performed by ANSES for the refinement of recommended health-based guidance values for Cd exposure in France, a review of the scientific literature regarding Cd toxicity was performed (ANSES, 2017). The effect of Cd exposure on bones was selected as the critical effect, based on the results of an epidemiological study indicating a relationship between Cd internal exposure in women and enhanced risk for osteoporosis or bone fractures.

Among the studies identified from the literature search, several epidemiological studies reported associations between low-level Cd exposure and bone demineralization and fracture risk (Akesson et al., 2006; Engström et al. 2011, 2012; Gallagher et al., 2008; Thomas et al., 2011; Wallin et al., 2016). The ANSES panel of experts considered bone effects as the most sensitive effect associated to long-term intake of Cd, based particularly on the findings of an epidemiological study by Engström et al. (2011; 2012). This study was conducted among 2680 women, aged 56–69 years, within the Swedish Mammography cohort. The study population was categorised into three groups of exposure (U–Cd concentrations from first morning voids at <0.50, 0.50–0.75 and ≥ 0.75 µg Cd/g of crea), with the lower exposed group being used as the reference group for calculating odds ratios (ORs) and 95% Confidence Intervals (95% CI). Results indicate that, starting from 0.50 µg/g crea, U–Cd is inversely associated with bone mineral density (BMD), and associated with elevated risk of osteoporosis and fracture. The calculated ORs, according to measured U–Cd levels, of hip or spine BMD-defined osteoporosis among 400 women aged between 56 and 69 years were 1.61 (1.20–2.16) for 0.50–0.75 µg U–Cd/g crea and 1.95 (1.30–2.93) for ≥0.75 µg U–Cd/g crea (cfr. Supplementary Data - Table S1) (Engström et al. 2011, 2012). These data were subsequently used to perform the calculations on the Cd attributable number of osteoporosis cases within similar study populations of women (>55–70 years).

In order to apply the ORs to women of younger age (<55 years), age-dependent U–Cd so-called "alert" values were derived, reflecting values not to be exceeded at various ages to avoid being at risk for osteoporosis at age over 55 years. Indeed, previous lifetime modelling of the U–Cd levels indicates that, when exposure to Cd remains constant, the body burden increases in a linear manner with age up until approximately 55 years, after which it reaches a plateau (or declines slightly) (ATSDR 2012). To predict the evolution of U–Cd levels assuming different lifetime constant intakes and considering the exposure levels referred by Engström et al. (2011; 2012) and their corresponding ORs, a PBPK modelling was considered. Therefore, the 8-compartments human PBPK model by (Kjellström and Nordberg 1978) for Cd, that was recently refined by the introduction of equations describing the French general population mean body weight and creatinine excretion evolutions according to age, was considered (Leconte et al., 2021). Two scenarios of intake were considered for derivation of the U–Cd alert values of at each age class, for the population groups aged <55 years:

- **Scenario 1** - Starting from age 0, lifetime constant Cd intakes (obtained from PBPK modelling) leading, at age over 55, to the two levels of exposure (U–Cd) for which ORs were calculated by Engström et al. (2011; 2012): 0.50 Cd μg/g crea (i.e. assumed threshold value for bone effects) and 0.75 μg Cd/g crea;
- **Scenario 2** - A constant Cd intake at 0.24 μg/kg bw/d (i.e. the middle bound mean lifetime average Cd exposure of European adults estimated by EFSA) for the average age of each selected age group of women in the HBM studies (EFSA 2012).

2.2. Exposure assessment: selection of HBM datasets reflecting Cd exposure at different ages

Available HBM datasets on U–Cd were identified from the HBM4EU data repository (a database where HBM data are gathered). A bibliographic search on Medline, Scopus and Web of Science was also carried out, in order to select HBM datasets complying with the following parameters:

- Study population: living in Europe, same gender, same smoking status and similar levels of Cd exposure as the population considered in the Engström studies, in which a significant relationship between osteoporosis and U–Cd levels was observed;
- HBM data: availability of measured U–Cd levels distribution (percentiles P10 to P95) and geometric mean (GM); availability of the number of samples, these being sufficient numerous for reconstructing the U–Cd levels distribution using a mathematical function in R version 3.5.1 (2018-07-02); availability of HBM datasets covering age categories over and below 55 years within selected EU countries, allowing for estimating respectively the current burden of osteoporosis attributable to Cd exposure, and prospective burden by including upcoming cases according to different hypothetical Cd intake scenarios (through the use of PBPK modelling).

2.3. Estimation of the fractions of the HBM study populations at risk for osteoporosis

The fractions of the HBM study populations from various age categories at risk for osteoporosis were estimated based on the relationship between the U–Cd levels and health effects on bone, as indicated by Engström et al. (2011; 2012).

The distribution of the U–Cd concentrations from the HBM studies were assumed to be log-normal. Based on available data (percentiles), distributions were fitted using the cumulative distribution function under the software R.

For the population groups aged >55 years, the risk fractions were determined from the U–Cd level distributions, by calculating the percentages included in the 0.50–0.75 μg/g crea range or exceeding 0.75 μg/g crea.

For the population groups aged <55 years, the risk fractions were estimated by the percentage of the U–Cd levels included or exceeding the exposure categories as determined by the alert values predicted by the PBPK model. These alerts values corresponds to the lowest U–Cd concentrations for which there is a risk of exceeding 0.50 or 0.75 μg/g crea at latter ages (>55 years) according to the U–Cd levels trajectories, as modelled for the two different Cd intake scenarios.

2.4. Estimation of the cadmium attributable burden of osteoporosis

The study population, among which the Cd attributable burden of osteoporosis (ABO) can be estimated, has to fit in terms of gender, age and exposure level to the study population in which a Cd exposure-response relationship for bone effects is determined. These conditions allow for estimating the ABO by directly applying the selected exposure-response relationship for osteoporosis to the corresponding population. The general methodology for the Cd ABO calculations follows the

comparative risk assessment approach (Hänninen and Knol 2011; Prüss-Üstün et al., 2003). Data on the prevalence of osteoporosis in women aged 50 years or more was retrieved from the literature: 22.4%, 22.6% and 22.5% in Belgium (including Flanders), Spain and France, respectively (Hernlund et al., 2013).

Recognizing that osteoporosis is not rare and that application of the OR could lead to overestimation, we estimated relative risks (RRs) at different Cd levels based on ORs indicated in the epidemiological study of Engström et al. (2011) as well as the osteoporosis prevalence in the reference group (i.e. women having U–Cd level <0.5 μg/g crea) from the same study using the following formula of Zhang and Yu (1998):

$$RR = \frac{OR}{(1 - prev) + (prev \times OR)}$$

*with **RR** = relative risk; **OR** = odds ratio; **prev** = baseline prevalence of disease.*

Based on the estimated RRs for osteoporosis at specific U–Cd levels and the calculated fractions of the HBM studies population's at risk for osteoporosis, Cd attributable fractions (AFs) were calculated according to the following formula:

$$AF = \frac{f.(RR - 1)}{f.(RR - 1) + 1}$$

*with **AF** = attributable fraction; **RR** = relative risk at a specific exposure level; **f** = fraction of the population exposed at a specific exposure level.*

Finally, the attributable number of cases (i.e. the ABO) in each country was obtained by multiplying the AF by the number of cases (C) among the target population (which was obtained by multiplying the national disease prevalence by the size of the target population), as with the following formula:

$$AC = AF.C$$

*with **AC** = attributable number of cases; **AF** = attributable fraction; **C** = number of cases among the target population.*

For the sake of the inter-country comparison of the estimated Cd attributable number of osteoporosis cases, the number of women of the exact same age range was retrieved in each selected country from Eurostat (thereby deviating somewhat from the age range of female samples from the HBM studies). Thereby, it was assumed that the AFs calculated for the women study populations aged >55 years are representative of women aged between 55 and 70 years, given that Cd accumulation in the kidney cortex (and thus U–Cd) is reaching a plateau by around 55 years of age. For the population groups aged <55 years for which HBM data were available for France, Spain and Belgium, the attributable number of osteoporosis cases were calculated at country level for almost the same age range as observed in the HBM studies, i.e. 35-45 years.

2.5. Costs assessment

Based on the estimated attributable number of osteoporosis cases, the costs associated with the osteoporosis-related fractures were assessed for the population of women above 55 years old in the targeted countries. The assessment included three types of costs: direct costs, indirect costs and intangible costs. Direct and indirect costs were based on Borgström et al. (2006) and values were inflation adjusted to 2019. A range of costs per osteoporotic fracture, (wrist, hip and vertebrae), i.e. 2500€ to 17,000€, was applied. Intangible costs were based on reduction in quality of life (quality-adjusted life years (QALYs) lost) from Peasgood et al. (2009), Hernlund et al. (2013) and Ström et al. (2011) (between 0.04 and 0.41 for wrist, hip and vertebrae). The QALY value was set at 62,000€ (see further discussion). The range of costs per case (based on the range of direct, indirect and intangible costs) was set identical for all the countries and were expressed in 2019 Euros (i.e. 4980–42,420€).

The low and high values of this range were used to build two (low/high) scenarios for the costs assessment.

The attributable cases estimated in the female population groups aged >55 are assumed to be already at risk and are likely to incur osteoporosis-related fractures anytime. They stand for 'current' cases.

Regarding the estimated attributable cases in the female population groups aged <55 years (based on PBPK modelling), we assumed that those groups will keep on being at risk during their lifetime, assuming that they will keep on being exposed to Cd, and may incur osteoporosis-related fractures after the age of 55. They stand for 'future' cases. Population numbers used to assess current (2019) and future (2040, considered as the earliest year the youngest women in the sub-group <55 years old are likely to incur an osteoporosis-related fracture) costs are those for the year 2010. To both groups, we assigned values of lifetime risks of incurring a major osteoporosis fracture (MOF), based on literature: lifetime risks values in women at age 50 for France from the IOF Report (2018) and for Spain from Borgström et al. (2020); lifetime risks in women at age 60 from Hiligsmann et al. (2008) for Belgium (22% for France, 20% for Spain and 44.3% for Belgium) (Borgström et al., 2020; Hiligsmann et al., 2008; IOF 2018). The number of attributable cases were multiplied by these lifetime risks values to get the number of osteoporosis-related fractures. The costs for future cases were discounted at a rate of 2.5%. Using a discount rate of 4% is common practice to estimate present values for typical financial assessments. However, when dealing with health costs assessment, a decreasing discount rate of 4% the first 30 years, and then 2% (for health adverse effects occurring after 30 years) can be used in order to take into consideration intergenerational equity when human health effects occur over long-term beyond 30 years. This is common practice at European level, for example in ECHA's human health impact assessments for hazardous chemicals (ECHA 2016). Moreover, it is considered that the value of preventing a fatality has a constant utility value over time and it is therefore uprated in real terms each year by real GDP (gross domestic product) per capita growth. An uprating factor, usually based on GDP growth and income elasticity, estimated around 1.5%, based on OECD forecasts[1] was used in our assessment. Therefore, when combined with a 4% discount rate (2% for adverse effects occurring after 30 years), it gives an 'effective' discount rate of 2.5% for effects occurring over 2020–2050 and 0.5% beyond. Since the costs for future cases were assessed for 2040, they were discounted at 2.5%.

3. Results

3.1. Hazard characterisation

The Cd exposure-response relationship for bone effects from the Engström et al. (2011) study was selected. It relates to never- and ever-smoking women aged over 56 years. Based on the Engström et al. results, the U–Cd concentration of 0.50 µg/g crea was selected as the toxicological threshold value for bone effects due to Cd exposure.

According to the selected PBPK model, constant lifetime Cd dietary intakes of 0.36 and 0.54 µg/kg bw/d are the lowest doses that would lead to exceed the U–Cd values of 0.50 µg and 0.75 µg Cd/g crea at age around 55 years, respectively (starting from 0 µg Cd/g crea at birth, see Fig. 1). The estimated U–Cd alert values for each specific age (from 0 to 62 years) and age ranges of interest as well, are presented respectively in Supplementary Data - Table S2 and in the results section.

3.2. Exposure assessment: selection of HBM datasets reflecting cadmium exposure at different ages

HBM studies focussing on Cd concentrations in elderly women were

reported in France, Flanders (Belgium), and Spain. The French National Nutrition and Health Survey (ENNS) ran from 2006 to 2007 and included 18-74-year-old adults. Distributions of U–Cd concentrations in 421 women aged 60–74 years are available (Fréry et al., 2011). The third Flemish Environment and Health Study (FLEHS) reported among others Cd levels in 111 women aged 50–65 (Schoeters et al., 2017). The Spanish BIOAMBIENT. ES study was conducted in 2009 among 119 women aged 50–65 years (López-Herranz et al., 2016).

HBM data for Cd in adult women under 55 years (on average) were also available for these three EU countries: the DEMOCOPHES (DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale) study ran from 2010 to 2012 and implied 17 European countries. Participating women were aged 18–45 years (Den Hond et al., 2015) and measured U–Cd levels are available according to the following age stratification: 18–35 years, 35–40 years and >40–45 years (Berglund et al., 2015). Results of U–Cd concentrations for Spanish and Belgian women were considered from this study. As France did not participated to DEMOCOPHES, the French ELFE (French Longitudinal Study since Childhood) cohort describing levels of U–Cd among 162 pregnant women aged 35–47 years having given birth in continental France in 2011 was considered (Dereumeaux et al., 2016; SPF 2017).

In summary, three EU countries for which distributions of urinary levels of Cd for adult women were available at comparable age ranges (on average) below and over 55 years were identified: Belgium, Spain and France. The characteristics of the selected studies and of the study populations are indicated in Table 1.

3.3. Estimation of the population's fraction at risk for osteoporosis

3.3.1. Risk of bone effects attributable to cadmium in the study population groups > 55 years

Table 2 presents the results for estimation of the percentage of the population exceeding the U–Cd threshold value for bone effects of 0.50 µg/g crea. Additionally, the fractions of studied populations presenting U–Cd concentrations between 0.50 and 0.75 µg/g crea and above 0.75 µg/g crea were estimated.

In the three countries, more than or equal to 40% of the women have a concentration > 0.50 µg Cd/g crea and are at risk for osteoporosis by Cd exposure. Largest exceedance was found for Flanders (48%), but 57% of these were situated in the U–Cd category 0.50–0.75 µg Cd/g crea. For Spain, 42% of the women exceeded 0.50 µg Cd/g crea but 61% of these were situated in the U–Cd category ≥0.75 µg Cd/g crea. For France, 40% exceeded 0.50 µg Cd/g crea with 56% of these in the category 0.50–0.75 µg Cd/g crea, similarly to Flanders.

3.3.2. Risk of bone effects attributable to cadmium in the study population groups <55 years and estimation of alert levels

Distributions of U–Cd levels measured in women aged under 55 years from the DEMOCOPHES Belgium, DEMOCOPHES Spain and ELFE studies are indicated in Table 3. Geometric mean values are close together, although age differences between the different groups.

Using the Cd lifetime biokinetic model, the evolution of U–Cd levels for these women was simulated assuming two different scenarios regarding their Cd intake until they reach approximately 55 years. Therefore, the estimation of the Cd attributable number of osteoporosis cases constitutes in these cases a prospective estimation.

Regarding the scenario 1, where constant Cd intakes of 0.36 µg/kg bw/d for those reaching 0.50 Cd µg/g crea and 0.54 µg/kg bw/d for those reaching 0.75 µg Cd/g crea starting from age 0 were considered, the U–Cd alert levels for the different age categories of women from the selected HBM studies were determined by using the alert value corresponding to the median of the age interval. The alert values differ by HBM campaign due to age interval differences (Table 4). For the DEMOCOPHES women from Spain and Belgium, the U–Cd alert values estimated to avoid exceedance of 0.50 µg Cd/g crea and 0.75 µg Cd/g

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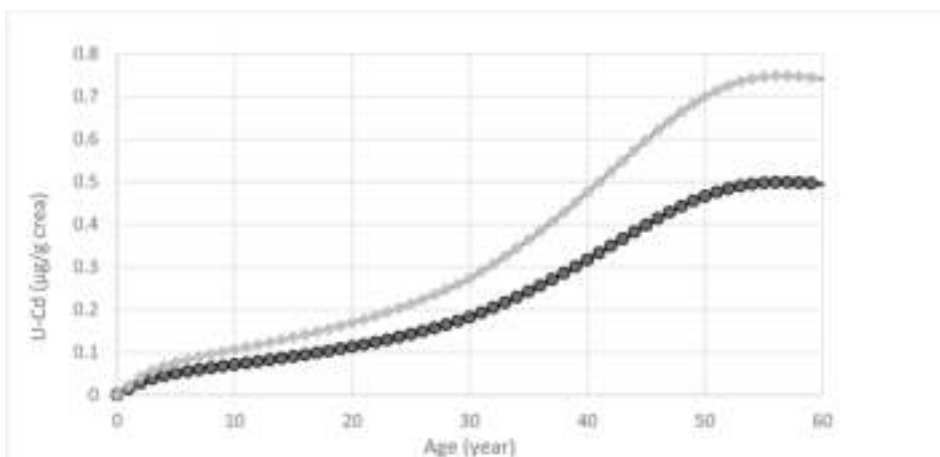[1] https://knoema.fr/iuacek/euro-area-gdp-growth-forecast-2019-2024-and-up-to-2060-data-and-charts.

Fig. 1. Predicted urinary Cd (U–Cd) concentrations (µg/g crea) as a function of age (year), reaching or exceeding at age around 55 years the values of: 1) dotted curve - 0.50 µg U–Cd/g crea (constant dietary intake of 0.36 µg/kg bw/d estimated from PBPK modelling); 2) curve with squares - 0.75 µg U–Cd/g crea (constant dietary intake of 0.54 µg/kg bw/d estimated from PBPK modelling).

Table 1

Characteristics of the HBM datasets reporting the distribution of urinary Cd (U–Cd) concentrations (adjusted for crea) among women from EU countries. All women (smoking and non-smoking) in all studies were selected.

Country	Study	Timeframe	Age of selected study population in years (mean)	Number of individuals	Type of sampling
Belgium	DEMOCOPHES	2011	35-40 (37.5)	58	Morning urine
			>40–45 (43)	45	
	FLESHIII	2012–2015	50-65 (57.5)	111	Spot-urine
Spain	DEMOCOPHES	2011	35-40 (37.5)	49	Morning urine
			>40–45 (43)	57	
	BIOAMBIENT.ES	2009	50-65 (57.5)	119	Morning urine
France	ELFE	2011	35-47 (41)	162	Spot-urine
	ENNS	2006–2007	60-74 (67)	421	Spot-urine

Table 2

Percentages of women aged over 55 years from the FLESHIII, BIOAMBIENT and ENNS studies exceeding the urinary Cd (U–Cd) threshold value for bone effects (0.50 µg/g crea), either for U–Cd concentrations between 0.50 and 0.75 µg/g crea or ≥ 0.75 µg/g crea, according to the U–Cd concentration distributions.

Study	U–Cd concentrations (µg Cd/g crea)								% of the study population at risk		
	GM	95% CI GM	P10	P25	P50	P75	P90	P95	% > 0.50 µg Cd/g crea	% at 0.50–0.75 µg Cd/g crea	% ≥ 0.75 µg Cd/g crea
FLESH III	0.49	0.44–0.53	0.27	0.37	0.48	0.64	0.96	1.12	48.5	**27.8**	**20.7**
BIOAMBIENT.ES	0.42	0.34–0.52	–	0.29	0.46	0.69	1.27	1.82	42.3	**16.3**	**26.0**
ENNS	0.43	0.40–0.46	0.20	0.29	0.42	0.65	0.99	1.15	40.1	**22.4**	**17.7**

With **GM** = Geometric mean; **CI** = Confidence Intervals; **P10** = Percentile 10; **P25** = Percentile 25; **P50** = Percentile 50; **P75** = Percentile 75; **P95** = Percentile 95.

Table 3

Distributions of urinary Cd (U–Cd) levels in the selected HBM studies population of women aged under 55 years.

Study	Age (mean) in years	U–Cd concentrations (µg/g crea)							
		GM	95% CI GM	P10	P25	P50	P75	P90	P95
DEMOCOPHES - Belgium	**35–40 (37.5)**	0.18	0.15–0.28	0.10	0.12	0.17	0.31	0.41	0.54
	41–45 (43.0)	0.19	0.15–0.23	0.09	0.14	0.21	0.27	0.37	0.46
DEMOCOPHES - Spain	**35–40 (37.5)**	0.21	0.17–0.27	0.09	0.14	0.19	0.39	0.58	0.64
	41–45 (43.0)	0.22	0.19–0.27	0.09	0.14	0.21	0.32	0.57	0.75
ELFE - France	**35–47 (41.0)**	0.21	0.19–0.24	<LOQ*	0.15	0.20	0.31	0.41	0.57

With **GM** = Geometric mean; **CI** = Confidence Intervals; **P10** = Percentile 10; **P25** = Percentile 25; **P50** = Percentile 50; **P75** = Percentile 75; **P95** = Percentile 95.

crea at latter age were respectively 0.28 µg/g crea and 0.42 µg/g crea for the 35–40 years age group and 0.33 µg/g crea and 0.50 µg/g crea for the 41–45 years age group). For the ELFE study women (35–47 years), the U–Cd alert values estimated to avoid exceedance of 0.50 µg Cd/g crea and 0.75 µg Cd/g crea at latter age were 0.33 µg/g crea and 0.50 µg/g crea, respectively. Overall, based on the modelling, between 10 and 34%

of the women will have a concentration exceeding 0.50 µg Cd/g crea and thus are at risk for developing osteoporosis due to Cd exposure.

For the scenario 2, where an intake of Cd equivalent to the lifetime average Cd dietary intake of European adults was considered, it was assumed that the women younger than 55 years of the selected HBM studies would have a constant intake of Cd of 0.24 µg/kg bw/d from

Table 4

Percentage of the study populations at risk for osteoporosis considering their modelled urinary Cd (U–Cd) levels at age over 55 years, according to two different scenarios of Cd intake (Scenario 1: constant Cd intake of 0.36 µg/kg bw/d for those reaching 0.50 Cd µg/g crea and 0.54 µg/kg bw/d for those reaching 0.75 µg Cd/g crea, starting from age 0; Scenario 2: constant Cd intake at 0.24 µg/kg bw/d from their age on). Urinary Cd "alert" values estimated for each age group of each study are also presented.

Study	Age (mean) in years	Scenario 1				Scenario 2			
		% of the study population at risk for osteoporosis depending on their measured Cd exposure				% of the study population at risk for osteoporosis depending on their measured Cd exposure			
		U–Cd alert value	U–Cd ≥ 0.50 µg/g crea at age over 55y	U–Cd alert value	U–Cd ≥ 0.75 µg/g crea at age over 55y	U–Cd alert value	U–Cd ≥ 0.50 µg/g crea at age over 55y	U–Cd alert value	U–Cd ≥ 0.75 µg/g crea at age over 55y
DEMOCOPHES-Belgium	**35–40** (37.5)	0.28	**25.2%**	0.42	10.0%	0.32	**19.2%**	0.62	3.0%
	41–45 (43.0)	0.37	**10.4%**	0.55	2.20%	0.44	**5.6%**	0.68	0.8%
DEMOCOPHES-Spain	**35–40** (37.5)	0.28	**34.0%**	0.42	16.1%	0.32	**27.4%**	0.62	6.1%
	41–45 (43.0)	0.37	**24.4%**	0.55	11.1%	0.44	**17.8%**	0.68	6.6%
ELFE - France	**35–47** (41.0)	0.33	**22.6%**	0.50	7.40%	0.41	**13.2%**	0.66	2.8%

their age on. Fractions of the study populations likely to have U–Cd levels between >0.50 µg/g crea and ≥0.75 µg/g crea at later age (at 55 years) were calculated. Results are indicated in Table 4 under scenario 2. Based on the modelling, between 6 and 27% of the women will have a concentration >0.50 µg Cd/g crea and are thus at risk for developing osteoporosis by Cd. The fraction of the population at risk, i.e. exceeding 0.50 µg Cd/g crea in scenario 2, is on average a factor 1.5 lower than for scenario 1. In general (considering scenario 1 and 2), between 6 and 34% of the considered populations < 55 years were at risk for osteoporosis.

3.4. Cadmium attributable burden of osteoporosis

Based on the multivariable-adjusted ORs for hip or spine osteoporosis in women aged over 55 years calculated by Engström et al. (2011) according to specific U–Cd levels (OR of 1.61 [95% CI (1.20–2.16)] for U–Cd at 0.50–0.75 µg/g crea; OR of 1.95 [95% CI (1.30–2.93)] for U–Cd ≥ 0.75 µg/g crea) and considering the 13% prevalence of hip or spine osteoporosis in the 2067 women with a U–Cd level < 0.50 µg/g crea (i.e. the prevalence of the outcome in the reference group considered as non-exposed), following RRs were estimated: 1.49 [95% CI (1.17–1.88)] for U–Cd levels at 0.50–0.75 µg/g crea and 1.74 [95% CI (1.25–2.34)] for U–Cd levels ≥ 0.75 µg/g crea.

3.4.1. Cadmium attributable burden of osteoporosis in the study population over 55 years

The Cd attributable risks (i.e. the AFs) for osteoporosis in the target countries population of women over 55 years were calculated based on

the fractions of the population at risk for osteoporosis according to their U–Cd levels as indicated in Table 2, and the U–Cd levels-depending estimated RRs as previously indicated.

Multiplying the AFs by the calculated number of osteoporosis cases (themselves obtained by multiplying the country-specific disease prevalence by the size of the target population in the corresponding country), the Cd ABO in women aged between 55 and 70 years depending on their U–Cd levels were obtained and are presented in Table 5. The number of osteoporosis cases in each country was finally obtained by summing these U–Cd levels-depending number of cases.

From Table 5, it becomes clear that around 23% (sum of the AFs) of the osteoporosis cases in women aged 55–70 years can be due to Cd exposure which is a relatively significant proportion. Largest burden per 1000 women is in Flanders followed by Spain and France, however confidence intervals overlap.

3.4.2. Cadmium attributable burden of osteoporosis in the study population at younger ages than 55 years

Attributable prospective number of cases is given in Table 6. For scenario 1, seeing the consistency of the prevalence of osteoporosis in the three countries (around 22% according to Hernlund et al., 2013) and the relatively larger U–Cd concentrations in the Spanish DEMOCOPHES data compared to the Belgian and French HBM data, the largest attributable number of cases is estimated for Spain. Confidence intervals overlap. Regarding scenario 2, as the fraction of the population exceeding 0.50 µg Cd/g crea is on average a factor 1.5 lower than for scenario 1 for all countries (see above), this is also reflected in the attributable number of cases which are somewhat lower for scenario 2

Table 5

Cd attributable burden of osteoporosis (ABO) for the female populations between 55 and 70 years in the target-countries.

Country (HBM Study)	U–Cd levels (µg/g crea)	AF (lower and upper limits)	Country-specific target population aged 55-70y in 2010	Cd ABO depending on U–Cd levels (lower and upper limits)	Total Cd ABO for women aged 55-70y (lower and upper limits)	Attributable number of cases per 1000 women 55-70y (lower and upper limits)
Belgium - FLESH III	0.50–0.75	**0.12** (0.05–0.2)	960942	25830 (10763–43050)	**54243** (19157–89760)	**56** (20–93)
	≥0.75	**0.13** (0.04–0.22)		28413 (8395–46709)		
Spain -BIOAMBIENT. ES	0.50–0.75	**0.07** (0.03–0.13)	3815137	63804 (23280–107778)	**202622** (75875–331093)	**53** (20–87)
	≥0.75	**0.16** (0.06–0.26)		138818 (52595–223315)		
France - ENNS	0.50–0.75	**0.10** (0.04–0.16)	5804897	129304 (48326–214201)	**279506** (104488–464972)	**48** (18–80)
	≥0.75	**0.12** 0.04–0.19)		156732 (56162–250772)		

Table 6

Cd attributable burden of osteoporosis (ABO) for the female populations at younger ages than 55 years in the target-countries, assuming in Scenario 1: lifetime constant Cd intake of 0.36 μg/kg bw/d, if reaching 0.50 μg Cd/g crea at age around 55 years or 0.54 μg/kg bw/d, if reaching 0.75 μg Cd/g crea around 55 years; in Scenario 2: constant intake of 0.24 μg/kg bw/d from the average women age at which the U–Cd levels were measured until about 55 years.

Country (HBM study)	Age range (mean) in years	U–Cd level (μg/g crea)	AF (lower and upper limits)	Age range considered for Cd ABO calculation (years)	Country-specific target population in 2010	Scenario 1				Scenario 2			
						Cd ABO depending on U–Cd level category	Total Cd ABO according to age (lower and upper limits)	Total Cd ABO for women aged 35-45y (lower and upper limits)	Attributable number of cases per 1000 women aged 35-45y (lower and upper limits)	Cd ABO depending on U–Cd level category	Total Cd ABO according to age (lower and upper limits)	Total Cd ABO for women aged 35-45y (lower and upper limits)	Attributable number of cases per 1000 women aged 35-45y (lower and upper limits)
Belgium - DEMOCOPHES	35-40 (37.5)	0.50–0.75	**0.07** (0.03–0.12)	35–40	446086	6995	**13889** (4996–23582)	**18770** (6682–32100)	**22** (8–38)	7394	**8105** (3397–16287)	**12166** (4285–20813)	**14** (5–25)
		≥0.75	**0.07** (0.03–0.12)			6895				2198			
	41-45 (43.0)	0.50–0.75	**0.04** (0.01–0.07)	41–45	396135	3461	**4880** (1686–8518)			2041	**2168** (887–4525)		
		≥0.75	**0.02** (0.01–0.03)			1420				532			
Spain - DEMOCOPHES	35-40 (37.5)	0.50–0.75	**0.08** (0.03–0.14)	35–40	2306223	42218	**97466** (35442–163659)	**157252** (56983–266526)	**37** (13–63)	49515	**60884** (26060–121441)	**115008** (41446–196174)	**27** (10–46)
		≥0.75	**0.11** (0,04–0.18)			55248				22412			
	41-45 (43)	0.50–0.75	**0.06** (0.02–0.10)	41–45	1945154	26816	**59786** (21541–102868)			22859	**36410** (15386–74733)		
		≥0.75	**0.08** (0.03–0.13)			32970				20222			
France - ELFE	35-47 (41)	0.50–0.75	**0.07** (0.03–0.12)	35–45	5020856	79078	**137822** (48577–234976)	**137822** (48577–234976)	**27** (10–47)	55355	**65682** (27113–135563)	**77949** (27113–135563)	**16** (5–27)
		≥0.75	**0.05** (0.02–0.09)			58744				22594			

Table 7
Number of major osteoporotic fractures (MOF) and costs related to Cd exposure in Belgium, Spain and France.

	Women aged >55 years (Current cases | Current costs)					Women aged < 55 years (Future cases | Future costs)	
						Scenario 1[a]	
	# Cd attributable cases likely to incur osteoporosis-related MOF (lower and upper limits)	Cost associated to burden of Cd osteoporosis-related MOF (not discounted, in million €) (lower and upper limits)		Cost associated to burden of Cd osteoporosis-related MOF (not discounted, in million € per 1000 women) (lower and upper limits)		# Cd attributable cases likely to incur osteoporosis-related MOF (lower and upper limits)	Cost associated to burden of Cd osteoporosis-related MOF (discounted at 2.5% in 2040, in million €) (lower and upper limits)
		Low cost	High cost	Low cost	High cost		Low cost
Belgium	24030 (8487–39764)	119.7 (42.3–198.0)	1019.3 (360.0–1686.8)	0.125 (0.044–0.206)	1.061 (0.375–1.755)	8315 (2960–14220)	25.3 (9.0–43.2)
Spain	40524 (15175–66219)	201.8 (75.6–329.8)	1719.0 (643.7–2809.0)	0.053 (0.020–0.086)	0.451 (0.169–0.736)	31450 (11397–53305)	95.6 (34.6–162.0)
France	61491 (22987–102294)	306.2 (114.5–509.4)	2608.5 (975.1–4339.3)	0.053 (0.020–0.088)	0.449 (0.168–0.748)	30321 (10687–51695)	92.1 (32.5–157.1)

aScenario 1: PBPK model used to derive alert values, starting from no internal Cd exposure at age 0 and assuming a lifetime constant dietary Cd intake of 0.36 µg Cd/kg bw/d, if reaching 0.50 µg Cd/g crea at age ~55 years and 0.54 µg Cd/kg bw/d if reaching 0.75 µg Cd/g crea at age ~55 years. Exceedance of the alert values means an increased risk of osteoporosis related to Cd exposure at latter age.

bScenario 2: PBPK model used to derive alert values, starting from observed internal Cd exposure (U–Cd levels in the selected HBM studies) at considered age and assuming from then on a constant dietary Cd intake of 0.24 µg/kg bw/d. Exceedance of the alert values means an increased risk of osteoporosis related to Cd exposure at latter age.

than 1.

In the women >55 years the largest burden is observed for Belgium (although results of different countries per 1000 women lay close together) (Table 5), whereas in the women < 55 years the number of cases per 1000 women stands out for Spain compared to the other countries (Table 6). Per 1000 women, the number of cases is lower in the two scenario calculations (Table 6) than in the female population > 55 years (Table 5).

3.5. Costs assessment

Based on the attributable cases estimated above, the costs for the first year after a major osteoporotic fracture (MOF) associated to Cd exposure for the three countries targeted in this paper were assessed and are presented in Table 7. The range of costs between the low/high scenarios reflects the range of severity of the different types of fractures (wrist, hip or vertebrae). The absolute first year (future) cost associated to Cd osteoporosis-related MOF in women aged < 55 years is lower for all scenarios for Belgium given that the attributable cases in Belgium are much lower than in Spain and France (factor of 3–4). Future costs for Spain and France are comparable for this same age category of population. Current absolute costs in women aged >55 years are significantly higher for France (factor of 1.5–2.5) proportionally to the higher attributable cases in this country (Table 7). In general, absolute costs ranged between 0.1 (low estimate Belgium) and 2.2 billion Euros (high estimate France). The relative first year (future) cost (per 1000 women) associated to Cd osteoporosis-related MOF in women aged < 55 years is the highest for Belgium (factor of 1.5 on average). Current costs in women aged >55 years are also significantly the highest for Belgium (factor of 2.3) and are identical for Spain and France (Table 7). Reasons may come from a larger probability for a MOF in Belgium (44% in Belgium versus around 20% in the other countries). Moreover, for women <55 years, the difference in costs per 1000 women between Belgium and the other countries becomes smaller when compared to women >55 years old. This is mainly because for women >55 years the number of osteoporotic cases per 1000 women was largest for Belgium

(Table 5) compared to the other countries, whereas for women < 55 years this was the smallest (Table 6).

4. Discussion

This work, which focuses on the estimation of the effects of human Cd exposure on osteoporosis, suggests a significant societal impact. As also other health effects are known or suspected to be caused by Cd at low-level exposure, the associated costs are probably underestimated (Li et al., 2016; Satarug et al., 2010; Tellez-Plaza et al., 2013). The renal toxicity of Cd for example is well-known and most of the regulatory guidance or limit values derived for this substance are based on it (ATSDR 2012; EFSA 2009). An HBM-guidance value of 1 µg/g crea for U–Cd in adults, based on kidney effects, was recently proposed under the HBM4EU project (Lamkarkach et al., 2021). The literature review performed thereby underlined that studies' results released within the past ten years support also causal associations between Cd low-level exposure and bone or cardiovascular effects. However, for the time being, the weight of evidence relating to these effects was not considered sufficient within the HBM4EU project to select them as critical effects for the derivation of an internal reference value. Due to some missing information when interpreting epidemiological findings on bone effects (e.g. nutritional factors, toxicodynamics associated with Cd tissue levels that cause bone decalcification), the causal relationship between low-level exposure to Cd (i.e. low concentrations of U–Cd) and decreased BMD is debated by the scientific community. Despite this, multiple recent publications provide evidence of effects of Cd on bones at low-doses occurring below the U–Cd critical value of 1 µg/g crea set by EFSA, as well as associated exposure-response relationships (Buha et al., 2019; Engström et al. 2011, 2012; Wallin et al., 2016). Buha et al. (2019) showed that bone mineral health is significantly related to environmental Cd exposure based on experimental and human data. Human data, including direct bone analysis, i.e. measurement of Cd concentration in the bone, showed that bone health is extremely sensitive to even background levels of environmental Cd, supporting the epidemiological evidence. Besides human studies, toxicity studies in rats showed

Women aged < 55 years (Future cases | Future costs)							
Scenario 1[a]			Scenario 2[b]				
Cost associated to burden of Cd osteoporosis-related MOF (discounted at 2.5% in 2040, in million €) (lower and upper limits)	Cost associated to burden of Cd osteoporosis-related MOF (discounted at 2.5% in 2040, in million € per 1000 women) (lower and upper limits)		# Cd attributable cases likely to incur osteoporosis-related MOF (lower and upper limits)	Cost associated to burden of Cd osteoporosis-related MOF (discounted at 2.5% in 2040, in million €) (lower and upper limits)		Cost associated to burden of Cd osteoporosis-related MOF (discounted at 2.5% in 2040, in million € per 1000 women) (lower and upper limits)	
High cost	Low cost	High cost		Low cost	High cost	Low cost	High cost
215.3 (76.6–368.1)	0.030 (0.011–0.051)	0.256 (0.091–0.437)	5390 (1898–9220)	16.4 (5.8–28.0)	139.5 (49.1–238.7)	0.019 (0.007–0.033)	0.166 (0.058–0.283)
814.2 (295.0–1380.0)	0.022 (0.008–0.038)	0.192 (0.069–0.325)	23002 (8289–39235)	69.9 (25.2–119.2)	595.5 (214.6–1015.7)	0.016 (0.006–0.028)	0.140 (0.050–0.239)
784.9 (276.7–1338.3)	0.018 (0.006–0.031)	0.156 (0.055–0.267)	17149 (5965–29824)	52.1 (18.1–90.6)	443.9 (154.4–772.1)	0.010 (0.004–0.018)	0.088 (0.031–0.154)

that effects on femur phosphor and zinc levels were observed at lower dietary Cd levels than the corresponding safe intake level for Cd currently based on renal effects. In the year 2000, the EC categorised Cd as not having sufficient evidence for endocrine activity (BHK/TNO 2000). However, in recent decades, more and more evidence has shown that Cd has endocrine disrupting properties (Kortenkamp 2011). considered Cd as an estrogen mimic. Cadmium exposure has also been linked with prostate cancer although the associations are weak which would merit further work to confirm them (WHO/UNEP 2013). Cadmium is capable of disrupting osteoblasts homeostasis by altering the Wnt/β-catenin pathway thus influencing bone health (Papa et al., 2015). The Wnt/β-catenin pathway promotes bone formation and suppresses bone resorption (Baron and Kneissel 2013). This mechanism is worth considering in endocrine disrupting chemical action (Üstündağ and Emekli-Alturfan 2020). Noteworthy is that bone effects were recently selected by a panel of experts as endpoint for deriving an oral toxicological reference value of 0.35 μg/kg bw/d for conducting a health risk assessment of Cd dietary exposure in France (ANSES, 2017). Thereby, the threshold value of 0.50 μg Cd/g crea, based on the Engström et al. (2011; 2012) results, was considered in the present study. Women ≥50 years of age with U–Cd levels between 0.50 and 1.00 μg/g crea based on the US NHANES (National Health and Nutrition Examination Survey) results were also found to be at 43% greater risk for hip-BMD–defined osteoporosis, relative to those with levels ≤ 0.50 μg/g (OR = 1.43; 95% CI, 1.02–2.00; p = 0.04) (Gallagher et al., 2008), supporting this threshold value of 0.50 Cd μg/g crea in urine for bone effects. Ximenez et al. (2020) examined the association of heavy metals & metalloids with BMD loss based on data of three NHANES cycles using a data mining approach (Ximenez et al., 2020). The model was able to identify arsenic, Cd and tungsten as having critical importance on BMD loss. Cadmium concentrations in urine were below 0.50 μg/L. Stronger correlations between these elements and BMD loss were found as compared to smoking or diabetes, which are important predictors for bone loss and fracture risk. A low concentration of Cd is thus important for bone health, but there may be a complex interplay between metals and other elements influencing bones.

Efforts for estimating the Cd attributable disease burden already exists, but differences in approaches can be raised. As an example, Zang et al. (2019) estimated the global burden of late-stage chronic kidney disease resulting from dietary exposure to Cd, including in WHO regions (Belgium, France and Spain) (Zang et al., 2019). However, their estimation is based on PBPK simulated U–Cd levels arising from an assumed Cd dietary intake (0.31 μg/kg bw/d in Belgium and 0.30 μg/kg bw/d in France and Spain). Despite the fact that food is the most significant source of exposure to Cd, other sources contribute nevertheless to the total Cd human exposure as e.g. inhalation of tobacco smoke or particulate matter from ambient air (ATSDR 2012; Satarug et al., 2017). Therefore, using HBM data reflecting the aggregated exposure to Cd seems more reliable for calculating the Cd attributable disease burden.

Based on this knowledge and in the framework of the precautionary principle, a calculation of the ABO was merited. We further think that large scale human studies measuring Cd in urine together with Cd in bone and bone health are supportive for unravelling the effect of Cd on osteoporosis and setting safe biomarker concentrations.

Uncertainty in our estimation of the Cd ABO is related in particular to the assumptions and extrapolations underlying the calculations as well as the PBPK modelling, including the chemical-specific, human physiological and HBM scaling parameters, described in more detail below.

- Uncertainty related to the estimation of the Cd aggregated exposure in our target country populations: we relied on limited-sized female samples from HBM studies, which we considered as representative (in terms of sex and age) of our population of interest (the population for which exposure to Cd and associated osteoporosis was identified). Thereby, uncertainty may exist in the reconstruction of the U–Cd concentration distributions from the study samples based on the publicly available GMs and percentiles P10–P95, and to the subsequent extrapolation of these study samples distribution to the corresponding total population of the same age in the respective country. Predicting whether an under- or overestimation of the ABO cases may result from a possible difference in the U–Cd distributions between the HBM study samples and the total population is however not possible.
- Uncertainty related to the OR: the uncertainty on the OR and consequently the estimated RR is in general categorised as parameter uncertainty (Knol et al., 2009). The applied ORs from Engström et al. (2011) were significantly higher than 1 but the uncertainty is considerable (cfr. Table S1). This is reflected in the attributable cases

estimations, which are about 2.6 times lower when considering the lower limit of the 95% CI of the ORs and 1.6 times higher when considering the upper limit of the 95% CI of the ORs than the estimations using the OR central values. A detailed sensitivity analysis to estimate the impact of the uncertainty related to the ORs on the results would still be necessary.

- Uncertainty related to the threshold at which the effect of Cd on bones start: this is still debated in the scientific community (cfr. discussion above).
- Uncertainty related to the modelling of lifelong internal exposures to Cd: changes in body weight and in kidney function (urinary creatinine excretion) with age were considered in the biokinetic PBPK model. However, these two age-related physiological parameters were scaled on physiological datasets measured in the French population (Leconte et al., 2021). These physiological parameters may differ among the European populations, nonetheless we assume that the impact on the results is quite limited. As the PBPK model used is only integrating the oral route, Cd intake *via* smoking was not considered in the age-related U–Cd levels predictions. As U–Cd levels are raising more steeply in current/former smokers than in non-smokers with age (López-Herranz et al., 2016; Mortensen et al., 2011), an underestimation of the Cd attributable future burden (osteoporosis cases) is likely.

Regarding costs assessment, direct costs include medical care (hospitalization, outpatient care and pharmaceuticals) based on the study of Hernlund et al. (2013). They evaluated the first-year cost of fracture (hip, vertebral, forearm and others) for EU 27 countries including France, Spain and Belgium. For those countries, costs are comparable and range from about 1000€ to 12,000€ per fracture (therefore considered as "costs per case"). These values are consistent with other values available in the literature for France and Spain, such as the ones from (Ström et al., 2011). Regarding Belgium, there seems to be no study estimating cost per case to be compared with but only evaluations for the whole Belgian population (such as Svedbom et al., 2013). Indirect costs refer to the losses of labour and productivity that are incurred in addition to the direct costs of an illness. In the present case, the costs of fracture-related productivity losses were not included in the Hernlund et al. (2013) evaluation given that they are only incurred in patients below the retirement age —median age 60 years in Europe— and have previously been estimated to be limited in osteoporosis in literature (Hernlund et al., 2013; Ström et al., 2011). However, Borgström et al. (2007) estimated that below the age of 65 years the indirect costs were approximately 9% of the total costs for hip fractures and 23% for wrist fractures. We consider that although these costs may be not the most significant in the overall cost of osteoporosis burden, they should be included in the assessment. Borgström et al. (2007) estimated (direct and indirect) costs of osteoporosis for Sweden at 2000€-14,000€, standing for the cost the year after a hip, vertebral and wrist fracture. This range is slightly higher than the one from Hernlund et al. (2013), which only accounts for direct costs. We thus used Borgström et al. (2007) values (inflation-adjusted to 2019) in our assessment in order to account for both direct and indirect costs. Intangible costs are based on a monetary valuation of QALYs lost, due to the lower quality of life in patients suffering from osteoporosis. QALYs lost associated to osteoporosis-related fractures were taken from the literature Peasgood et al. (2009), Hernlund et al. (2013) and Ström et al. (2011) and estimated between 0.04 and 0.41. To value these QALYs lost, Borgström et al. (2007) suggested to assign a Willingness To Pay of 2 x GDP/capita for industrialised countries, such as the ones targeted in this paper, as a proxy to the societal value of a QALY (based on WHO's own

recommendations, 2001) (Borgström et al., 2007; WHO 2001). In 2019, the GDP per capita in the EU27 was around 31,000€ (Eurostats[2]), therefore a value of 62,000€ per QALY was used.

Uncertainty in the costs assessment may be due to several elements: firstly, the lifetime risks of incurring a MOF for France and Spain is available in women at 50 years old whereas it is in women at 60 years old for Belgium. The HBM data sets used in the paper starts from 55 years old which does not entirely fit the lifetime risks. However, no other data on lifetime risks were available. Secondly, the years of reference used in the costs calculation may show some discrepancy: the HBM dataset is from 2010 and the disease burden costs are estimated for 2019 (current costs) and 2040 (future costs). The population numbers are assumed to stay steady regardless the period. Thirdly, one source of overestimation of the costs assessed may be due to differences in country-specific costs data: for example, Borgström et al. (2020) indicate lower (but only direct) costs for Spain. However, one source of underestimation of the costs assessed is due to the fact that we don't take into account costs of consecutive and multiple fractures (i.e. long term costs) but only the first year costs associated to the first fracture. Finally, Borgström et al. (2020) provide a loss of QALYs of 0.16 for forearm fracture which is higher than the lower bound used herein for QALYs lost (wrist). Using 0.16 as a sensitivity parameter in our assessment would increase the low cost scenarios results with a factor of 2.5.

Compared to total cost of osteoporosis such as reported in the IOF 2018 report (in 2017, annual cost associated to new fragility fractures in the EU6 were estimated at €37 billion), the costs assessed in this paper show that Cd exposure stands for a major contribution.

HBM datasets meeting the parameters for being relevant for our calculations were available only for three EU countries. Therefore, we refrained from extrapolating the estimated costs to whole Europe. Extrapolating our results to a larger scale may be of interest in the future, provided that comparable HBM data relating to a similar target population becomes available for more countries.

5. Conclusion

Current calculation on the ABO cases due to Cd exposure shows that in women aged 55 years or older, around 20% of the osteoporosis cases are associated with Cd aggregated exposure. PBPK modelling shows that for the future generation, between 6 and 34% of the women are at risk of osteoporosis due to Cd. Seeing the relatively large prevalence of osteoporosis and its associated costs, these results should be of great interest particularly to risk managers and policy makers, to define priorities and mitigation strategies to reduce Cd exposure and the subsequent health costs. In this regard, Schaefer et al. (2020) have reviewed current or new mitigation efforts that may reduce dietary exposure to Cd and therefore the global burden on human health (Schaefer et al., 2020).

Finally, more efforts are needed to reduce the uncertainty related with bone effects at low environmental Cd concentrations. Measurements of U–Cd in HBM studies coupled with assessment of indicators of bone status reflecting short-term and long-term effects can help in targeting this issue.

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[2] https://ec.europa.eu/eurostat/tgm/refreshTableAction.do?tab=table&plugin=1&init=1&pcode=tec00001&language=en; retrieved on June the 2nd 2020.

(UIDP/50017/2020+UIDB/50017/2020), through national funds.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113747.

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The author regrets that in Table 6, titled "Time spent indoors and outdoors by children at different life stages" the mean time outdoors for children 6–12 months old is incorrectly shown as 139 minutes. It should be corrected to 39 minutes.[1]

The author would like to apologize for any inconvenience.

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Effects of climate variables on the COVID-19 outbreak in Spain

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ABSTRACT

An outbreak of the novel COVID-19 virus occurred during February 2020 onwards in almost all the European countries, including Spain. This study covers the correlation found between weather variables (Maximum Temperature, Minimum Temperature, Mean Temperature, Atmospheric Pressure, Daily Rainfall, Daily Sun hours) and the coronavirus propagation in Spain. A strong relationship is found when correlating the virus spread to the mean temperature, minimum temperature, and atmospheric pressure in different Spanish provinces. In this analysis we have used the ratio of the PCR COVID-19 positives with respect to the population size. A linear regression model using the mean temperature is implemented. Moreover, an analysis of variance is used to confirm the influence of mean temperature on the spread of virus. As a second measurement of the COVID-19 outbreak we have used the results of the antibodies tests carried out in Spain that provide an estimation of the heard immunity achieved. Based on this analysis, an estimation of the asymptomatic population is performed. All these results exhibit significant correlation with weather variables. The most affected provinces were Soria, Segovia and Ciudad Real, which are the coldest. On the opposite side, places such as Southern Spain, the Baleares, and Canary Islands showed a lower rate of spread. This might be related to the warmer climate and the insularity of these islands. Besides, the coastal influence and the daily sun hours might also influence the lower rates in the east and west regions in Spain. This analysis provides a deeper insight of the influence of weather variables onto the COVID-19 spread in Spain.

1. Introduction

This paper aims to understand the influence of the weather variables in the COVID-19 spread in Spain. Many studies have only focused on the importance of the density of population in major cities with high connectivity worldwide. Nevertheless, there are other factors that could influence the transmission. For instance, it has been seen in South Korea that only one COVID-19 infected person, known as "patient 31", transmitted the virus to 4482 persons in the first stage of the pandemic (Shim et al., 2020). Therefore, the potential spread of the COVID might not be only related to the number of infected people and other factors might have a great influence in the strength of the transmission. Several weather variables have been mentioned among the various potential factors that might have played a crucial role in the spread of this virus. One of the first studies regarding the effect of the temperature showed that the COVID-19 survived better with mean temperatures in the range between −10 and 15 °C (Bannister-Tyrrell, M et al., 2020). Moreover, a robust negative association with high temperature and high humidity as factors, that reduced the COVID-19 transmission, was found over for

Chinese and USA cities (Wang et al., 2020). Following these statements, neither extreme cold nor hot conditions would favour the virus transmission. Regarding humidity, for Iran cities seems it has a negative correlation with COVID-19 propagation (Ahmadi, M et al., 2020). However, the authors of that study say the correlations are not enough significant. Another study focused on the main Turkish cities concluded that the climate factors that best correlate with COVID-19 spread are the mean temperature on the day of positives measurement and the wind speed 14 days before the positive detection (Şahin, M., 2020). Nevertheless, this result should be interpreted with caution. Another study, focused on Brazil pointed that high mean temperatures (around 27.5 °C) favoured COVID-19 propagation (Auler, A.C. et al., 2020). The same authors pointed that other demographic and social-economic factors, such as health conditions, might be crucial. Beside Brazil is located in the tropical zone with temperatures that are approximately steady along the year. In addition, the distance among large metropolitan areas are considerable. In another study, concluded that mean and minimum temperatures correlated well with COVID-19 pandemic in New York (Farhan Bashir et al., 2020). Since the major affected cities in Spain are

not such air polluted the role of air quality has been not focused in this study. Regarding a high populated country as India, air temperature was mentioned as a non-significant factor for the virus spread, as well as the lockdowns were said to have not a correlation to the reduction of the virus spread (Gupta, A et al., 2020). However, the virus spread was reduced by a 45% in Italy during the lockdown (Gatto, M et al., 2020).

This paper analyses the influence of the main meteorological variables on the Covid spread, treating these variables as spatial-temporal processes, analyzing their correlations through time and space in the Iberian Peninsula. Although the methodology that we used is well-known in applied mathematics and statistics, this serves to clarify and shed light on the importance of meteorological variables in the spread of the pandemic in all the Spanish provinces.

2. Methodology and data

2.1. Data sources

Climate variables values were obtained from the Spanish Agency of Meteorology (AEMET) platform OpenData (AEMET OpenData). Appendix I shows the list of used meteorological stations. The range of dates covers from 31st January to 20th June. The percentiles for each climate variable were computed for each meteorological station representative of each Spanish province. COVID-19 positives data was collected from Carlos III, 2020 Institute website. Spain has notified 237 096 COVID-19 cases and has conducted 2 536 234 COVID-19 PCR Tests by the May 28, 2020. At the start of the pandemic, Spain notified 2968 COVID-19 cases by the March 12, 2020. The distribution of PCR positives is not uniform along the country. Finally, the population data is obtained from the Spanish National Institute of Statistics (INE, 2020).
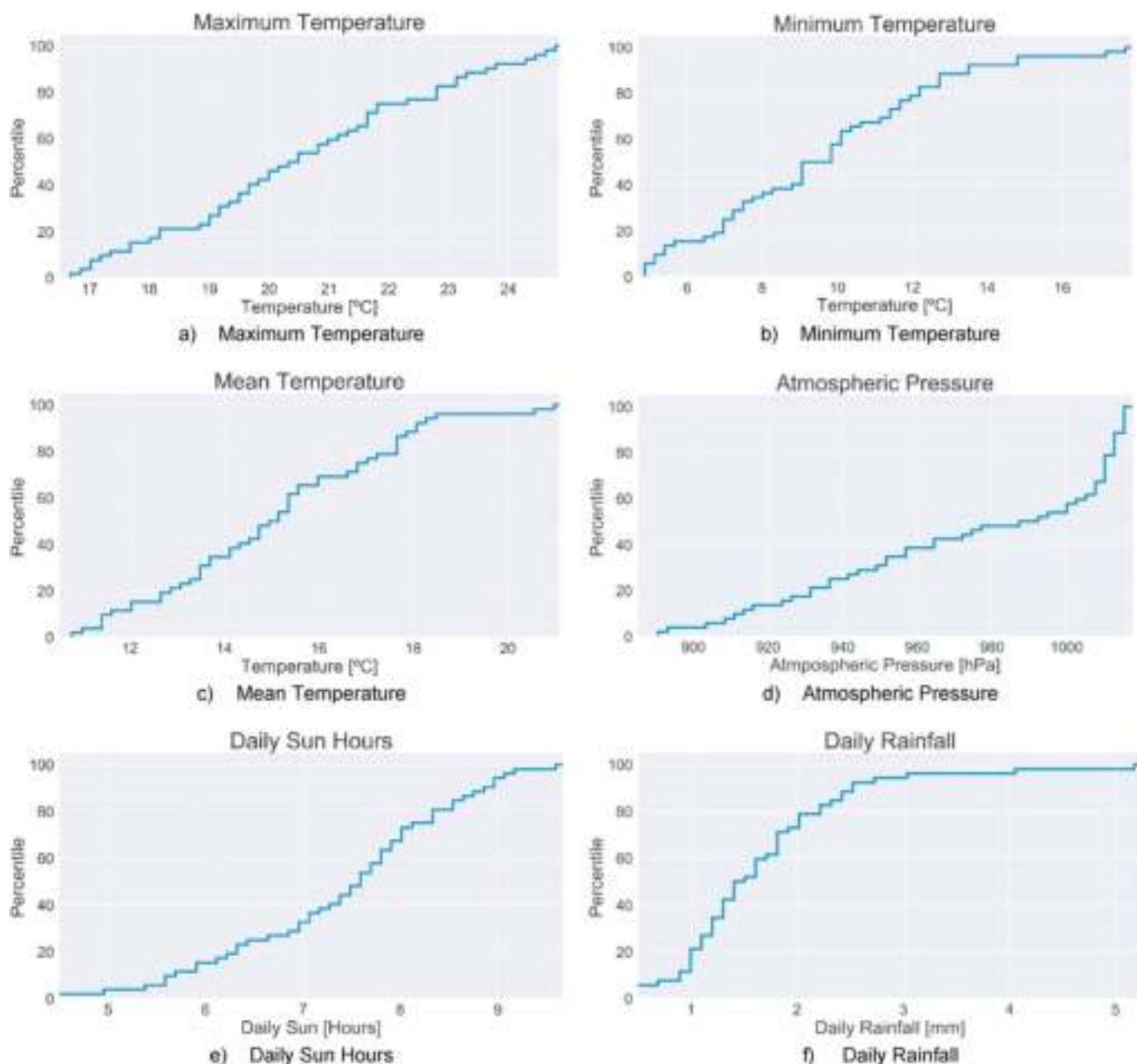

Fig. 1. Cdf (percentile curves) of the Climate Variables.

2.2. Climate variables

Six climate variables are used in this project: Maximum Temperature, Minimum Temperature, Mean Temperature, Atmospheric Pressure, Daily Sun Hours and Daily Rainfall. All these variables are obtained from AEMET for each Spanish province.

- Maximum, Minimum and Mean Temperatures are expressed in Celsius degrees [°C].
- Atmospheric Pressure is expressed in hectopascals [hPa] and is the mean of the minimum and maximum atmospheric pressures during the day.
- Daily sun hours are obtained directly from insolation.
- Daily rainfall is expressed in millimeters [mm] and it is the total rain gathered during the day.

Data about wind speed is available, although wind direction data is not available. From our point of view, wind direction data is quite as important as wind speed data. Hence, if both data are not available, we decided using only one is not a right approach. Another reason is that wearing a mask outside is compulsory. Therefore, most of the virus transmission happens in indoor scenarios, in which wind speed is not as important. We also think that in an urban scenario, the wind speed and direction can be modified by the buildings or the factories. Fig. 1 shows the Cumulative Distribution Function (CDF) of these climate variables. Table 1 shows the percentiles of these variables. Appendix II also shows the descriptive analysis of the climate variables for all the Spanish provinces.

2.3. COVID-19 variables

The cumulative number of COVID-19 tests carried by each Autonomous Community shows the total number of PCR tests done, positive or negative. It is estimated as a "density of tests" (*Testd*), defined as follows:

$$Testd = \frac{PCR}{Total\ Population}, \tag{1}$$

The cumulative number of COVID-19 positives by province is taken from the data provided by the organization responsible for Carlos III, 2020 Spanish Institute, which is responsible to gather and cure this data. To estimate a "density of positives", the population data from each province is needed.

The density of positives by province (*PCRd*) is defined as follows:

$$PCRd = \frac{PCR+}{Total\ Population} \tag{2}$$

which gives the ratio of the PCR positive over the total population.

To estimate a "daily density of positives", the population data from each province is needed. The density of positives by province (*Daily PCRd*) is defined as follows:

$$Daily\ PCRd = \frac{PCR + (in\ a\ single\ day)}{Total\ Population} \tag{3}$$

which gives the ratio of the number of PCR positive in a single day over the total population.

To estimate a "daily increment of the density of positives", the population data from each province is needed and the density of positives by province (*Daily PCRd*) for two consecutive days is required. The density of positives by province (Δ*Daily PCRd*) is defined as follows:

$$\Delta\ Daily\ PCRd = \frac{PCR + (in\ a\ single\ day) - PCR + (the\ day\ before)}{Total\ Population} \tag{4}$$

which gives the evolution of the daily density of PCR positives.

The Spanish Ministry of Health carried out a prevalence of COVID-19 IgG Antibodies in all the provinces and the autonomous cities (Ceuta and Melilla). The result is an estimation of the percentage of inhabitants of each province who could have been infected. Three rounds were done to obtain the percentage of population for each province who had been infected (*IgGd* [%]). Theoretically, the IgGd is computed following equation (2). The *IgGd* [%] values are given in Appendix III.

$$IgGd = \frac{IgG+}{Total\ Population} \tag{5}$$

By using the data from the COVID-19 density of positives and the results from the IgG antibodies research study, an estimation of the percentage of asymptomatic population can be computed. Two assumptions are made. First, it is assumed all people who were in serious or critical conditions were tested with a PCR. Second, it is assumed all the infected people has generated IgG antibodies after recovering from the virus. Therefore, the percentage of asymptomatic population (*APr*) is the percentage of population with antibodies minus the percentage of positives:

$$APr[\%] = IgGd\ [\%] - PCRd\ [\%] \tag{6}$$

These metrics (density of tests, density of positives, increment of the density of positives, density of antibodies and percentage of asymptomatic population) provide different insights about the impact of COVID-19 in each Spanish province. The aim is to avoid any type of bias due to the number of inhabitants.

To reduce the impact of noise in data, we have computed for each day the moving averages for five days for each of these variables. Therefore, the correlation is performed between smoothed versions of these temporal series. This is particularly important for *PCRd*, since this kind of information is sometime delayed by the public health authorities. Then, the correlation between both temporal series is computed via the Pearson's and Spearman's correlation coefficients. That way is possible to produce regionalized variables using these coefficients for all the provinces.

3. Results

This section shows the results obtained in this study. First, the correlation between the COVID-19 density of positives *PCRd* and the climate variables is found, in subsection 3.1. Second, a linear regression model using the mean temperature as independent variable and the COVID-19 density of positives *PCRd* as dependant variable is presented, in subsection 3.2. Third, an Analysis of the Variance (ANOVA) between the mean temperature and the daily density of new COVID-19 positives. *Daily PCRd* is computed in subsection 3.3. Fourth, the results from the massive antibodies study *IgGd* carried out in Spain are correlated with the climate variables in subsection 3.4. An estimation of the percentage of Asymptomatic Population *APr* [%] is computed, and the correlation of this result with climate variables is also tested, in subsection 3.5. Last, a comparison between the *PCRd* and *Testd* is shown for each Autonomous Community in subsection 3.6.

These variables are considered spatial-temporal processes $X(t, \mathbf{x})$, where $\mathbf{x}$ stands for the spatial (or geographic) coordinates that depend on each province and t for the temporal dependency. Fixing a time t_0,

Table 1
Percentiles 10, 25, 50, 75 and 90 of the climate variables.

Variable	P10	P25	P50	P75	P90
Maximum Temperature [°C]	17.48	19.13	20.54	22.06	23.72
Minimum Temperature [°C]	5.44	7.26	9.62	11.86	13.45
Mean Temperature [°C]	11.74	13.48	15.15	17.00	18.22
Atmospheric Pressure [hPa]	915.34	941.26	991.03	1011.66	1015.40
Daily Sun Hours [h]	5.77	6.62	7.63	8.18	8.89
Daily Rainfall [mm]	0.99	1.12	1.53	2.05	2.55

$X(t_0, \mathbf{x})$ is a regionalized variable. In this case $X(t_0, \mathbf{x})$ is a discrete sample of dimension 52, which is the total number of provinces and autonomous cities in Spain. On the other side fixing $\mathbf{x}$, provides a temporal series $X(t, \mathbf{x}_0)$ that provides the temporal evolution of $X(t)$ in the province $\mathbf{x}_0$. Both aspects (spatial and temporal) are important in understanding the Covid-19 spread.

Summarizing, we have concluded that the mean and minimum temperatures are correlated to the density of PCR positives for Spanish provinces. Besides, the maximum temperature has a lower correlation than the minimum temperature. Therefore, the areas where the pandemic has been stronger are the cold regions of the Spanish "Meseta" (plateau) that are characterized by a continental climate. This fact has been also observed in New York (Farhan Bashir et al., 2020). Nevertheless, in the New York study there was not any evidence that the warm weather could suppress COVID-19 transmission. This paper shares this conclusion: climate plays a very important role in the COVID-19 transmission, mainly in the cold areas, reducing or amplifying its spread, but it cannot eliminate the outbreak, as it has been observed lately on the second wave, when mobility restrictions have been abandoned. Besides, we have also observed that atmospheric pressure has a negative correlation with the density of COVID-19 positives in all the Spanish provinces. Low atmospheric pressure is a quite significant factor and could be one of the most related with the virus transmission. In Spain the low pressures are typical of spring and autumn due to the Azorean anticyclone moving intermittently south. In winter high pressures predominate because of marine polar anticyclones, the Scandinavian anticyclone and its connection with that of the Azores. Finally, in summer the weather is controlled by the Azorean anticyclone again, resulting in warm, dry and stable weather with low surface pressures that reverse with altitude and generate summer storms. Therefore, the climate in Spain is very different from the rest of Europe, and is dominated by low pressure throughout the year, which would favour the spread of the COVID virus. We have also analyzed the correlation between PCR positives and the antigen tests, showing a very important correlation between the PCR tests that originally were only performed on people with hospital symptoms and the number of people with antibodies, which also takes into account those who are asymptomatic. Basically, the count is simple: the number of infected is approximately six times the number of PCR positive tests. This would explain partly the higher rates of mortality observed in Spain referred to the PCR tests with respect to other countries that initially made more massive use of rapid tests. This situation would also explain the higher number of positives observed in the second wave because of the greater mobility and increased testing of the population.

In conclusion, the climate variables can have a great influence in the propagation of the COVID virus and could be taken into account in the process of public health decision making to define the areas with a higher potential risk of COVID transmission.

3.1. COVID-19 density of positives analysis

A correlation analysis was performed among the different climate variables and the density of COVID-19 positives (*PCRd*). Two correlation coefficients were used: the Pearson's and the Spearman's correlation coefficients. Pearson's coefficient measures the degree of the linear correlation between variable, whereas Spearman's coefficient is a measure of the rank correlation. It serves to assess how well the relationship between both temporal series can be described using a monotonic function (linear or not).

For two series X_1, X_2 of size n, both coefficients are defined as follows:

$$\rho_P = \frac{cov(X_1, X_2)}{\sigma_{X1}\,\sigma_{X2}} \tag{7}$$

$$\rho_S = \frac{cov(r(X_1), r(X_2))}{\sigma_{r1}\,\sigma_{r2}} \tag{8}$$

The Pearson coefficient ρ_P is related to the slope of the regression line between these variables (X_1, X_2). Spearman's correlation coefficient is the Pearson coefficient between the corresponding rank variables. Ranking is the data transformation in which the numerical values of X_1 and X_2 are replaced by their rank $(r(X_1), r(X_2))$, when the data are sorted.

● **Modelling as regionalized variables: spatial variability**

Table 2 shows the relationship between the climate variables and the density of COVID-19 positives on 1st June when most of the pandemic had already taken place. The correlations with the different temperatures, the atmospheric pressure and the daily sun hours are negative. Besides, the correlations with mean, minimum temperatures, and atmospheric pressure are the most important. The correlation with the daily sun hours is very low. The analysis shows no correlation between the density of COVID-19 positives and daily rainfall. Daily sun hours and daily rainfall seem to be non-significant factors in the COVID-19 spread.

Fig. 2 shows the evolution of the correlation of the *PCRd* values with the climate variables, starting from 12th March. In this case for each date, the correlation between the corresponding spatial variables is performed. The minimum temperature (Fig. 2b) has a stronger correlation with the density of COVID-19 positives *PCRd*, than the maximum temperature (Fig. 2a). Mean temperature (Fig. 2c) is the temperature which best correlates with *PCRd*. The correlation is always negative and decreases with time from - 0.66 to - 0.78. Fig. 2d shows the correlation with the mean range atmospheric pressure, while Fig. 2e and f shows the evolution for the daily sun hours and rainfall. The conclusions are similar to those previously commented for the 1st June, that is, the analysis is consistent along the duration of the outbreak. Particularly, in the case of the atmospheric pressure, both correlation coefficients show similar and very close trajectories.

The virus propagation is also a temporal process $X(t, \mathbf{x}_0)$ when the spatial variable (province) is fixed. To show a detailed view of the Spain geography, Fig. 3 shows a map recorded using QGIS and Natural Earth Data. The map features latitude and longitude coordinates, a scale bar, and a north arrow. Then, the correlation between the climate variables and the density of daily positives can be computed for each province. For that purpose, first the right time span to analyze the outbreak must be selected. The analysis was performed between 11th March to 20th May where most of the infections have occurred. Fig. 4 shows the absolute value of the correlation between *PCRd* and the maximum, minimum and mean temperatures, using maps created with the online tool Mapchart (Mapchart). The daily increment of the density of positives has a negative correlation with the maximum, minimum and mean temperatures for all provinces. Pearson and Spearman's correlation coefficients show the same tendency, with a few changes in the value of the correlation in some cases. Catalonia, La Rioja, two provinces of Comunidad Valenciana (Valencia and Castellón) and in some cases Madrid are strongly negative

Table 2
Correlation coefficients (climate variables, density of positives).

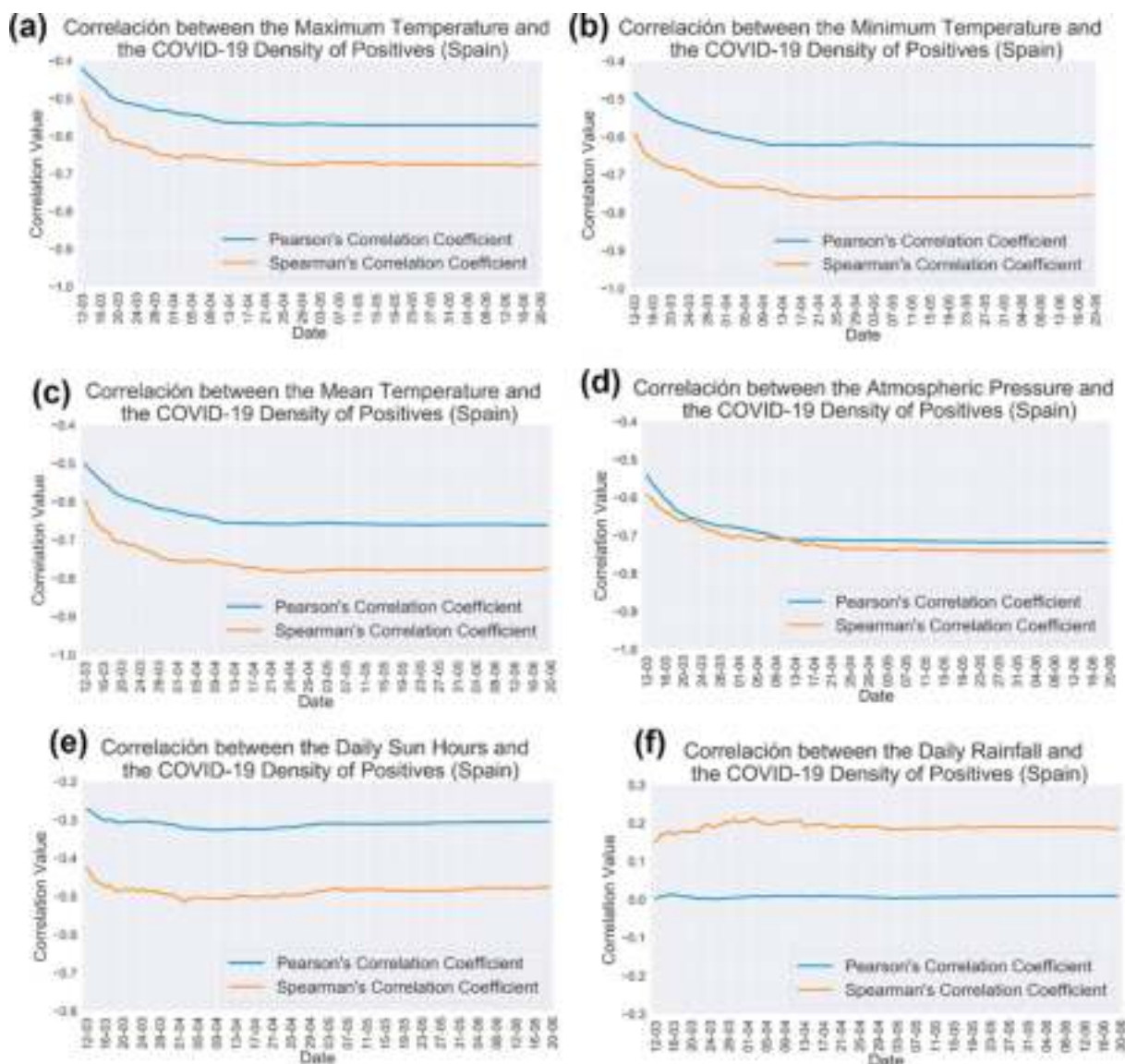| Variable	Correlation Coefficient with the Density of Positives (June 1st)			
	Pearson's		Spearman's	
	Value	p-value	Value	p-value
Maximum Temperature	- 0.57	1.14×10^{-5}	- 0.67	6.09×10^{-8}
Minimum Temperature	- 0.62	1.06×10^{-6}	- 0.75	1.10×10^{-10}
Mean Temperature	- 0.65	1.65×10^{-7}	- 0.78	10^{-11}
Atmospheric Pressure	- 0.70	6.14×10^{-9}	- 0.73	6.3×10^{-10}
Daily Sun Hours	- 0.30	0.03	- 0.42	1.78×10^{-3}
Daily Rainfall	0.06	0.69	0.21	0.14

Fig. 2. Temporal evolution of the Correlation coefficients between the climate variables and the COVID-19 Density of Positives (*PCRd*).

- **Modelling as temporal series**

correlated with the daily increment of the density of positives, with absolute values higher than 0.7. However, most places of Northwest Spain are less strongly correlated, showing absolute correlation values lower than 0.5. Southern Spain, Ceuta, Melilla, and the Canary Islands show an absolute correlation coefficient value close to 0.6. Therefore, in the places that show strong correlated results it is easier to predict the daily number of cases based on their mean temperature.

The same analysis was performed to study the correlation with the temporal evolution of the atmospheric pressure. The atmospheric pressure has a high dependence on the terrain elevation. Given the terrain elevation is a constant value over time, the results of the temporal analysis for all provinces are close to 0, some of them positive and others negative. In other words, atmospheric pressure does not influence the daily evolution of the pandemic in a province but can affect the differences in the number of positives observed among different provinces.

The main reason for using as a metric for measuring the virus outbreak the daily increment of positives and not the daily number of positives is that this measure shows more information. The number of positives only shows the virus impact during a day, but the daily increment also shows if the virus spread is increasing or decreasing.

3.2. Linear regression model (mean temperature and the COVID-19 density of positives PCRd)

Mean temperature is the most correlated variable with the density of positives, according to the Spearmans' correlation coefficient (Table 2). Thus, a linear regression model, using the density of positives metric *PCRd* as the dependent variable can be constructed:

$$PCRd = a_0 + a_1 \times T_{mean} \qquad (9)$$

Fig. 5 shows the correlation between COVID-19 density of positives metric *PCRd* and mean temperature T_{mean} for two weeks timestep. In this plot the atmospheric pressure variable is only used to colour the points, but not for the linear regression. The correlation is plotted for the March 15th, April 1st, April 15th, May 1st, and May 15th. The negative correlation between the density of positives and the mean temperature is found in all the plots. Table 3 shows the linear equation and the RMSE error for each regression. Hence, a coefficient which enables us to establish a relationship between temperature and the COVID-19 spread was found. The slope is always negative and is closer to zero at the beginning of the outbreak. From April 15th, the slope value stabilizes

Fig. 3. Spain Map (it includes a North arrow on the left side and a scale bar on the right side).

between - 0.0012 and - 0.0014, that is, a decrease between 1.2 and 1.4 new PCRs per 1000 inhabitants and every degree of increase in the average temperature. The RMSE error is also very low in all the cases.

3.3. ANOVA (mean temperature and the daily COVID-19 density of positives DailyPCRd)

ANOVA serves to demonstrate that the mean temperature is correlated with the COVID-19 propagation. The virus expands in a similar way in two different cities, provided the daily mean temperature values are the same. As the reader can expect, there is always an error margin.

In this analysis, we have taken all the daily data of mean temperatures and density of COVID-19 positives between the 12th March and 20th June. To reduce the measurement error, the average mean of 10 days is computed for both the mean temperature and the daily new density of COVID-19 positives.

Fig. 6 shows a plot of the daily density of positives and the moving average of the mean temperature. To produce this plot, the mean temperature varies from 11.74 °C (P10) to 18.22 °C (P90). P10 and P90 were taken from Table 1. Within this range with step of 0.1 °C, the *Daily PCRd* was found for all the days between the 12th March and 20th June. For each temperature we scan the days were this temperature has occurred and we take the mean of the corresponding *Daily PCRds*. An average of these values along all provinces is finally taking place. Before doing these calculations, a moving average of 10 days of the daily mean temperatures and PCRd is performed to smooth the observed data and enforcing the dynamics of the virus propagation.

This figure shows a negative correlation between mean temperature

and COVID-19 density of positives (independently of the provinces). The equation of the linear regression is:

$$Daily\ PCRd = 1.517 \times 10^{-4} - 8.329 \times 10^{-6} \times Mean\ Temperature\ [°C]$$

$$(10)$$

that is, a decrease of round 8.3 cases by million every degree of increment in the mean temperature.

For example, 0.00005 (5 $\times 10^{-5}$) *Daily PCRd* means 50 new daily positive cases that for 1 million habitants. Therefore, a decrease of 10° in the mean temperature during the winter implies around 83 new daily positive cases for 1 million habitants. This might be one the factors of the increase of cases in January 2021 during the impact of Filomena storm in the Spanish peninsula. Obviously, the mobility and the social networks are also very important in the dynamic of COVID spread.

For the ANOVA analysis, the null hypothesis H_0 is that the mean temperature has not an effect in the COVID-19 propagation. The result of the ANOVA test is 11.60, with 51 (number of provinces-1) and 1661 (number of data – number of provinces) as degrees of freedom. The zero values in the data (temperatures in which there are not notified COVID-19 positives) are removed. The critical value is 1.32–1.39 for a significance level of 0.05. Therefore, the null hypothesis H_0 is rejected and the conclusion is that mean temperature has a relationship with the COVID-19 propagation.
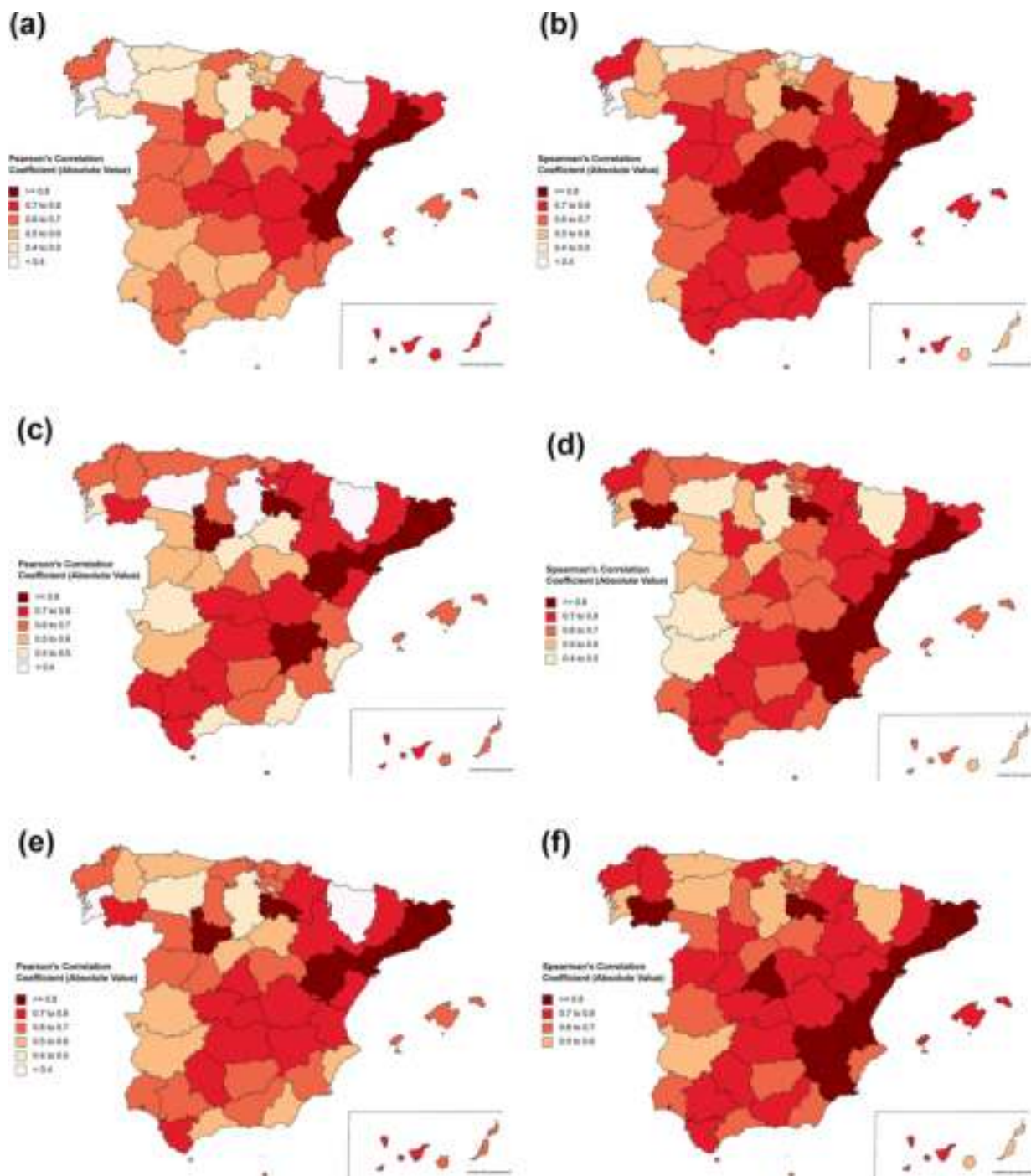
$$f(51, 1661) = 11.60 > 1.39 \qquad (11)$$

Fig. 4. Correlation between the temporal series for each province and the maximum, minimum and mean temperatures.

3.4. Antibodies analysis

The percentage of population with antibodies data *IgGd* [%] has also been correlated with the climate variables. First round analysis was carried from the 27th April to the 11th May. Second round analysis was carried from the 18th May to the 1st June. Third round analysis was carried from the 8th June to the 22nd June. Correlations with the climate variables are shown for each round: First Round (Table 4), Second Round (Table 5), Third Round (Table 6). As could be expected, correlation coefficients are quite similar when compared with the ones obtained from the COVID-19 density of positives metric *PCRd*.

Third Round analysis correlates better than the second and first round antibodies results *IgGd* [%] with the climate variables. Mean and

minimum temperature are more significant factors than the maximum temperature. Daily sun hours and rainfall seem not to be very significant factors, showing almost no correlation. Atmospheric pressure is the climate variable that correlates with *IgGd* [%] showing a bigger difference with respect to the temperature. These differences were smaller for the *PCRd* correlation analysis. The atmospheric pressure seems to impact more the COVID-19 transmission and the low temperatures with the new detected cases by PCR, which are more related to the severity of the infection, since it does not include the asymptomatic. Nevertheless, the impact of these two indicators must be implicit in the mean temperature (more daily sun hours, higher temperature) and in the atmospheric pressure (storms in Spain are usually associated to the entry of low pressures fronts from the North and West of the Spanish peninsula).
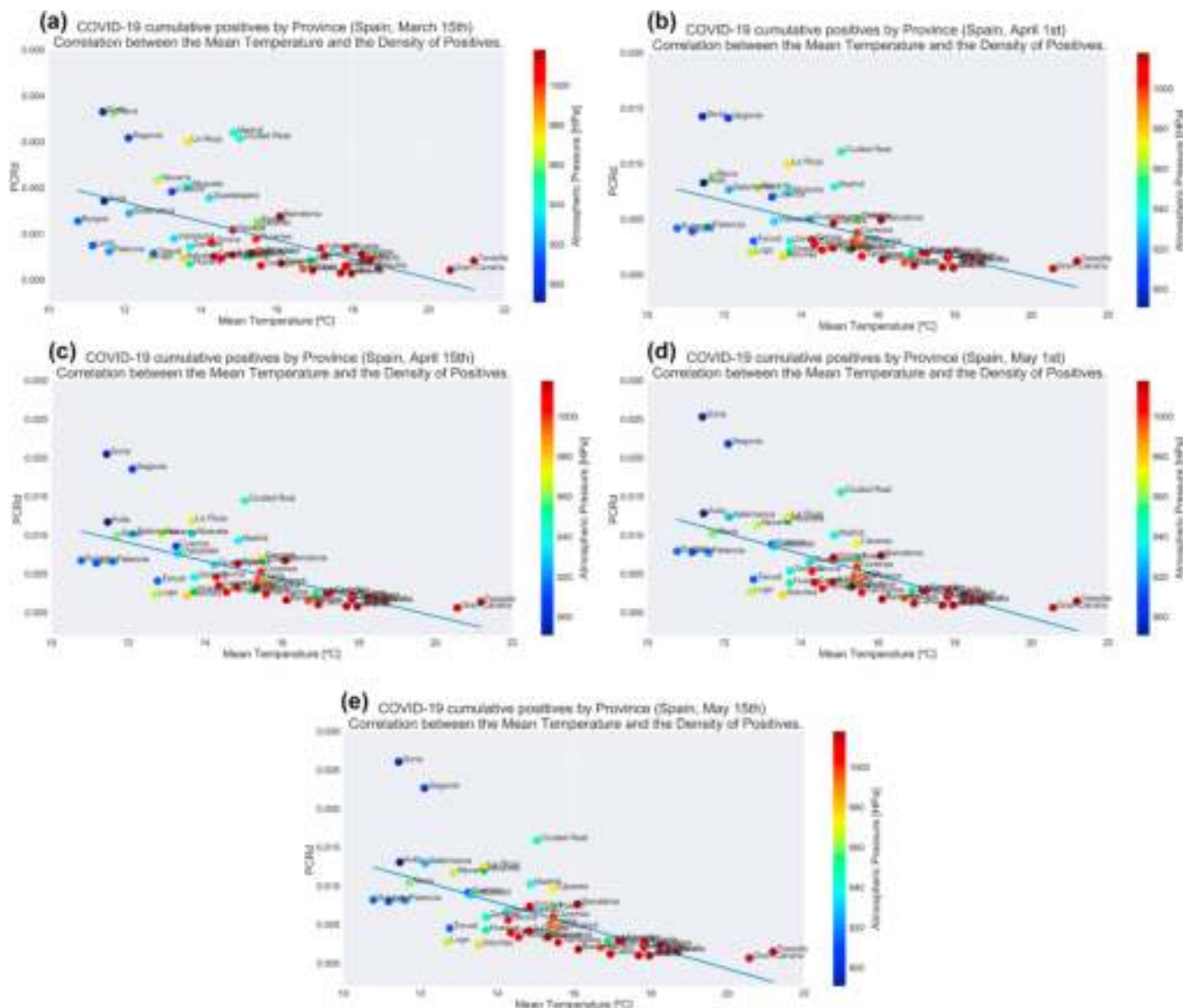
Fig. 5. Correlation between the COVID-19 density of positives *PCRd* and the mean temperature.

Table 3
Linear Regression (Mean Temperature, Density of Positives) coefficients.

Measurement Day	a_0 (Constant)	a_1 (Slope)	RMSE
March 15th	0.004161	- 0.000206	10^{-6}
April 1st	0.016826	- 0.000848	7×10^{-6}
April 15th	0.023314	- 0.00119	1.1×10^{-5}
May 1st	0.026891	- 0.00138	1.5×10^{-5}
May 15th	0.027824	- 0.001427	1.6×10^{-5}

Therefore, the virus could propagate along the whole year, more in winter than in summer, and the infections in summer would be more benevolent than in winter. These results suggest that during clear sky days, there would be lesser possibility of increment of positive cases, furthermore rainy winter could be very susceptible weather for Spain for the spread of this virus.

3.5. Estimation of the percentage of asymptomatic population

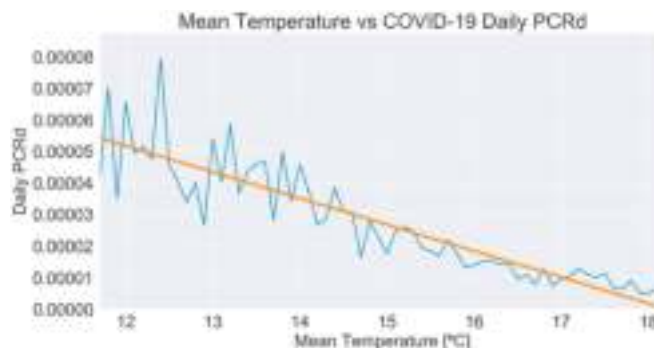The linear regression between *PCRd* and *IgGd* is:

Fig. 6. Expected *Daily PCRd* depending on the mean temperature.

$$IgGd\ [\%] = 1.605 + 5.17 \times PCRd\ [\%] \tag{12}$$

This relation tells the percentage of herd immunity is between 5 and 6 times *PCRd*. Therefore, an estimation of the asymptomatic people can be computed based on the number of PCR positives known. To produce

Table 4
Correlation coefficients (climate variables, percentage of population with antibodies (first round)).

| Variable | Correlation Coefficient with the Percentage of Population with Antibodies (First Round) | | | |
| | Pearson's | | Spearman's | |
	Value	p-value	Value	p-value
Maximum Temperature	- 0.45	9.37×10^{-4}	- 0.48	3.64×10^{-4}
Minimum Temperature	- 0.54	3.64×10^{-5}	- 0.64	3.64×10^{-7}
Mean Temperature	- 0.55	2.43×10^{-5}	- 0.62	1.22×10^{-6}
Atmospheric Pressure	- 0.75	1.40×10^{-10}	- 0.71	5.20×10^{-9}
Daily Sun Hours	- 0.19	0.17	- 0.26	0.06
Daily Rainfall	- 0.11	0.44	- 0.05	0.74

Table 5
Correlation coefficients (climate variables, percentage of population with antibodies (second round)).

| Variable | Correlation Coefficient with the Percentage of Population with Antibodies (Second Round) | | | |
| | Pearson's | | Spearman's | |
	Value	p-value	Value	p-value
Maximum Temperature	- 0.44	1.17×10^{-3}	- 0.44	9.61×10^{-4}
Minimum Temperature	- 0.53	5.53×10^{-5}	- 0.59	3.90×10^{-6}
Mean Temperature	- 0.54	3.68×10^{-5}	- 0.57	1.09×10^{-5}
Atmospheric Pressure	- 0.77	2×10^{-11}	- 0.71	4.69×10^{-9}
Daily Sun Hours	- 0.16	0.26	- 0.19	0.18
Daily Rainfall	- 0.14	0.33	- 0.11	0.43

Table 6
Correlation coefficients (climate variables, percentage of population with antibodies (third round)).

| Variable | Correlation Coefficient with the Percentage of Population with Antibodies (Third Round) | | | |
| | Pearson's | | Spearman's | |
	Value	p-value	Value	p-value
Maximum Temperature	- 0.46	6.97×10^{-4}	- 0.44	9.78×10^{-4}
Minimum Temperature	- 0.56	1.52×10^{-5}	- 0.60	2.59×10^{-6}
Mean Temperature	- 0.57	1.13×10^{-5}	- 0.57	9.42×10^{-6}
Atmospheric Pressure	- 0.80	2×10^{-12}	- 0.72	1.51×10^{-9}
Daily Sun Hours	- 0.15	0.30	- 0.18	0.20
Daily Rainfall	- 0.16	0.27	- 0.15	0.29

this result, we have used the IgG data from the third round. Fig. 7 shows a plot of both data and the linear regression.

The percentage of asymptomatic people Apr [%] is the subtraction of the percentage of population with antibodies $IgGd$ [%] and the percentage of positives for each province $PCRd$ [%]. Therefore, we have:

$$Apr \ [\%] = 1.605 + 4.17 \times PCRd \ [\%] \tag{13}$$

According to this result, for each positive case there are more than 4 people that have stayed in mild conditions or even asymptomatic. This result does not depend on the population. These data are quite important to evaluate possible approaches that could be adopted in case of a second wave next autumn. It also helps to estimate the spread of the COVID-19 virus in a territory. Table 7 shows the correlation between the climate variables and this estimation of the percentage of asymptomatic population, based on the $PCRd$ [%] from the 3rd June, which was used to find the Apr [%] for each province. Spearman's correlation coefficient shows that the mean temperature is the strong correlation variable with the Apr [%], followed by the minimum temperature and the atmospheric pressure. This result was also expected based on previous analysis and the linear relationships among variables.

3.6. Relation between PCRd and Testd

We also show the relationship between $PCRd$ and $Testd$, which the quotient between the amount of PCR Tests carried out and the population of each Autonomous Community. Appendix IV shows the number of PCR tests carried out in each Spanish Community since the start of the pandemic until the May 28th. These data were released by the Spanish Ministry of Health. These data were not provided for each province individually.

Fig. 8 shows the correlation between $PCRd$ and $Testd$. Pearson's

Table 7
Correlation coefficients (climate variables, estimation of the percentage of asymptomatic population).

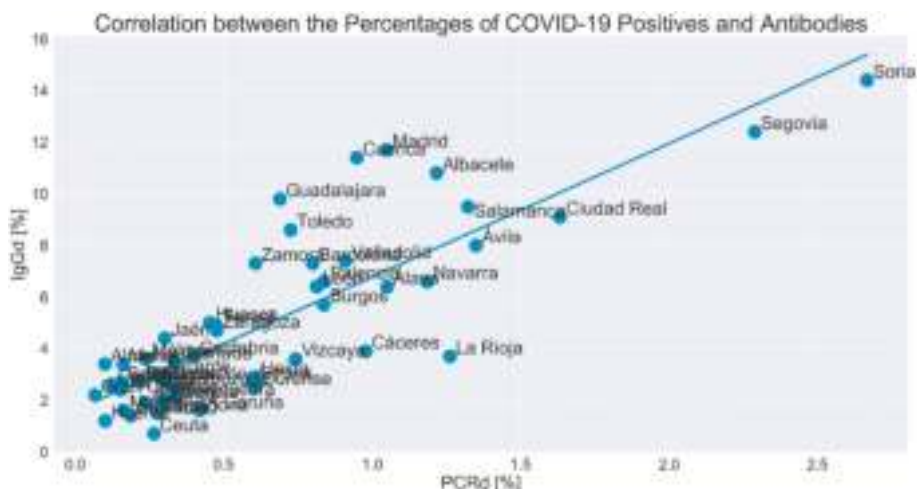| Variable | Correlation Coefficient with the Estimation of the Percentage of Asymptomatic Population | |
	Pearson's	Spearman's
Maximum Temperature	- 0.57	- 0.68
Minimum Temperature	- 0.62	- 0.76
Mean Temperature	- 0.66	- 0.78
Atmospheric Pressure	- 0.72	- 0.74
Daily Sun Hours	- 0.31	- 0.48
Daily Rainfall	0.01	0.19

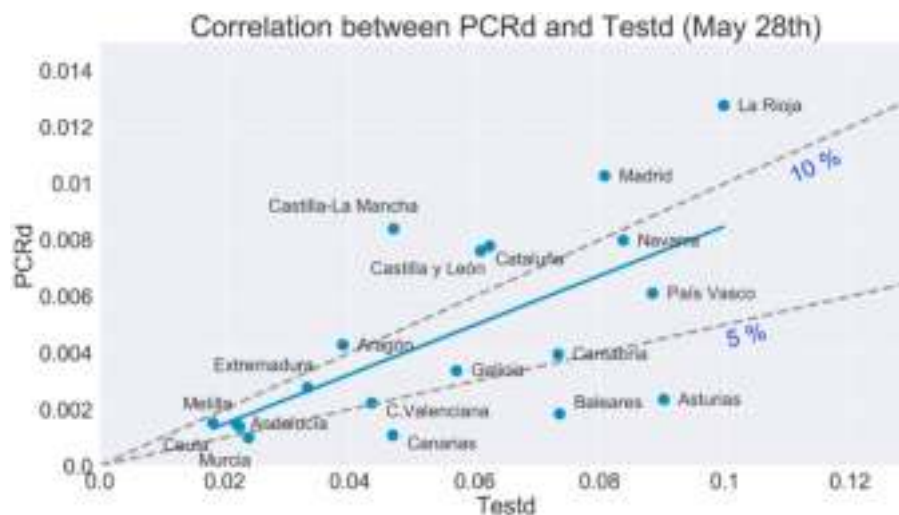**Fig. 7.** Relationship between the positives $PCRd$ [%] and antibodies $IgGd$ [%].

Fig. 8. Relationship between the positives *PCRd* and tests *Testd*.

Correlation Coefficient value is 0.64, whereas Spearman's Correlation Coefficient is 0.66. Two thresholds were included. Both represent the ratio between *Testd* and *PCRd*. A 5% threshold indicates that 1 out of 20 PCR tests provides a positive result. Similarly, a 10% threshold indicates that 1 out of 10 PCR tests is positive Fig. 8 can also be divided into four quadrants. The top left one would be Autonomous Communities with low *Testd* and high *PCRd* (Worst scenario). The bottom right quadrant would be Autonomous Communities with high *Testd* and low *PCRd* (Best scenario). The other two quadrants show low *Testd* and low *PCRd* (Second best scenario) and high *Testd* and high *PCRd* (Third best scenario).

4. Conclusions

Climate variables provide an explanation for the COVID-19 propagation. Spain is a suitable country for this analysis since it exhibits four different types of climate: oceanic, Mediterranean, sub-tropical and continental. Temperature is an important factor to explain the COVID-19 propagation behaviour. The mean temperature is the one what best correlates with the density of positives *PCRd*. The correlation between the mean temperature and the density of COVID-19 positives *PCRd* increased from March to April, and then it is stabilized. Moreover, the minimum temperature correlates better than the maximum temperature with the density of positives *PCRd*. In colder places, the virus propagation can be extremely fast. Atmospheric pressure has a negative correlation with the density of COVID-19 positives *PCRd* in Spain provinces. Atmospheric pressure is a quite significant factor and could be one of the most related with the virus transmission. Daily sun hours and rainfall seem to be non-significant factors, although their contribution might be implicit in the mean temperature and also in the atmospheric pressure. Both values are considered not important to explain the COVID-19 spread behaviour. The ANOVA test has also confirmed the existing relationship between the mean temperature and the COVID-19 propagation. We also provide a plot for the Expected Daily PCR density depending on the mean temperature. According to our results there would be lesser possibility of increment of positive cases under good weather conditions, whilst cold weather is more susceptible for the COVID spread. Besides, the mild coastal weather conditions show an important influence in the Eastern and Western parts of Spain.

Finally, we have performed the estimation of the asymptomatic population based on the results of the antibodies analysis, showing that for each PCR positive case there are more than 4 people that have stayed in mild conditions or even asymptomatic. Besides we have shown a positive correlation between the density of the PCR positives and the

number of tests that have been performed in each state in Spain.

In conclusion climate variables explain some important aspects in the COVID transmission and its strength but cannot be used to justify the disappearance/vanishing of the outbreak without taken into consideration epidemiological measures of social distance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113723.

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Exposure Load: Using biomonitoring data to quantify multi-chemical exposure burden in a population

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A B S T R A C T

People are often concurrently exposed to numerous chemicals. Here we sought to leverage existing large bio-monitoring datasets to improve our understanding of multi-chemical exposures in a population. Using nationally-representative data from the 2012–2015 Canadian Health Measures Survey (CHMS), we developed Exposure Load, a metric that counts the number of chemicals measured in people above a defined concentration threshold. We calculated Exposure Loads based on five concentration thresholds: the analytical limit of detection (LOD) and the 50th, 75th, 90th and 95th percentiles. Our analysis considered 44 analyte biomarkers representing 26 chemicals from the 2012–2015 CHMS; complete biomarker data were available for 1858 participants aged 12–79 years following multiple imputation of results that were missing due to sample loss. Chemicals may have one or more biomarkers, and for the purposes of Exposure Load calculation, participants were considered to be exposed to a chemical if at least one biomarker was above the threshold. Distributions of Exposure Loads are reported for the total population, as well as by age group, sex and smoking status. Canadians had an Exposure Load between 9 and 21 (out of 26) when considering LOD as the threshold, with the majority between 13 and 18. At higher thresholds, such as the 95th percentile, the majority of Canadians had an Exposure Load between 0 and 3, although some people had an Exposure Load of up to 15, indicating high exposures to multiple chemicals. Adolescents aged 12–19 years had significantly lower Exposure Loads than adults aged 40–79 years at all thresholds and adults aged 20–39 years at the 50th and 75th percentiles. Smokers had significantly higher Exposure Loads than nonsmokers at all thresholds except the LOD, which was expected given that tobacco smoke is a known source of certain chemicals included in our analysis. No differences in Exposure Loads were observed between males and females at any threshold. These findings broadly suggest that Canadians are concurrently exposed to many chemicals at lower concentrations and to fewer chemicals at high concentrations. They should assist in identifying vulnerable subpopulations disproportionately exposed to numerous chemicals at high concentrations. Future work will use Exposure Loads to identify prevalent chemical combinations and their link with adverse health outcomes in the Canadian population. The Exposure Load concept can be applied to other large datasets, through collaborative efforts in human biomonitoring networks, in order to further improve our understanding of multiple chemical exposures in different populations.

1. Introduction

Individuals are ubiquitously exposed to chemicals that can vary both spatially and temporally in diverse microenvironments. Although humans are exposed to a variety of different chemicals and stressors from many sources in the environment, either concurrently, sequentially or both, there is a lack of understanding about the prevalence and magnitude of multi-chemical exposures and the associated health impacts (Lee et al., 2017; Løkke et al., 2013). Accordingly, it is recognized that there is an urgent need to develop approaches that enable the assessment of multiple exposures and their potential health impacts (Drakvik et al., 2020; HBM4EU, 2020; Hernandez et al., 2019). A better understanding of the nature and magnitude of exposures, their interactions, and combined and cumulative effects on health would

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provide information relevant to cumulative risk assessment and inform risk management strategies (HBM4EU, 2020; Meek et al., 2011; OECD, 2018). Appreciating the breadth of real-world exposures is made manifest in a concept that has been attracting increasing attention from environmental health researchers: the exposome, which is the totality of environmental exposures from conception onward (Wild, 2005). Exposome variability extends across populations residing in different locations with varying diets, lifestyles and exposure sources. Whereas genetic influences on health have been extensively studied, the complexities of environmental exposures encompassed by the exposome and its potential health impacts are still largely unknown (Dennis et al., 2017). The importance of this research question is reflected in a number of ongoing large-scale exposome projects, including the Human Early-Life Exposome (HELIX) project, which integrates a range of exposure assessment methods to characterize early-life exposure to multiple environmental factors and associate these with omics biomarkers and child health outcomes, and project HEALS (Health and Environment-wide Associations based on Large Population Surveys), which has identified biomarkers of exposure for a large number of environmental stressors for use in supporting environment-wide association studies (Bopp et al., 2018; Steckling et al., 2018; Vrijheid et al., 2014).

Addressing real world multi-chemical exposures is a persistent problem in health evaluation. Though chemical exposures do not typically occur one at a time in isolation, they are traditionally evaluated this way in risk assessment. Researchers and risk assessors have long recognized the need to take into account multi-chemical exposures when considering the potential impacts of real-world exposures. Progress has been slow due to experimental limitations, scarce funding, and a lack of knowledge about the complex exposure patterns of individuals and populations (Løkke et al., 2013). Various approaches have been used to assess chemical mixtures, many of them based on toxicological responses and confronting issues such as shared mode of action and component interactions (Bopp et al., 2019; Drakvik et al., 2020; More et al., 2019; Hernandez et al., 2019; OECD, 2018). New conceptual approaches are needed that move from a chemical-by-chemical assessment paradigm towards considering the cumulative effects of multi-chemical exposures and characterizing the exposome.

Biomonitoring provides a means to examine how internal chemical concentrations vary because of ongoing changes in exposure sources (Rappaport, 2011). It reflects exposure from all sources (including water, air and food) and all routes (such as ingestion and inhalation), and is indicative of real-world chemical exposures. As such, biomonitoring data offer a way to quantify multi-chemical exposures and understand how chemical burdens vary within a population. Biomonitoring datasets have previously been used to quantify multi-chemical exposures within a population, though with variations in method and purpose (Hendryx and Luo, 2018; Kapraun et al., 2017; Porta et al., 2010). In the present study, we used biomonitoring survey data to evaluate the profile of multi-chemical exposures in a population using a metric we have termed Exposure Load. We define Exposure Load as the number of chemicals measured in an individual above a defined concentration threshold. The present study describes the methodology used to calculate Exposure Load and provides the initial results of our analysis for the Canadian population, including an examination of differences between age groups, sexes and smoking habits. We propose that Exposure Load provides an indication of overall exposure burden in a population, and that it can be used to identify potentially vulnerable subpopulations that may be disproportionately exposed to multiple chemicals, as well as to characterize intrinsic and extrinsic factors that may contribute to heterogeneity in chemical exposure profiles across a population.

2. Material and methods

2.1. Survey design and biomonitoring data

The Canadian Health Measures Survey (CHMS) is an ongoing national survey led by Statistics Canada, in partnership with Health Canada and the Public Health Agency of Canada, which collects information about the general health of Canadians. Survey design and sampling are described in detail elsewhere (Statistics Canada, 2014, 2016). Briefly, a complex multi-stage sampling strategy is used to select approximately 5800 participants for each 2-year cycle from 16 collection sites across Canada. Each participant is assigned a survey weight such that results are nationally-representative of Canadians aged 3–79 years living in the ten provinces. The survey consists of an interview at each participant's household, which obtains demographic and self-report health information, followed by a visit to a mobile examination center (MEC), where physical measurements and biological specimens, including blood and urine samples, are obtained. Ethics approval was obtained from the Health Canada and Public Health Agency of Canada Research Ethics Board for all components of the CHMS. Informed written consent for the MEC portion of the CHMS was obtained from participants older than 14 years of age. For younger children, a parent or legal guardian provided written consent, and the child provided assent.

The present study used data from cycles 3 (2012–2013) and 4 (2014–2015) of the CHMS. These cycles were chosen because the same chemicals were measured across each cycle, allowing for an increased sample size of participants having the same measured chemical data, whereas the use of other survey cycles for which there was more variation between chemicals did not offer the same optimal sample size and number of chemicals. Selection of chemicals for inclusion in CHMS cycles 3 and 4 was based on a number of factors, including health risks, evidence of human exposure, existing data gaps and availability of laboratory analytical methods; further information on these chemicals (including sources, exposure pathways and health effects) can be found in the Third and Fourth Reports on Human Biomonitoring of Environmental Chemicals in Canada (Health Canada, 2015, 2017).

The full survey sample size was 5785 participants in cycle 3 and 5794 participants in cycle 4. The survey design stratified participants by six age groups (3–5, 6–11, 12–19, 20–39, 40–59, and 60–79 years) and by sex (except for 3–5 years). Except for some core chemicals that are measured in the full sample, biomonitoring analyses are routinely conducted in a subsample that is approximately half the sample in size, such that only around 2500 participants in each cycle (5000 participants total) would be eligible for inclusion in the current study. Although individuals aged 3 to 11 are included in the CHMS, volatile organic compounds were not measured for this youngest age group, and therefore those participants were excluded. This yielded a final sample size of 1858 consisting of those participants aged 12 to 79 that were selected for each biomonitoring analysis subsample and that had results for most, if not all, chemicals considered in the present study. Biomonitoring results for various chemicals were divided among different data files based on the nature of the sample (i.e. full sample or subsample) and biological matrix (i.e. urine or blood). The present analysis considered chemicals included in the following data files: environmental lab blood and urine full sample (EL), environmental urine subsample (EU), acrylamide blood subsample (ACRY), and volatile organic compounds person level blood subsample (VOCP). Since the CHMS was not designed to combine non-overlapping subsamples in this way, the representativeness of the reduced sample was compared to the full sample.

2.2. Chemical analysis

Analyses of environmental chemicals and creatinine were performed at analytical laboratories within Health Canada and at the National Public Health Institute of Quebec (*Institut National de Santé Publique du Québec*). In most cases, laboratories developed analytical protocols

Table 1
Chemicals and associated analytes, matrices, LODs and sample files.

Chemical	Analyte	Matrix	Limit of Detection (LOD)	Sample File[a]
1 Cadmium	Cadmium	blood	0.08 µg/L	EL
2 Lead	Lead	blood	0.16 µg/dL	EL
3 Mercury	Mercury (total)	blood	0.42 µg/L	EL
	Mercury (inorganic)	urine	0.16 µg/L	EL
4 Nicotine/Tobacco Products	Cotinine (free)	urine	1.1 µg/L	EL
5 Bisphenol A	Bisphenol A	urine	0.23 µg/L	EU
6 Triclosan	Triclosan	urine	4.8 µg/L	EU
7 Inorganic Arsenic	Arsenate (arsenic [V] acid)	urine	0.75 µg As/L	EU
	Arsenite (arsenous [III] acid)	urine	0.75 µg As/L	EU
	Dimethylarsinic acid (DMA)	urine	0.75 µg As/L	EU
	Monomethylarsonic acid (MMA)	urine	0.75 µg As/L	EU
8 Benzo[a]pyrene	3-Hydroxybenzo[a]pyrene	urine	0.0029 µg/L	EU
9 Chrysene	2-Hydroxychrysene	urine	0.0054 µg/L	EU
	3-Hydroxychrysene	urine	0.0026 µg/L	EU
	4-Hydroxychrysene	urine	0.0023 µg/L	EU
	6-Hydroxychrysene	urine	0.0025 µg/L	EU
10 Fluoranthene	3-Hydroxyfluoranthene	urine	0.008 µg/L	EU
11 Fluorene	2-Hydroxyfluorene	urine	0.0064 µg/L	EU
	3-Hydroxyfluorene	urine	0.002 µg/L	EU
	9-Hydroxyfluorene	urine	0.0045 µg/L	EU
12 Naphthalene	1-Hydroxynaphthalene	urine	0.021 µg/L	EU
	2-Hydroxynaphthalene	urine	0.031 µg/L	EU
13 Phenanthrene	1-Hydroxyphenanthrene	urine	0.0024 µg/L	EU
	2-Hydroxyphenanthrene	urine	0.0025 µg/L	EU
	3-Hydroxyphenanthrene	urine	0.0021 µg/L	EU
	4-Hydroxyphenanthrene	urine	0.0031 µg/L	EU
	9-Hydroxyphenanthrene	urine	0.004 µg/L	EU
14 Pyrene	1-Hydroxypyrene	urine	0.0029 µg/L	EU
15 Benzene	S-Phenylmercapturic acid (S-PMA)	urine	0.08 µg/L	EU
	trans,trans-Muconic acid (t,t-MA)	urine	0.61 µg/L	EU
	Benzene	blood	0.007 µg/L	VOCP
16 Ethylbenzene	Ethylbenzene	blood	0.011 µg/L	VOCP
17 Styrene	Styrene	blood	0.012 µg/L	VOCP
18 Tetrachloroethylene	Tetrachloroethylene (perchloroethylene)	blood	0.02 µg/L	VOCP
19 Toluene	Toluene	blood	0.011 µg/L	VOCP
20 Trichloroethylene	Trichloroethylene (1,1,2-trichloroethylene)	blood	0.027 µg/L	VOCP
21 Bromodichloromethane	Bromodichloromethane	blood	0.012 µg/L	VOCP
22 Dibromochloromethane	Dibromochloromethane	blood	0.007 µg/L	VOCP
23 Tribromomethane	Tribromomethane (bromoform)	blood	0.01 µg/L	VOCP
24 Trichloromethane	Trichloromethane (chloroform)	blood	0.014 µg/L	VOCP
25 Xylenes	m-Xylene & p-xylene	blood	0.023 µg/L	VOCP
	o-Xylene	blood	0.009 µg/L	VOCP
26 Acrylamide	Acrylamide haemoglobin adduct	blood	11 pmol/g Hb	ACRY
	Glycidamide haemoglobin adduct	blood	23 pmol/g Hb	ACRY

[a] Sample file abbreviations are as follows: EL, environmental lab blood and urine full sample; EU, environmental urine subsample; VOCP, volatile organic compounds person level blood subsample; ACRY, acrylamide blood subsample.

based on methods that were published in peer-reviewed literature or implemented in large scale biomonitoring studies, and validation studies were carried out to demonstrate the accuracy and precision of the analytical methods (Haines et al., 2017). Among chemicals measured in blood samples, metals/trace elements and volatile organic compounds and their metabolites were measured in whole blood, while acrylamide and glycidamide were measured as haemoglobin adducts. Details on laboratory analysis procedures and quality assurance and quality control protocols are described in the Third and Fourth Reports on Human Biomonitoring of Environmental Chemicals in Canada (Health Canada, 2015, 2017). The chemicals included in the present analysis are listed in Table 1.

2.3. Exposure Load and statistical methods

We define Exposure Load as the number of chemicals for which analyte biomarkers of exposure exceeded a defined concentration threshold (i.e. the analytical limit of detection (LOD) or a population-based exposure cut-off such as a percentile concentration). Related chemical analytes were grouped together, reflecting that a given chemical can be measured in different forms or through different metabolites in the body. For example, benzene includes the following analytes: benzene measured directly in blood, and the benzene

metabolites S-phenylmercapturic acid and trans,trans-muconic acid measured in urine. Similarly, mercury includes two analytes: methyl mercury and total mercury. Importantly, each chemical was treated as an equal contributor to Exposure Load regardless of the number of chemical analytes for a given chemical, as will be explained below. There were 26 chemicals, which are listed in Table 1 along with their associated analytes, matrices, LODs and sample files.

In the present study we used the analytical LOD, as well as the 50th, 75th, 90th and 95th percentiles as thresholds for calculating Exposure Loads of the Canadian population. To account for differences in dilution among spot samples, percentiles were calculated using creatinine-adjusted concentrations for chemicals measured in urine (Barr et al., 2005). Percentile concentrations for each analyte are listed in Supplemental Table 1. For the Exposure Load calculation for each participant, if an analyte was measured at or above a given threshold (analyte-specific LOD or population percentile), the participant was assigned a "1" for that analyte. If the analyte was not detected or measured below a percentile threshold, the participant was assigned a "0". For analytes with low detection rates, the 50th, 75th, 90th and even 95th percentiles could be below the LOD. In those cases, participants were only assigned a "1" if the analyte was measured at or above the LOD. For example, for an analyte with an 8% detection rate, those 8% of participants would be

assigned a "1" for the LOD threshold and the 50th, 75th and 90th percentile thresholds, while the remaining 92% of participants with measures below the LOD would be assigned a "0". As expected, only the 5% of participants with the highest concentrations would be assigned a "1" for the 95th percentile threshold. The next step was to assign a "1" or "0" to each chemical based on the scores of the individual analytes. If one or more individual analytes were "1", then the chemical score was "1". If all individual analytes were "0", then the chemical score was "0". Once each chemical was assigned a "1" or "0", the scores of all chemicals were added to determine the Exposure Load of each participant. Therefore, in this analysis the minimum Exposure Load was zero and the maximum was 26. An exception was analysis by smoking status, where Nicotine/Tobacco Products was removed as a chemical since urinary cotinine was used as a variable to determine this status, and the maximum Exposure Load was 25 in this case.

Data for individual analytes can be missing for several reasons, including insufficient sample volume obtained during collection, problems with sample storage or shipping, or issues with laboratory analysis. Since the Exposure Load combined information from 44 analytes, a missing value for any analyte could result in a missing Exposure Load for that participant if no other analyte for a given chemical was above the threshold, and the participant would need to be removed from all further analyses. Of the 1858 participants in the dataset, 728 would have needed to be excluded. However, there is still useable information on these participants. For example, if someone had an Exposure Load of 13 with one missing analyte, then their true Exposure Load could have been either 13 or 14 since the missing value was either a "0" or "1". Initial analysis showed that all analytes had ≤10% missing values, with the majority having <2% missing, and 626 of the 728 participants with missing data were missing only one or two chemicals (Supplemental Tables 2 and 3). For this reason, we decided that imputing missing values would be valid in order to create a complete dataset so that all participants could be retained.

Imputation for each of the 44 analytes was performed using an updating EM (Expectation-Maximization)-type algorithm where 0/1 responses were randomly generated using a probability of success P equal to the probability that the analyte is larger than its threshold (Schafer, 1997). The first estimate of P was obtained using the non-missing data for each analyte, and each missing observation was imputed by a random Bernoulli trial with probability of success equal to P. Then an updated estimate of P was computed on the newly completed dataset, and the missing values were again imputed using the new estimate of P. The process was repeated until stable estimates of P were achieved, which was determined in this case to be 10 iterations. Since this is a stochastic process, the data imputation procedure was repeated 10 times using the final P of each analyte, resulting in 10 complete datasets. The process of creating multiple complete datasets via imputation of missing values is called 'multiple imputation' (Schafer, 1999). An underlying assumption of this method is that the missing value pattern is "Missing Completely at Random," (Little and Rubin, 1987) which was verified by visual examination and Little's test (Roderick, 1988). Analytes were grouped after imputation.

Although this is not complete imputation of missing Exposure Load scores since partial information exists for all observations, this nevertheless imputes the response variable. To verify the results, all analyses were also carried out for only those individuals with non-missing Exposure Loads (n = 1130), and no demonstrable difference was found. For the purpose of imputation, all quantiles and Ps were computed without using the survey weights in order to simplify interpretation of results. For example, with a 90th percentile cutoff, we wanted approximately 10% of sample values to be above the cutoff. If we had used weighted quantiles to derive cutoffs, then this would no longer hold.

The prevalence of each Exposure Load score (= 0, 1, 2, …) was computed using population proportions. Tests of significance of age group, sex and smoking status were conducted using ordinal logistic

Table 2
Descriptive information for the Exposure Load study sample and the total CHMS sample.

		Exposure Load Study Sample		Total CHMS Sample	
		N[a]	% (95% CI)[b]	N[a]	% (95% CI)[b]
CHMS Cycle	3	942	51 (46, 56)	2527	49 (49, 49)
	4	916	49 (44, 54)	2527	51 (51, 51)
Participant Sex	Male	923	49 (43, 55)	2514	50 (50, 50)
	Female	935	51 (45, 57)	2540	50 (50, 50)
Participant Age Group	12–19	719	16 (14, 19)	1470	11 (11, 11)
	20–39	387	31 (26, 36)	1157	33 (33, 33)
	40–59	344	33 (27, 39)	1229	35 (35, 35)
	60–79	408	20 (18, 23)	1198	21 (21, 21)
Participant Region	Atlantic	271	5.6 (4.8, 6.6)	722	6.8 (6.8, 6.8)
	British Columbia	249	16 (13, 19)	651	13 (13, 13)
	Ontario	614	36 (32, 41)	1739	39 (39, 39)
	Prairies	251	18 (13, 25)	688	18 (18, 18)
	Quebec	473	24 (21, 28)	1254	23 (23, 23)
	Total	1858		5054	
Participant Smoking Status[c]	Nonsmoker	1557	81 (74, 86)	4146	81 (78, 83)
	Smoker	300	19 (14, 26)	828	19 (17, 22)
		1857[d]		4974[d]	

[a] N is unweighted.
[b] Percentages were calculated using CHMS cycle 3 and cycle 4 combined weights.
[c] Smoking status was defined by smokers having ≥50 ng/ml urinary cotinine and nonsmokers having <50 ng/ml.
[d] Total number of nonsmokers and smokers is less because of missing smoking status.

regression, collapsing adjacent Exposure Load groups when necessary to maintain a minimum cell size of 10. The proportional odds assumption required for this model was assessed using Brant's test (Brant, 1990). In cases where Brant's test failed, testing was also performed using multinomial logistic regression. The Bonferroni multiple comparison technique was used to identify differing groups where overall tests were significant. All estimates and tests were conducted using the survey weights and bootstrap weights from the combined cycle 3 and 4 VOCP weights file, since this was the subsample with the fewest participants and therefore largest weights, along with 22 denominator degrees of freedom for testing (Statistics Canada, 2016). Weighted analysis was conducted separately on all 10 imputed datasets and Rubin's rule was used to combine estimates and associated standard errors across imputation runs (Rubin, 1987), although no further adjustment was made to the denominator degrees of freedom (Research Triangle Institute, 2012). Hypothesis tests used the combined estimates and standard errors. Statistical analysis was performed using SAS (Statistical Analysis System) Enterprise Guide 7.1 and R (R Core Development Team). Unless otherwise indicated, a 5% significance level (α = 0.05) was implemented throughout.

3. Results

Demographic characteristics for the Exposure Load study sample and the total CHMS sample are shown in Table 2. There were no major differences in the compositions of the study sample and total CHMS sample in regards to participant sex, age group and region, or being sampled as part of cycle 3 or cycle 4. As a starting point to consider how the definition of exposure will impact the Exposure Load profile of the population, we first looked at overall chemical detection. The percent detection frequency in the sample population for each chemical analyte is shown in Fig. 1. There were a small number of analytes with a detection frequency just above zero. Chemicals for which all constituent chemical analytes were at this very low level, notably benzo[a]pyrene, chrysene, fluoranthene and trichloroethylene, will have little to no
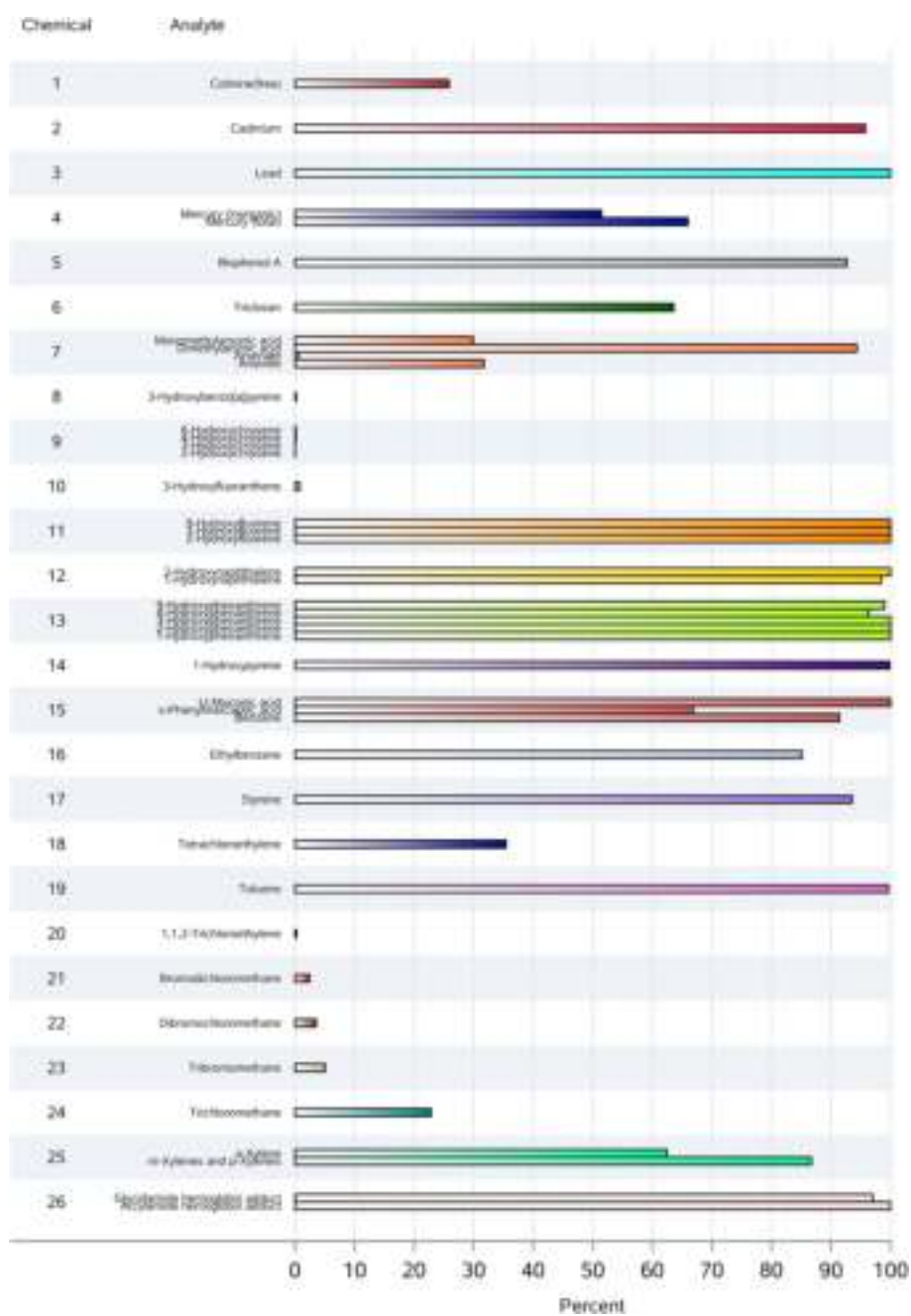
Fig. 1. Detection frequency of each measured chemical analyte in the Exposure Load study sample (imputed data).

influence on Exposure Loads at any exposure threshold for the majority of the population. By contrast, there were several analytes with a detection frequency of 100% or nearly 100%; when using the LOD as the exposure threshold, these chemicals will increase Exposure Load for almost the entire population, but they will not influence the differences in Exposure Load between individuals since everyone has detectable levels. Notable among these high detection chemicals were lead, fluorene, naphthalene, phenanthrene, pyrene, benzene, toluene and acrylamide.

The estimated Exposure Load distribution for the Canadian population using analytical LOD as the exposure threshold for each analyte is shown in Fig. 2, panel A. The Exposure Load distribution was relatively tightly clustered for the population when using LOD as the exposure threshold, with 98% of the Canadian population having an Exposure Load between 13 and 18. There were a number of chemicals that were measured above the LOD for the entire study sample, and accordingly

the Exposure Load distribution is shifted to the right: no individual had an Exposure Load lower than 9, while the greatest proportion of people had an Exposure Load of 16. The most highly exposed 10% of the Canadian population had an Exposure Load between 18 and 21.

We then examined how Exposure Load varied by age with LOD as the exposure threshold. Exposure Loads for four different age groups (12–19, 20–39, 40–59 and 60–79 years) are depicted in Fig. 2, panel B. This figure shows the Exposure Load distribution of the youngest age group (aged 12–19 years) shifted to the left compared to other ages, with greater proportions of this group having lower Exposure Loads compared with older age groups. Bonferroni multiple pair-wise comparisons revealed that 12–19 year olds had a significantly lower Exposure Load than 40–59 and 60–79 year olds. In contrast, there was no significant difference in Exposure Load by sex (Fig. 2, panel C) or by smoking status (Fig. 2, panel D) when using LOD as the exposure threshold. Outcomes of the ordinal logistic regressions for sex, age and
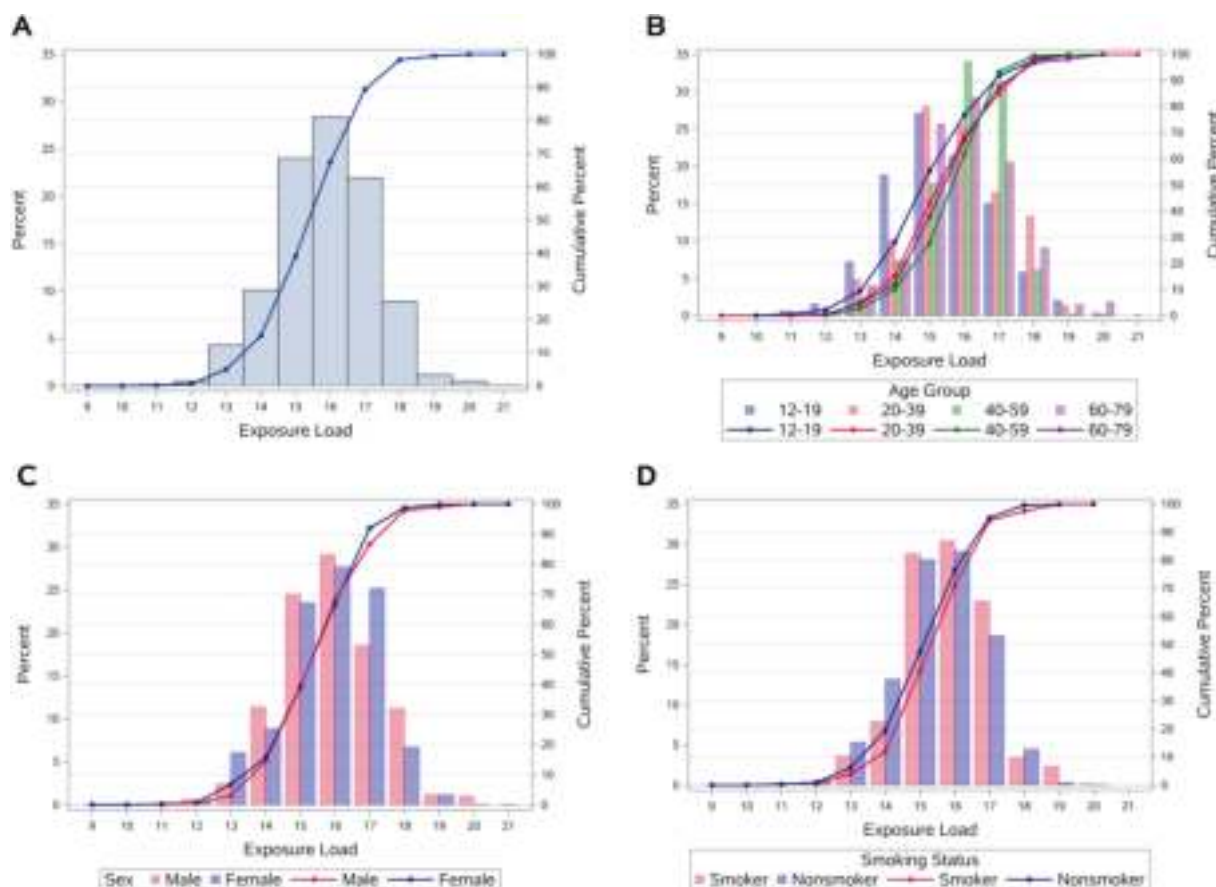
Fig. 2. Exposure Load as population percentage using LOD as the exposure threshold for the total population (A), by age group (B), by sex (C), and by smoking status (D).

smoking status are listed in Supplemental Table 4.

We next explored the influence of using other threshold cutoffs to define exposure (Fig. 3). As expected, Exposure Loads in the Canadian population decreased as exposure thresholds increased from the 50th to the 95th percentile, with distributions noticeably shifting left to lower totals (Fig. 3, panels A to D). At the 50th percentile exposure threshold, nearly 80% of the population had an Exposure Load of 13 or less, while at the 95th percentile exposure threshold this same proportion of the population had an Exposure Load of 2 or less (Fig. 3, panels A and D). The 95th percentile exposure threshold represents individuals with high chemical exposures in the population. Since only 5% of the population is above the 95th percentile for each individual analyte, Exposure Loads for the majority of Canadians were low for this threshold. For example, cumulatively 88% of the Canadian population had an Exposure Load of 3 or less at the 95th percentile exposure threshold (Fig. 3, panel D). However, 12% of the population had an Exposure Load ranging from 4 to 15 at the 95th percentile exposure threshold, indicating that there is a substantial subset of the population who are highly exposed to multiple chemicals.

Comparing Exposure Loads across population subgroups may provide insight on the extent to which biological parameters and lifestyle factors contribute to and are associated with multi-chemical exposures, and using different exposure thresholds may improve the sensitivity of this analysis. A comparison of Exposure Loads among different age groups using the 50th, 75th, 90th or 95th percentile as an exposure threshold is shown in Fig. 4. The greatest disparity in Exposure Load distributions was between the youngest age group (12–19 year olds) and older age groups (20–79 years old). Higher proportions of 12–19 year olds had lower Exposure Loads compared to older age groups, as shown in Fig. 4. For example, at the 75th percentile exposure threshold, the

proportion of 12–19 year olds in the Canadian population having the four lowest Exposure Loads (0 through 3) was the highest among all age groups (Fig. 4, panel B), and at the 95th percentile, the proportion of 12–19 year olds having an Exposure Load of 0 was almost twice that of each of the other three age groups (Fig. 4, panel D). These differences were reflected in the cumulative percentage curves, where for example at the 90th percentile exposure threshold a large majority (90%) of 12–19 year olds had an Exposure Load of 3 or less, while the same cumulative percentage of 40–59 and 60–79 year olds had Exposure Loads of up to 10 and 9, respectively (Fig. 4, panel C). Accordingly, Bonferroni multiple pair-wise comparisons showed that 12–19 year olds had a significantly lower Exposure Load than 20–39, 40–59 and 60–79 year olds at the 50th and 75th percentile exposure thresholds, and a significantly lower Exposure Load than 40–59 and 60–79 year olds at the 90th and 95th percentile thresholds. Exposure Load distributions based on percentile thresholds were also compared between sexes (Fig. 5). Similar to that observed when using LOD as the exposure threshold, Exposure Loads were similar between males and females with no statistically significant differences between sexes at any exposure threshold.

To illustrate how the Exposure Load approach with varying thresholds can be used to compare groups with known contrasts in chemical exposures, we next examined the impact of smoking habits (Fig. 6). As expected, smokers had significantly higher Exposure Loads compared to nonsmokers at all percentile exposure thresholds. Exposure Load distributions were noticeably shifted left towards lower scores for nonsmokers compared to smokers, and the divergence became more pronounced as the percentile exposure threshold increased. The striking difference between these subgroups was underscored by the cumulative percentage curves, where for example at the 95th percentile exposure threshold, 97% of nonsmokers had an Exposure Load of no more than 3
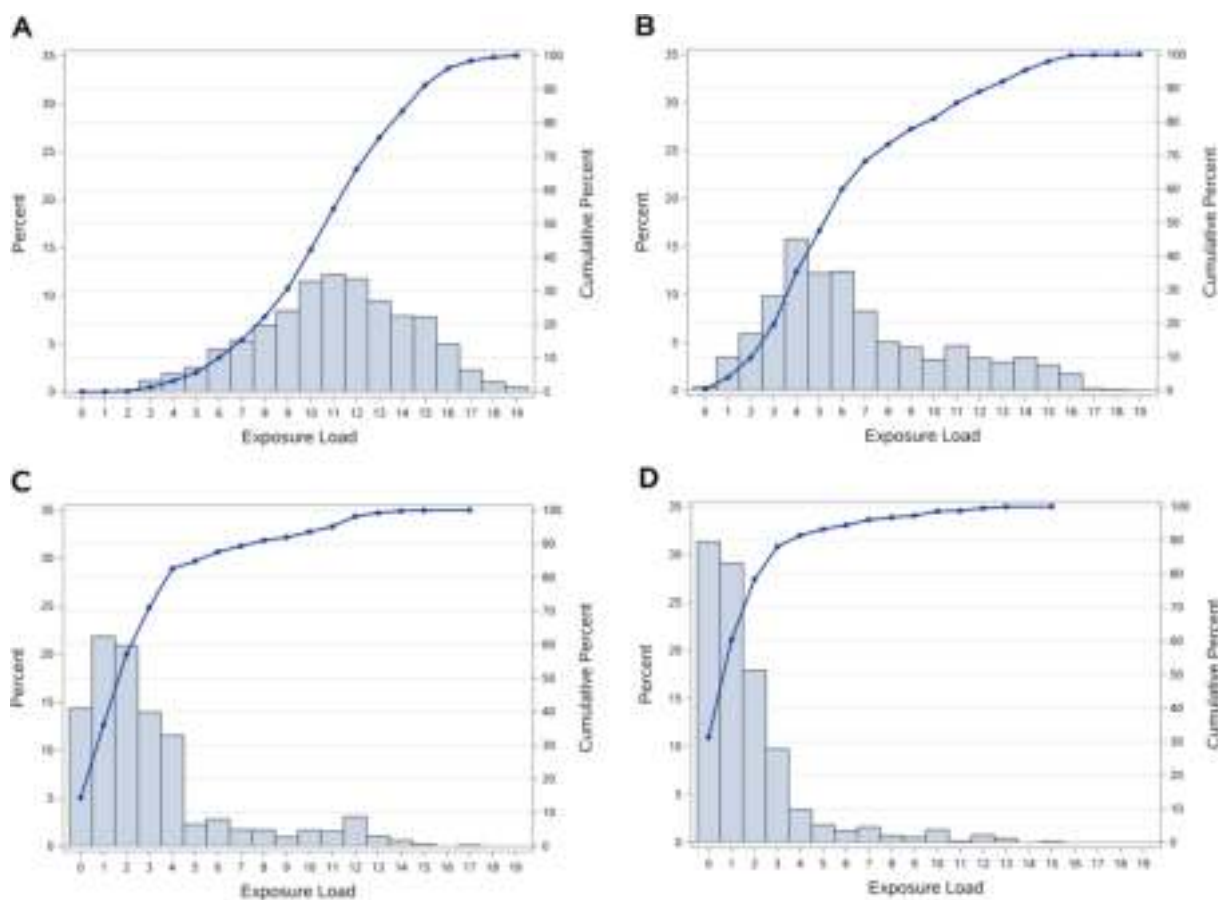
Fig. 3. Exposure Load as population percentage using the 50th (A), 75th (B), 90th (C) or 95th (D) percentile as the exposure threshold.

while the same proportion of smokers had an Exposure Load of up to 11 (Fig. 6, panel D).

4. Discussion

There has long been a need to evaluate exposure to real-world chemical mixtures. Various approaches have been used to assess health outcomes of chemical mixtures. Examples include mixture assessment using toxicological equivalency, which assumes the same mode of action for all mixture components; the hazard index, which sums hazard quotients for multiple chemicals having the same target organ or system; and, going beyond toxicological data, approaches such as death-adjusted life years (DALYs) and quality of life impacts, which utilize epidemiological data to combine chemical specific outcomes with quality of life indices. These methods have achieved varying degrees of success in assessing the health impacts of multi-chemical exposures, albeit with certain assumptions and limitations (Bopp et al., 2019; Drakvik et al., 2020; More et al., 2019; Hernandez et al., 2019; OECD, 2018; Prüss-Ustün et al., 2011). The Exposure Load method approaches the problem of multi-chemical assessment by concentrating on the exposure side of the risk equation. Exposure Load utilizes biomonitoring data, reflective of all sources and routes of exposure, to count the number of chemicals measured in individuals above a defined threshold in order to arrive at a measure of overall exposure burden in a population.

The initial results of our Exposure Load analysis using nationally-representative biomonitoring data based on 26 chemicals reveal the distribution of exposure burdens across the Canadian population. The Exposure Load distribution for the population was relatively tightly clustered when using LODs as a low concentration exposure threshold, with a majority of people having an Exposure Load between 13 and 18.

This result substantiates that exposures to multiple chemicals (simultaneously and/or sequentially) are a fact of life for Canadians. When employing higher exposure thresholds based on percentile concentrations there was more variability across the population, and Exposure Loads decreased as the threshold increased from the 50th to 95th percentile concentrations. At the highest exposure thresholds (90th and 95th percentile concentrations), the majority of people had very low Exposure Loads (e.g., 78% of the Canadian population had an Exposure Load of 2 or less at the 95th percentile) but there remains a minority of people who are highly exposed to multiple chemicals.

The identification of subpopulations more highly co-exposed to multiple chemicals is an important objective of Exposure Load analysis. Our initial work investigated potential differences in Exposure Loads based on age group, sex and smoking status. Our analysis revealed differences in Exposure Load between age groups. Most notably, the youngest age group (12–19 years) had a significantly lower Exposure Load than older age groups. Age is known to play a role in exposure to chemicals and the amounts of chemicals measured in people. People are repeatedly exposed to chemical toxicants, whether by occupation or within different microenvironments, and persistent pollutants can bioaccumulate, potentially remaining in tissues for decades (Genuis and Kelln, 2015). Many toxicants including solvents, petrochemicals, heavy metals and various synthetic chemical agents are persistent pollutants with long half-lives that can remain in the body for years. Changes in regulations and the phase out of chemicals can translate into younger people having less exposure to certain legacy chemicals, while older people can retain persistent chemicals from past exposures when their use was more widespread. Several of the chemicals included in Exposure Load are considered persistent in the body (cadmium, lead and mercury). Older adults may be affected by chemical exposures differently due to the slowing of metabolic processes or pathological alteration due
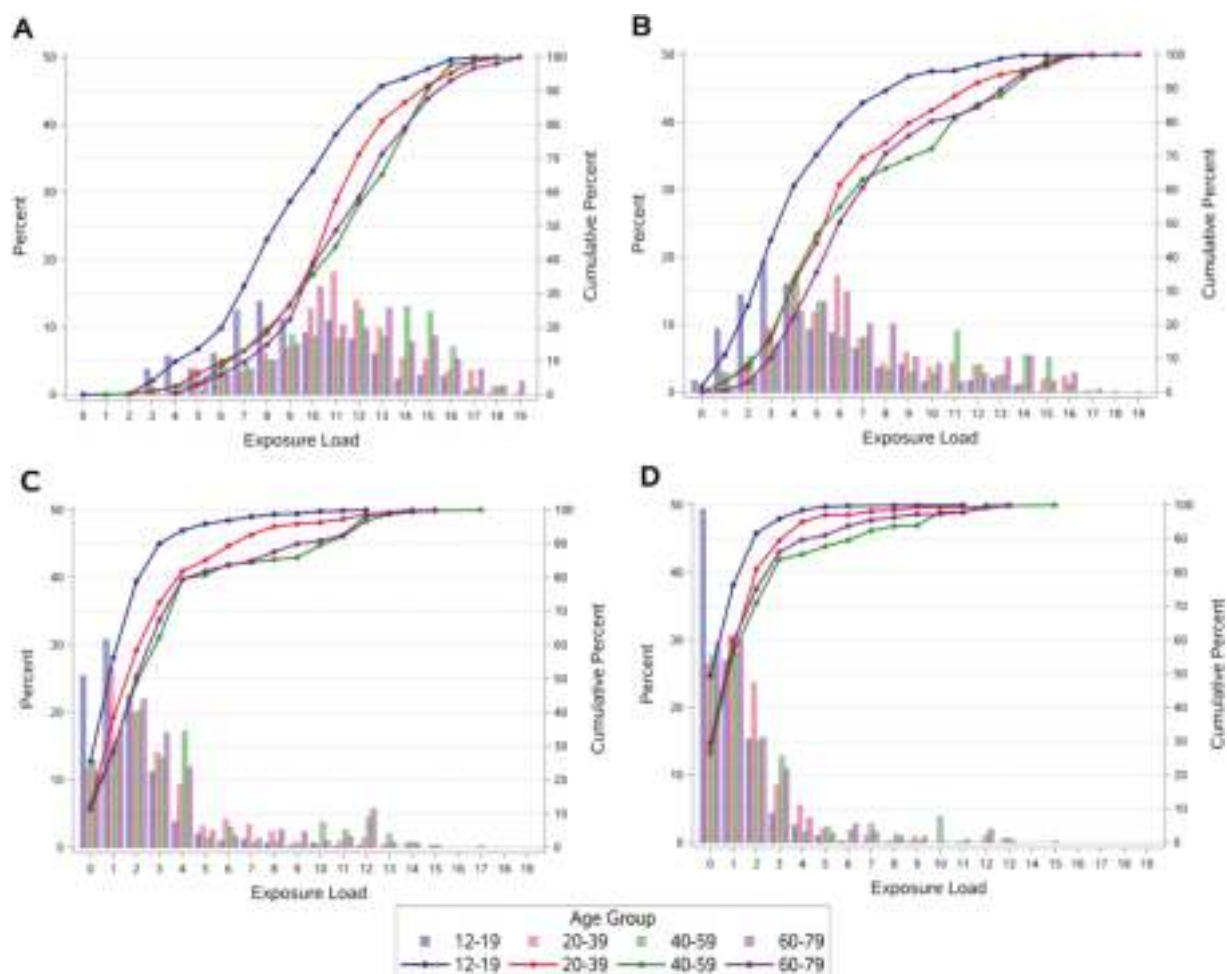
Fig. 4. Exposure Load as population percentage by age group using 50th (A), 75th (B), 90th (C) or 95th (D) percentile as the exposure threshold.

to disease, while longer half-lives of environmental chemicals in the body may result from the reduction of homeostatic reserves and decreased function of organ systems responsible for eliminating xenobiotics as a consequence of aging (Choi et al., 2017; Geller and Zenick 2005; Risher et al., 2010). Higher prevalence of smoking in the adult population may also lead to greater exposures to certain chemicals compared to adolescents. Given all of these factors we would expect age to be positively correlated with chemical levels in blood and urine and therefore higher Exposure Loads. Indeed, CHMS data show that levels of bioaccumulative metals tend to increase with age in the population. Blood lead measured over 5 cycles (2007–2017) was significantly higher in adults aged 20–79 years compared to children and adolescents aged 3–19 years (Health Canada, 2019). Similarly, cadmium and total mercury measured in blood were significantly lower in 12–19 year olds compared to adults aged 20–79 over the same five CHMS cycles. The lower Exposure Load of those aged 12–19 years compared with older age groups aligns with these broader trends for bioaccumulative metals in CHMS data.

Chemical exposures can be influenced by sex and gender differences. Examples include variations in physiological parameters (e.g., differences in body fat and storage of chemicals in tissues, hormonal influences resulting in differing absorption, metabolism, storage, and/or excretion); differences in characteristics associated with gender including diet, lifestyle, personal hygiene and use of cosmetic products; and differences in occupational exposures and time spent in different microenvironments (Arbuckle, 2006; Gochfield, 2007; UNDP, 2011). It would not be unreasonable to expect some differences in exposure burden between sexes, however in our analysis males and females displayed only a minimal and non-significant difference in Exposure Load, whether using LODs or percentile concentrations as the exposure threshold. Therefore our Exposure Load results suggest that when looking broadly at the Canadian population, males and females have similar exposure burdens. Despite little evidence of sex-based differences in Exposure Load, the body burden of individual chemicals may differ by sex. This points to the importance of considering not just the number of chemicals in the Exposure Load score, but also the composition. The importance of chemical constituents within overall Exposure Load is an avenue of exploration for future work.

It is well documented that smokers, via inhalation of cigarette smoke, incur repeated high level exposures to a wide variety of chemicals, many of which are known toxicants (Cunningham et al., 2011; Haussmann, 2012; St. Charles et al., 2013). Cigarette smoke is a complex mixture containing an estimated 5000 or more chemicals, with the type of tobacco and the design of the smoking product influencing chemical composition (Andreoli et al., 2003; Talhout et al., 2011). A comparison of Exposure Loads between smokers and nonsmokers was undertaken to test the *a priori* expectation of contrasting chemical burdens. A number of chemicals in cigarette smoke are part of the Exposure Load calculation in the present analysis, including acrylamide, cadmium, toluene, xylene and various PAHs (Health Canada, 2015, 2017). The expected higher Exposure Load in smokers versus nonsmokers was confirmed by our analysis, and the magnitude of the difference between the two subgroups was striking. As the threshold for defining exposure increased, the higher Exposure Loads incurred by smokers grew increasingly pronounced in comparison to nonsmokers, consistent with the expected higher level of exposures of smokers to multiple chemicals. Looking at
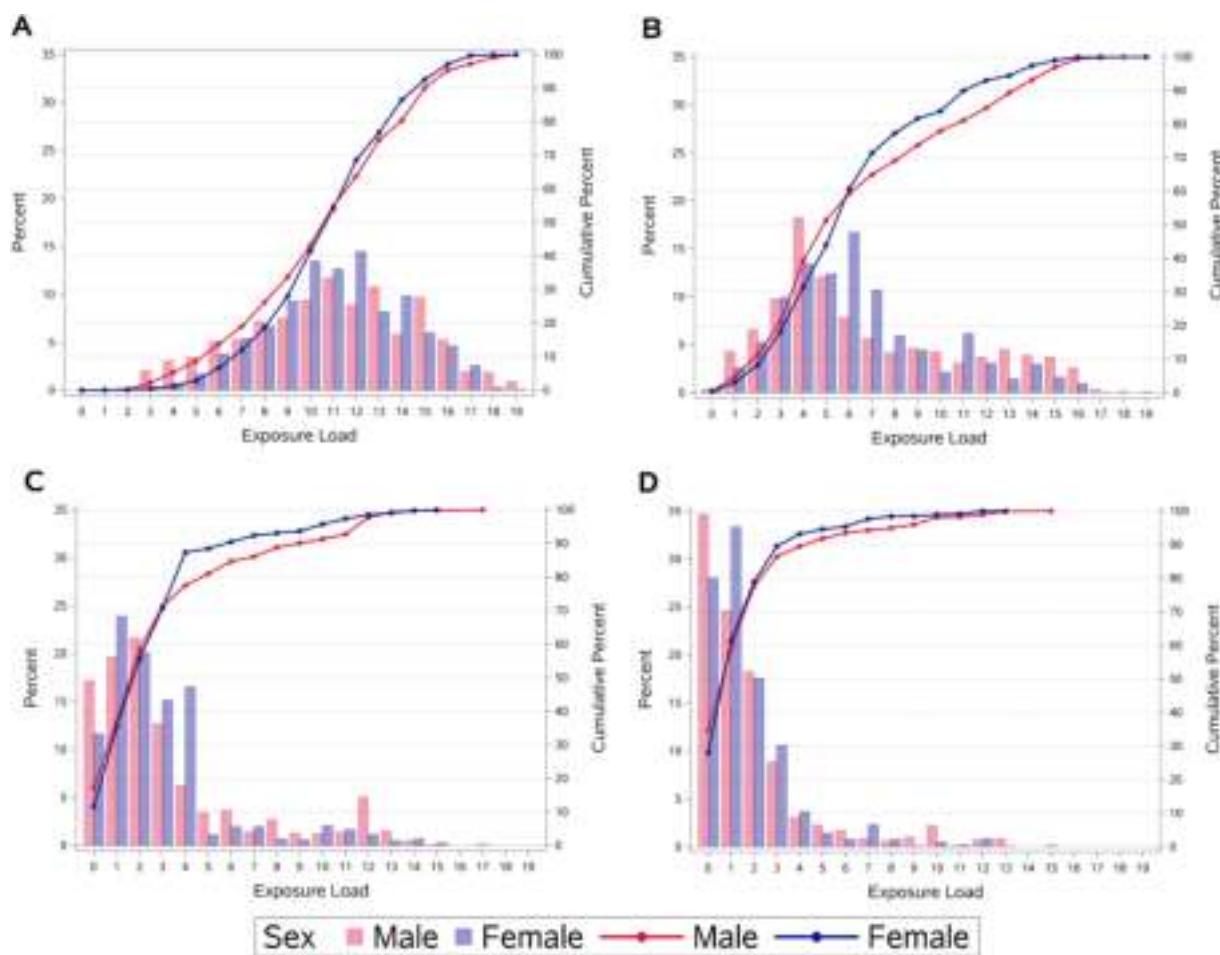
Fig. 5. Exposure Load as population percentage by sex using the 50th (A), 75th (B), 90th (C) or 95th (D) percentile as the exposure threshold.

95th percentile concentrations, more than one third (37%) of non-smokers had an Exposure Load of zero, while an Exposure Load of zero was found in only 10% of smokers. The results support the notion that smokers face markedly higher exposure burdens compared to non-smokers, with exposure to cigarette smoke overwhelming many other sources of chemical exposures. These results also demonstrate the utility of Exposure Load in exploring differences in exposure burdens based on lifestyle choices and by subpopulation.

The Exposure Load concept has been similarly explored using bio-monitoring data from other surveys. Kapraun et al. (2017) employed frequent itemset mining to identify prevalent chemical combinations using data from the 2009–2010 U.S. National Health and Nutrition Examination Survey (NHANES). Their histograms indicating proportions of the U.S. population for which a given number of chemicals were present are similar to Exposure Load distributions observed in the Canadian population. Biomonitoring data have also been used to quantify multi-chemical exposures for the population of Catalonia, Spain (Porta et al., 2010) and for children via 2003–2012 NHANES data (Hendryx and Luo, 2018). These studies employed a similar method to quantify exposures to multiple chemicals within an individual, however there are some notable differences in their approach. One important difference is that these studies only considered multiple exposures within defined subsamples of the population and/or for defined chemical groups. Concurrent exposures were reported for 106 analytes across 3 separate population subsamples by Kapraun et al. (2017), 36 analytes across 5 separate chemical groups by Hendryx and Luo (2018), and for 19 persistent organic pollutants (1 chemical group) by Porta et al. (2010). The limitation of considering defined subsamples and/or chemical groups is that concurrent exposures and prevalent chemical

combinations cannot be assessed broadly. In contrast, the CHMS sampling protocol measures all chemicals in each participant selected for the environmental chemical subsamples. Missing results are a common occurrence in population surveys for a variety of reasons, and a complete dataset is required to calculate reliable Exposure Loads. To alleviate this issue, we applied multiple imputation to preserve the sample size, statistical power, and national representativeness of the Exposure Load metric. The statistical imputation method for dealing with missing data presented here can be applied in similar analyses where the biomonitoring dataset may be incomplete, and where the sample size may not be large enough to accommodate excluding participants with missing data.

There are a number of limitations that should be considered when applying Exposure Load and interpreting the results. The 26 chemicals included in the present analysis based on CHMS cycles 3 and 4 are only a fraction of the potentially hundreds or even thousands of chemicals to which individuals may be exposed, from all sources and exposure routes. The chemicals used in this analysis do however include many chemicals of common concern (e.g., lead, arsenic and benzene), and the analysis can be expanded with data as they become available, for example by incorporating more participants and chemical data from additional survey cycles. The more chemicals that are included in Exposure Load, the more this measure will reflect the actual exposure burden in a population arising from multiple real-world exposures. It should be kept in mind that direct comparability of Exposure Load results across different studies or even across different years within the same study may present challenges due to differences in the chemicals surveyed, population sample demographics (for example the age range of participants), and other variables.
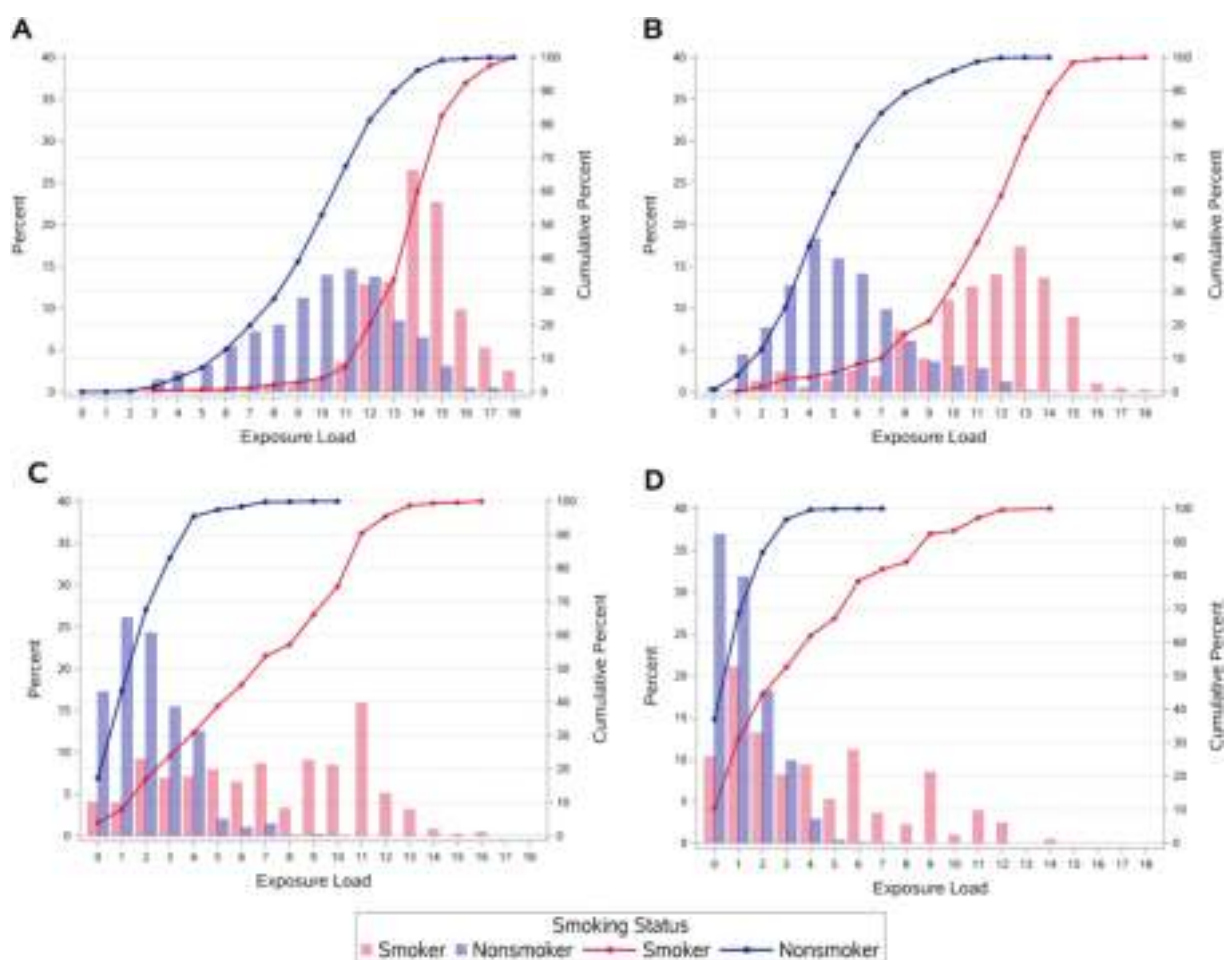
Fig. 6. Exposure Load as population percentage by smoking status using the 50th (A), 75th (B), 90th (C) or 95th (D) percentile as the exposure threshold.

Exposure Load is a method for characterizing the burden of exposure to multiple chemicals in a population, but it does not directly address the health impacts of these exposures. Since the method is based on measured concentrations in biological samples, the use of LOD as an exposure threshold is a reflection of the sensitivity of the analytical methods used, and therefore the results may not be directly comparable to those from other studies employing different analytical methods. Kapraun et al. (2017) point out that using the LOD or percentiles as an exposure threshold is somewhat arbitrary from the standpoint of health risk, and suggest that ideally the threshold for presence of a chemical in an individual would be a critical concentration or a point of departure (POD) associated with toxicity. However, such concentrations and PODs are typically derived from chemical concentrations in environmental media (e.g. air or water) based on animal studies or even *in vitro* studies, and not from blood or urine concentrations (Kapraun et al., 2017). Incorporating critical health effect concentrations as exposure thresholds is one potential way of tailoring Exposure Load to a more health-related measure. A biomonitoring equivalent (BE) is the concentration of an exposure biomarker for an environmental chemical in a biological medium such as blood or urine that corresponds to a chemical concentration in environmental media at which an exposure guidance value for that chemical has been set, with the value typically associated with health risk (Hays et al., 2007). Use of BEs as an exposure threshold is one way to link Exposure Load with health, but at present BEs have been developed for only a limited number of chemicals, and are not widely enough available to apply uniformly in Exposure Load analysis with a large number of chemicals (Faure et al., 2020). The feasibility of this approach will improve as more BEs become available.

The Exposure Load approach may be useful in identifying factors beyond age, sex and smoking status that may be associated with exposure burden, for example sociodemographic variables and diet. Further, the shape of the Exposure Load profile for these parameters may be meaningful in terms of understanding heterogeneity in population exposures, and could guide the important task of identifying potentially vulnerable subpopulations. In our example of looking at different age groups, while the younger age group may have lower overall numbers of chemicals detected, they may be more vulnerable for developmental reasons. Consequently, the more highly exposed within this subgroup (i. e. the right tail of the Exposure Load distribution) would be of considerable interest as a potential group of concern. Exposure Load could also be used to identify whether certain chemicals or chemical classes contribute disproportionately to higher exposure burdens. An important additional application of the Exposure Load concept is examining the relationship between the magnitude of exposure burdens and their bearing on health outcomes. These could be linked to specific health outcomes (e.g. biomarkers, physiological measures, cause-specific hospital admissions, mortality), or to more broad subclinical measures of physiological dysfunction such as allostatic load scores (Thomson et al., 2019).

5. Conclusions

A method is presented whereby chemicals with varying numbers of analyte biomarkers can be analyzed together against defined concentration thresholds in order to generate a metric termed Exposure Load, which is an overall measure of multi-chemical exposure in a population. The method employs statistical imputation for dealing with missing analyte data, so that participants can be retained in the analysis.

Exposure Load analysis has provided a picture of the overall exposure burden of the Canadian population; within this population the youngest analyzed (12–19 year olds) had a lower Exposure Load than older individuals, smokers incurred a much higher Exposure Load than non-smokers, while males and females did not substantially differ. Exposure Load has helped quantify and characterize multi-chemical exposure burdens in the Canadian population, advancing our knowledge of the exposome and population exposures. We believe the same objectives can be achieved for other countries and datasets by applying the Exposure Load method. The International Biomonitoring Network, a network of national biomonitoring programs for which Health Canada is currently providing chair and secretariat functions, has recently been initiated with the intention of promoting collaboration and information sharing. This network could facilitate Exposure Load analysis across different national programs, and comparisons between national datasets could yield interesting results.

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Declaration of competing interest

None to declare.

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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.2021.113704.

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From spontaneous to strategic natural window ventilation: Improving indoor air quality in Swiss schools

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ABSTRACT

Natural window ventilation is frequently employed in schools in Europe and often leads to inadequate levels of human bioeffluents. However, intervention studies that verify whether recommended ventilation targets can be achieved in practice with reasonable ventilation regimes and that are also suitable for countries with cold winters are practically non-existent. To explore the initial situation in Switzerland we carried out carbon dioxide (CO_2) measurements during the winter in 100 classrooms, most of which (94%) had natural window ventilation. In more than two thirds of those, the hygienic limit value of 2000 ppm specified for CO_2 in the Swiss Standard SN 520180 (2014) was exceeded. To improve ventilation behavior, an intervention was implemented in 23 class-rooms during the heating season. Ventilation was performed exclusively during breaks (to avoid discomfort from cold and drafts), efficiently, and only for as long as was necessary to achieve the ventilation objective of compliance with the hygienic limit value (strategic ventilation). The intervention included verbal and written instructions, awareness-raising via a school lesson and an interactive tool for students, which was also used to estimate the required duration of ventilation. CO_2 exposure was significantly reduced in pilot classes (Wilcoxon signed-rank test, p = $3.815e^{-06}$). Median CO_2 levels decreased from 1600 ppm (control group) to 1097 ppm (intervention group), and the average proportion of teaching time at 400–1400 ppm CO_2 increased from 40% to 70%. The duration of ventilation was similar to spontaneous natural window ventilation (+5.8%). Stricter ventilation targets are possible. The concept of the intervention is suitable for immediate adoption in schools with natural window ventilation for a limited period, pending the installation of a mechanical ventilation system. The easy integration of this intervention into everyday school life promotes compliance, which is particularly important during the COVID-19 pandemic.

1. Introduction

In heavily occupied rooms such as classrooms, ventilation is particularly important for indoor air quality (IAQ). Assuming good outdoor air quality and no major source of pollutants in the classroom, human bioeffluents are the most significant pollutants (Bekö et al., 2020; Braniš et al., 2005; Jacobs et al., 2013; Tang et al., 2016; Wargocki, 2004). Since this type of pollution is unavoidable, good IAQ can only be maintained by means of adequate ventilation. Because of the high occupancy in schools, the recommended ventilation rates are so high that

pollutants from indoor sources are also reduced to a certain extent (Rosbach et al., 2015; Salthammer et al., 1995). Adequate ventilation of classrooms thus improves IAQ when outdoor air quality is good. However, there is a great need for action with regard to ventilation. While the use of CO_2 concentrations as an indicator of ventilation in occupied rooms is well established (Pettenkofer, 1858; Sundell, 2004), numerous studies from various countries show that IAQ in classrooms is often unsatisfactory, and that the degree of ventilation specified in building codes and ventilation standards is often not achieved (Daisey et al., 2003; Fisk, 2017). In buildings with natural window ventilation, the

ventilation can be particularly poor. This has consequences, not only for health but also to a significant extent for intellectual performance (Bakó-Biró et al., 2011; Fisk, 2017; Haverinen-Shaughnessy et al., 2011; Strøm-Tejsen et al., 2016; Sundell et al., 2011; Wargocki and Wyon, 2006; Zhang et al., 2017).

While society has an obligation to take action, there is no generally accepted strategy on how to proceed in order to achieve the goal of good IAQ in all schools in a short time. Recommendations have, however, been made for European countries to strengthen national and local policies for healthy environments within schools, including some general guidance on reducing exposure to pollutants or eliminating pollutant sources, as well as on adequate ventilation (Kephalopoulos et al., 2014; Carrer et al., 2018; ECA Report No 30, 2020). In Switzerland, the Federal Office of Public Health (FOPH) is responsible for providing information on indoor air pollutants and can make appropriate recommendations for their reduction. The present study was conducted as part of the FOPH strategy to improve IAQ in Swiss schools. Swiss schools are mainly ventilated manually, and the outdoor air quality is mostly good (FOEN Report, 2020). Ventilation solutions that are more suitable than manual window ventilation are only required in cases of excessive ambient noise (average day traffic noise impact threshold 60 dB(A) for sensitivity level II, which usually applies to schools, Noise Abatement Ordinance NAO, 1986), or in cases of polluted outdoor air (limit value of 45 $\mu g/m^3$ as an annual average for realization of window ventilation according to Swiss Standard SN 520180, 2014). Replacement of natural window ventilation with mechanical ventilation in order to improve ventilation has been sluggish in renovated and even new schools. One reason for this could be barriers to the widespread adoption of energy-efficient solutions to which mechanical ventilation systems are assigned, as they are often perceived as a potential threat to comfort, well-being, and performance (Kozusznik et al., 2019). Although a sound data basis is currently lacking, we estimate that only about 40% of school buildings constructed during the past decade have been equipped with mechanical ventilation. For renovated buildings, the proportion is likely to be much lower. We therefore assume that ventilation in Swiss classrooms is often inadequate, particularly in winter because of discomfort due to low outdoor temperatures. Accordingly, the strategy has focused on raising awareness from the outset, not only among building owners (as builders/operators), but also among schools (as occupants/users), as well as giving recommendations aimed at improving ventilation.

The FOPH has set the long-term objective that CO_2 levels in classrooms should remain below 1400 ppm throughout the school day, irrespective of the ventilation system used (maximum values). This corresponds to the standard IAQ category RAL 3 (IDA 3 in the withdrawn European Standard EN 13779 (2007)) and to the minimum requirement for mechanically ventilated rooms specified in the Swiss Standard SN 546382/1 (2014). In every newly built or renovated school, a ventilation concept should be put in place that ensures that this specification is met on a permanent basis. Concepts involving demand-controlled mechanical ventilation with heat recovery and hybrid ventilation should mainly be considered. This measure will be slow to take effect and requires long lead times. Above all, it must be rigorously implemented in energy-saving building renovations, which are an important component of the Swiss Federal Council's Energy Strategy 2050 (Swiss Federal Office of Energy, 2020). The first part of the present study – in which CO_2 levels were measured during the heating season in 100 different classrooms in 96 public school buildings (including addition of stories) – was designed to explore the initial situation and, at the same time, to provide a basis for awareness-raising through communication. The aim of the second part was to propose, and test in practice, an intervention for winter ventilation that can be integrated into everyday school life. The impetus for this was the following: recommendations for good indoor air quality in schools are often limited to hints or advice and the specification of ambitious CO_2 targets (e.g. ventilation required at a CO_2 level of 700 ppm;

Kephalopoulos et al., 2014). No intervention studies have been reported that tested whether such targets can be achieved in practice with reasonable ventilation regimes. There is also no solution to the problem of significant comfort issues jeopardizing compliance in countries with cold winters. The few published intervention studies tested CO_2 sensors that indicated a need for ventilation, ideally in combination with measures to raise awareness of the issue (e.g. Wargocki and Da Silva, 2015). These studies took into account the finding that it is not sufficient simply to tell school classes to improve window ventilation (Ausschuss für Innenraumrichtwerte Ausschuss für Innenraumrichtwerte AIR, 2008; Rovelli et al., 2014). However, a disadvantage of using CO_2 sensors is that they might disturb classes, as the devices demand attention, and they lead to massive comfort problems in cold winters. Students close to a window are particularly exposed to discomfort; as a result, ventilation times in everyday school life are too short and the devices are often ignored (Park and Choi, 2019). It was thus decisive for the intervention that cold outside temperatures are taken into account, so that ventilation takes place exclusively during breaks between lessons, while the classrooms are empty.

The FOPH recommends a CO_2 level of 2000 ppm as the hygienic limit value, in agreement with the upper limit of CO_2 guideline values for occupied rooms in the Swiss Standard SN 520180 (2014) and the recommendation of the German Committee on Indoor Guide Values (Ausschuss für Innenraumrichtwerte Ausschuss für Innenraumrichtwerte AIR, 2008). It is applicable in the absence of substantial sources of indoor pollutants apart from human bioeffluents. For communication with students, the categories for CO_2 levels in schools with natural window ventilation were defined as follows: <1000 ppm ('excellent'), 1000–1400 ppm ('good'), 1400–2000 ppm ('satisfactory') and >2000 ppm ('unacceptable').

2. Material and methods

2.1. Data collection and cross-sectional study (S1)

A total of 100 different classrooms in 96 public school buildings (including addition of stories) were selected for CO_2 measurements. They were all primary and lower-secondary schools (i.e. compulsory education), for which the cantons and communes are responsible. The selection is reasonably representative of Switzerland in terms of political districts (cantons of Bern, Graubünden, Vaud), size and type of commune (8 size categories from <1000 to >100,000 inhabitants, urban or rural) and the two most common languages (German and French), covering 90% of the official spoken languages (Swiss Federal Statistical Office, 2012). Wealthy communities were excluded from selection. The aforementioned criteria influence school class size, readiness for renovations, and experience in school building construction. Thirty-two classrooms were in buildings located in a rural environment, 43 in a suburban and 25 in an urban setting. Almost all the classrooms had a single window frontage on the long side of the room, and most of them were located on the first (52) or ground floor (31). A nearby motorway was reported in 4 cases, a main road in 20 and a side road in 39 cases. Site visits showed that for 9 classrooms, the distance to a main road was less than 20 m. However, traffic was not heavy in most cases and measured NO_2 values in the outdoor air were <45 $\mu g/m^3$ for all buildings (n = 100 schoolrooms, median: 26 $\mu g/m^3$, range: 9–41 $\mu g/m^3$, measuring duration: 14 days during wintertime), i.e. less than the limit value for realization of window ventilation according to Swiss Standard SN 546382/1 (2014) (Palmes' tubes, measured at the front of the building near the schoolroom window). Delegates of the participating communes and schools were nominated, and an interdisciplinary technical support group was established, comprising experts in local administration from the areas of building, education and energy, and representatives of the Swiss Lung Association and the Federation of Swiss Teachers. Heads of schools and teachers from all classrooms voluntarily agreed to measurements for data acquisition for a

cross-sectional study on indoor air quality, without being fully aware of the impact of ventilation on measurement results. Teachers were asked to ignore measurement equipment and behave as usual.

Classrooms were analyzed for carbon dioxide (CO_2) concentrations using non-dispersive infrared (NDIR) sensors with 2-beam infrared measuring cell and data-logging on four consecutive school days (Almemo D6, digital carbon dioxide sensor FYAD 00 CO2B10, measuring range: 0 to 10,000 ppm, accuracy: $\pm$(100 ppm + 5% of measured value). The devices were calibrated before each series of measurements (2-point calibration with nitrogen (0 ppm) and CO_2 test gas (2700 ppm)) and checked for correct function. Measurements were made during the heating season if predicted outdoor temperatures were below 15 °C, minimizing the possibility of windows being fully open or tilted for prolonged periods. However, it was unavoidable that maximum outdoor temperatures occasionally exceeded 15 °C during a single week of measurements (for 5 classrooms, maximum outdoor temperature 17.4 °C). Data collection frequency was one data point every 2 min, and the data were visualized and processed with appropriate software (akrobit AMR WinControl 07/14). Periods with vacancy or with open windows and doors (air exchange) were identified using a mathematical algorithm ($dCO_2/10$ min > 20 ppm is considered to indicate occupancy) and by visually checking the shape of the CO_2 curve, together with the curves for temperature and relative humidity. During measurements, teachers recorded the number of persons in the classrooms. A single expert carried out all CO_2 measurements and determined the volumes of the classrooms and the numbers, dimensions and airtightness of windows. Windows containing rubber seals were defined as airtight, whereas those lacking rubber seals were termed leaky. Persons responsible for school buildings (caretakers, public building authorities) provided additional information concerning the type of building and year of construction, renovation events and type of ventilation. Buildings constructed in 2000 or later were defined as new buildings, since national building codes required airtight building envelopes by that date, as opposed to older buildings (up to and including 1999), for which a minimum air exchange rate through infiltration was required (Swiss Standard SN 520180, 1999; Swiss Standard SN 520180, 1970).

2.2. Intervention (S2) – determining factors

In our view, key prerequisites for a change in ventilation behavior in schools are increased awareness of the ventilation issue among the persons responsible and classes, and the possibility for those concerned to take appropriate decisions themselves. On this basis, we organized the intervention and developed aids to ensure that, in addition to verbal instructions, all teachers and students would receive the same information and guidance.

The general conditions below are applicable for the intervention:

(i) Ventilation essentially takes place in the empty classroom during breaks, as well as before and after a half school day for the following reasons: Ventilating during breaks takes comfort into account (cold outside temperatures in winter) and strict regularity is ensured by the ringing of the break bell. Ventilation before and after the half school day makes it possible not only to remove pollutants from indoor materials and earlier occupancy but also to start with good IAQ;

(ii) Ventilation is performed efficiently (all windows opened) not only to avoid unnecessary heat losses, but also because of the short break-time;

(iii) The hygienic limit value of 2000 ppm is complied with (CO_2 levels exceeding 2000 ppm for precisely 10% of teaching time per school day). The precise target allows ventilation plans to be reviewed. The modest target ensures that the requirements are not unrealistically high.

The long intervals between break-time ventilation events and the need to comply with the hygienic limit value mean that ventilation plans must provide for the best possible IAQ at the beginning of lessons. As a result, classes will spend a longer time with excellent CO_2 levels (400–1000 ppm range) than if, for example, with the same total ventilation time, short ventilation events took place when a CO_2 sensor indicated levels of e.g. 1400 or 2000 ppm.

The following aids were used:

a) **Illustrated flyer for school classes on the topic of ventilation**.

An explanation of the importance of good IAQ for performance, health and well-being is combined with a call for efforts to improve IAQ through appropriate ventilation. The flyer shows how to perform ventilation efficiently; mentions, as well as the duration of ventilation and the number of windows opened, the most important factors influencing ventilation, such as wind, outside temperatures, and window size and arrangement; and recommends use of the SIMARIA tool to determine an appropriate ventilation period for each break.

b) **Lesson on ventilation for school classes**.

The Bern University of Teacher Education and the Swiss Lung Association (a non-profit, non-governmental organization) developed a standardized school lesson to promote understanding of the need for ventilation. The lesson was adapted to different school levels. Assignments and experiments were used to illustrate the composition and quality of air. For example, the amount of oxygen available to a burning candle was limited by covering it with a glass. To impress upon younger students the fact that the quality of indoor air is not always satisfactory, they were sent outside to collect fresh air in paper bags.

c) **SIMARIA tool for school classes to generate ventilation plans and estimate CO_2 levels in the classroom**.

In classrooms with natural window ventilation and high occupancy, CO_2 levels rise sharply during lessons and decline sharply again during ventilation. Viewed over a number of lessons, a typical sawtooth pattern arises. The curve is actually non-linear and is influenced by numerous variables (Fig. 1). To raise students' awareness of the classroom ventilation issue in a straightforward and memorable way, we decided to represent the sawtooth curve in a heavily simplified manner (with a linear rise and fall) in a simulation tool, which can also be used to provide a rough estimate of the ventilation required.

To ensure that the tool was appropriate for students from third grade upwards, we focused on the visualization and considered only the most important constant input parameters (volume of classroom, number of persons, number of lessons per half day, number of lessons before the only long break of a half day, duration of planned ventilation during short and long breaks).

The simple equation used for the tool is as follows: the change of CO_2 level over t minutes is given by:

$$dCO_2(t) = \begin{cases} t \times rCO_2^l \times \dfrac{P}{V}, & during\ lesson, \\ \\ t \times rCO_2^v, & during\ ventilation, \end{cases} \quad (1)$$

where P denotes the number of persons in the classroom, V denotes the volume of the classroom, and rCO_2^l and rCO_2^v denote the calculated rates of change of CO_2 levels during lesson and ventilation, respectively. Given a point in time t_0, the CO_2 level at time $t_0 + t$ is:

$$CO_2(t_0 + t) = CO_2(t_0) + dCO_2(t). \quad (2)$$

The parameter rCO_2^l, as well as the CO_2 concentration at the start of school, was derived from highly reliable measurement data from the
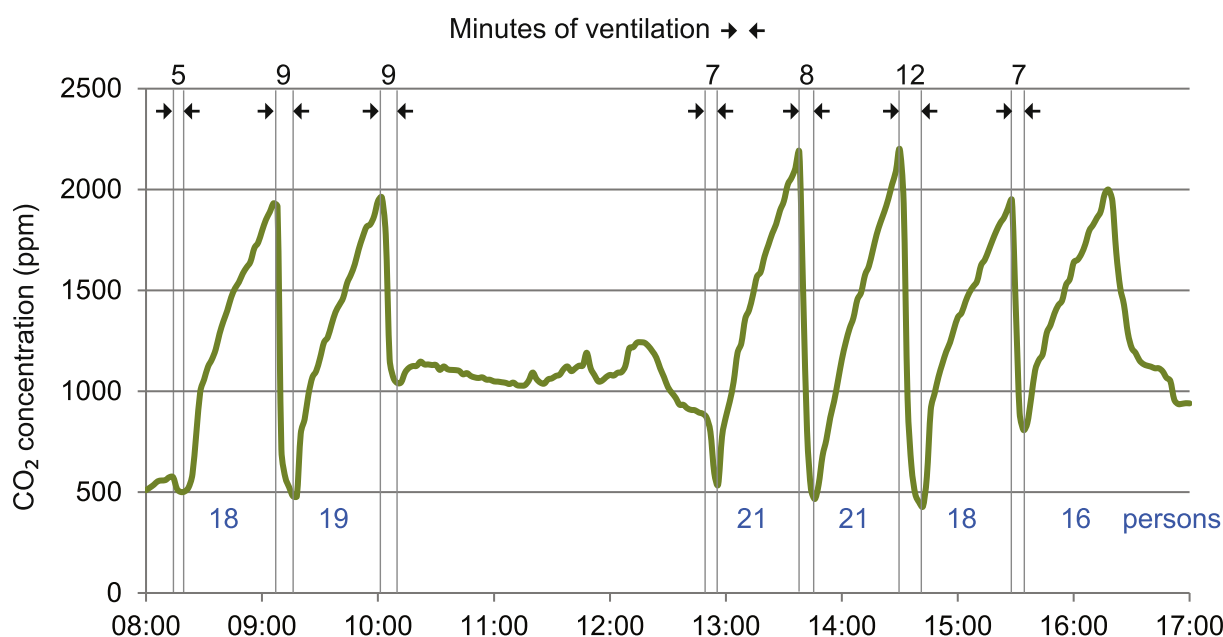
Fig. 1. Development of CO_2 levels in a classroom (volume: 169 m^3; occupancy: 16–21 persons) throughout the school day with strict manual window ventilation performed during breaks (arrows).

cross-sectional study (Table 1). This offered the advantage that all factors were taken into consideration. For periods without occupancy or ventilation (e.g. during midday break), the tool assumes constant CO_2 levels with no leakage (worst-case assumption). The simulation tool requires efficient ventilation as well as ventilation before the first lesson of the morning and the afternoon (i.e. during midday break). Use of the tool is restricted to typical classrooms; it cannot be used for rooms with markedly different dimensions, occupancy levels and ventilation conditions (e.g. apartments or offices).

The output chart shows in a simplified manner how CO_2 levels change in the course of an entire school day, based on the entries in the input screen. A second chart visualizes the effect of the inputs in terms of extrapolated air quality, i.e. the proportion of teaching time with good, satisfactory or unacceptable values (Fig. 2). By adjusting the duration of ventilation for the various breaks, the students can see the direct impact on CO_2 levels and can produce ventilation plans that meet their

requirements. The tool promotes an understanding of how key parameters influence CO_2 levels in the classroom. If, for example, the omission of a break-time ventilation is simulated, it can be seen that CO_2 levels for the half day concerned are too high as a result (Fig. 2).

d) **Written instructions for teaching staff**.

Teachers received the following written instructions for the intervention (measurement period):

(i) Doors and windows should be kept shut during lessons.
(ii) Ventilation should take place during breaks between lessons, as well as before and after a half school day.
(iii) The duration of ventilation is based on the ventilation plans, which are generated using the SIMARIA tool and are designed to achieve compliance with the hygienic limit value (CO_2 levels exceeding 2000 ppm for precisely 10% of teaching time per school day).
(iv) Students should stay outside the classroom during ventilation whenever possible.
(v) Ventilation should be performed efficiently, as illustrated in the flyer, by opening all windows wide and keeping doors shut in the case of single-sided ventilation; otherwise, by opening all windows wide in the classroom as well as the door and all windows on the other side of the corridor (cross-ventilation).

Table 1
Characteristics of rooms and parameters of the SIMARIA prototype.

Variable	SIMARIA (prototype)[a]	
	Median	Range
Openable window area (m^2)	5.8	1.2–13.8
Volume (m^3)	204.2	178.2–247.3
No. of persons	21.5	20–26
Temperature difference[b] (°C)	19.5	12.1–27.2
CO_2 at start of school[c] (ppm)	**554.0**	**386–986**
Rate of change of CO_2 during		
lesson (ppm/minute/person/m^3)	**1.1500**	**0.75–1.61**
ventilation[d] (ppm/minute)	**−100**	

[a] Data from 20 classrooms (16 airtight, 4 leaky) with single-sided spontaneous natural window ventilation, with high occupancy time (7 of 8 half school days during measurement) and high data reliability, i.e. reasonable consistency of CO_2 curves with teachers' occupancy records, as well as the absence of permanently open windows or doors.
[b] Indoor temperature minus outdoor temperature.
[c] CO_2 level before the first lesson in the morning, about 150 ppm above outdoor air levels.
[d] The CO_2 change during ventilation was arbitrarily set at −100 ppm/min since efficient ventilation was defined as a key prerequisite for the use of SIMARIA (Perino and Heiselberg, 2009).

2.3. Intervention study (S2) – practical implementation

Heads of schools and teachers from a quarter of the 100 classrooms participating in the cross-sectional study (S1) responded to our request to conduct an intervention study designed to improve CO_2 levels in the same classrooms as before by means of awareness-raising, ventilation instructions and ventilation plans. Nineteen of those who responded met the inclusion criteria, i.e. natural window ventilation, classes of 17 students or more, high attendance on at least 5 half-days during the 4-day measurement period, high motivation and voluntary participation of teachers. Three schools were in a rural setting, 13 in a suburban and 3 in an urban setting; one school had a distance of less than 20 m to a main road.
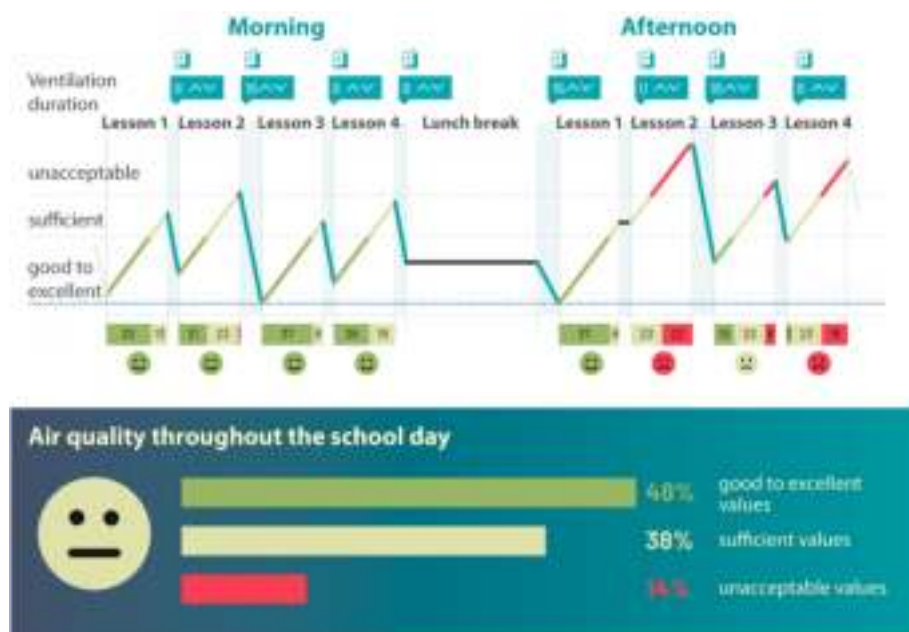
Fig. 2. Output chart of the SIMARIA tool, which is available in the Swiss national languages (French, German and Italian). The original designations in the printscreen have been overwritten with the English translation for this paper. For the morning (4 lessons on the left), adequate ventilation with levels <2000 ppm was simulated; for the afternoon (4 lessons on the right), the same ventilation periods were planned as for the morning, omitting the first ventilation after the midday break. Over the entire school day, the hygienic limit value is expected to be exceeded for 14% of teaching time. Simulation for 21 persons, 210 m^3 room volume.

The same classrooms with the previous measurements in the cross-sectional study of spontaneous window ventilation (S1) served as the control group.

Four additional classrooms in the city of Zurich were added to the intervention group for testing and validation of the SIMARIA prototype tool because of high occupancy (mean of 25.3 students, compared to 20.0 in the original group of 19 classrooms without classes from Zurich). Measurements of CO_2 concentrations, temperature and relative humidity were carried out as described above.

Before the measurement week, one of the two (either French- or German-speaking) assistants from the Swiss Lung Association visited the teachers of the pilot classes to provide personal and written instructions (including the flyer for all students). The assistant introduced teachers to the SIMARIA prototype tool and showed them how to use it to generate a ventilation plan that would result in acceptable CO_2 levels each day (exceeding 2000 ppm for precisely 10% of teaching time). Teachers set up a schedule, either alone or together with their classes that would meet the requirements. As the SIMARIA prototype did not yet have a user-friendly interface, the assistant offered support where needed. Next, the assistant gave pilot classes the standardized school lesson to promote understanding of the need for ventilation. Before the measurements began, the classes had approximately 2–3 weeks to familiarize themselves with the topic and the instructions for the intervention.

Shortly before the measurement period, teachers were reminded to ventilate their classrooms in strict accordance with the improvement procedures on the four consecutive school days, and to keep accurate records using a prepared form.

To assess the outcome of the intervention, only measurements carried out in the same classrooms as in the cross-sectional study were used (i.e. the S2 intervention group, n = 19, excluding the Zurich classes). Along with the teachers' records, CO_2 measurement data (i.e. CO_2 curves) from the intervention group were visually checked for deviations from the planned ventilation behavior (e.g. duration of ventilation events regularly too short or too long, missing ventilation, evidence of open doors or windows during lessons). Pilot classes that had followed the plan closely throughout the 4-day measurement period were assigned to a subgroup (intervention group with good compliance, n = 10).

3. Results

3.1. CO_2 exposure in Swiss schools (cross-sectional study S1)

Table 2 summarizes the CO_2 measurement data for 100 classrooms in 96 public school buildings (including addition of stories), together with the corresponding building characteristics. Of the 100 classrooms, 6 were mechanically ventilated (2 new buildings constructed in 2000 or later and 4 old & tight (retrofitted) buildings). Median CO_2 values for the mechanically ventilated classrooms ranged from 686 to 1320 ppm (median 1009 ppm) during lessons over 4 days. In 3 of the 6 classrooms, CO_2 levels consistently remained below 1400 ppm, thus meeting national technical standards (Swiss Standard SN 546382/1, 2014); in the other 3 classrooms, 1400 ppm was exceeded for 11%, 43% and 47% of teaching time and, in two cases, even the 2000 ppm level was exceeded, for 5% and 7% of teaching time.

In comparison, median CO_2 levels in the 94 naturally ventilated classrooms were about 520 ppm higher (median 1533 ppm, range 862–2898 ppm). Ventilation of these rooms was performed exclusively by opening windows manually; there were no additional installations, such as ventilation grids, which could provide continuous background ventilation. In 2 of these 94 classrooms, CO_2 levels were consistently below 1400 ppm during the 4–day measurement period; one of these had difficult conditions with regard to occupancy (23 students/160 m^3). In Switzerland, the Federal Office of Public Health considers CO_2 levels to be acceptable for existing school buildings with natural window ventilation if they are <2000 ppm (hygienic limit value) for more than 90% of teaching time. This was the case for 29 (31%) of the 94 classrooms in the study group. Thus, CO_2 levels would not be considered acceptable in about two thirds (69%) of the classrooms with natural window ventilation.

We analyzed exposure to different CO_2 levels (<1000, 1000–1400, 1400–2000 and > 2000 ppm) as a proportion of teaching time (Fig. 3). Poor IAQ as indicated by high CO_2 levels has a known impact on performance and health. As shown in Fig. 3, of the 94 classes, 41 (44%) were exposed to CO_2 levels >2000 ppm for at least 20% of teaching time, 28 (30%) for at least 30%, 18 (19%) for at least 40%, and 9 (10%) for at least 50%. One class was exposed to CO_2 levels >2000 ppm for 92% of the entire 4 days' teaching time. Given this situation, it is

Table 2
Building characteristics and CO_2 levels in the cross-sectional study (S1).

Type of ventilation	Type of building	Number of classrooms	Year built		Room volume (m³)		Openable window area (m²)	
			Median	Range	Median	Range	Median	Range
All	All	100	1966	1812–2010	204.2	138.9–572.2	6.1	1.2–42.6
Mechanical[a]	All	6	1985	1914–2010	228.2	204.0–273.4	5.2	2.0–42.6
	New[b]	2	2010	2009–2010	228.2	219.5–237.0	4.4	2.0–6.8
	Old & tight[c]	4	1977	1914–1988	240.3	204.0–273.4	5.2	3.1–42.6
Natural window	All	94	1965	1812–2008	202.1	138.9–572.2	6.1	1.2–16.5
	New	8	2004	2001–2008	216.2	189.8–269.4	6.6	4.6–13.8
	Old & tight	63	1967	1826–1999	202.0	138.9–572.2	5.9	1.2–16.5
	Old & leaky[d]	23	1951	1812–1976	197.4	141.1–250.0	6.2	2.5–11.8

Type of ventilation	Number of persons (P)		Volume per person (m³/P)		Indoor temperature (°C)		Outdoor temperature (°C)		Temp. diff. (°C)
	Median	Range	Median	Range	Median	Range	Median	Range	Median
All	20	3–26	10.5	6.6–80.0	22.0	18.6–24.8	3.1	−3.1 to 9.4	18.7
Mechanical	20	15–22	11.0	10.0–16.6	23.6	21.4–24.1	0.9	−3.1 to 5.6	22.7
Natural window	20	3–26	10.5	6.6–80.0	22.0	18.6–24.8	3.9	−3.1 to 9.4	18.5

Type of ventilation	Type of building	Median[e] CO_2 levels (ppm)		Maximum CO_2 levels (ppm)		Minimum CO_2 levels (ppm)		Overall CO_2 levels (ppm)	
		Median	Range	Median	Range	Median	Range	Minimum	Maximum
All	All	1496	686–2898	2707	1025–4853	492	401–1448	401	4853
Mechanical	All	1009	686–1320	1364	1025–2247	466	436–495	436	2247
Natural window	All	1533	862–2898	2754	1282–4853	493	401–1448	401	4853
	New	1757	1012–2115	3406	1962–4666	534	415–614	703	4001
	Old & tight	1556	862–2898	2768	1282–4853	490	401–1448	542	3836
	Old & leaky	1431	879–2311	2669	1509–4299	499	412–634	412	4299

Type of ventilation	Type of building	Percentage of teaching time[f] with CO_2 levels (ppm) at:							
		<1000		1000–1400		1400–2000		>2000	
		Mean[g]	Range	Mean	Range	Mean	Range	Mean	Range
All	All	25	0–99	25	0–58	28	0–55	21	0–93
Mechanical	All	59	22–99	24	1–58	15	0–40	2	0–7
Natural window	All	23	0–81	25	0–50	29	0–55	23	0–93
	New	23	4–57	21	10–35	25	8–44	31	0–50
	Old & tight	22	0–81	24	0–50	30	0–55	23	0–93
	Old & leaky	26	3–73	29	11–50	28	2–45	17	0–63

[a] Windows could also be opened.
[b] Building constructed in 2000 or later.
[c] Building constructed before 2000, windows with rubber seals.
[d] Building constructed before 2000, windows without rubber seals.
[e] Median of average CO_2 levels during lessons on four consecutive school days.
[f] Total teaching time over four consecutive school days = 100%.
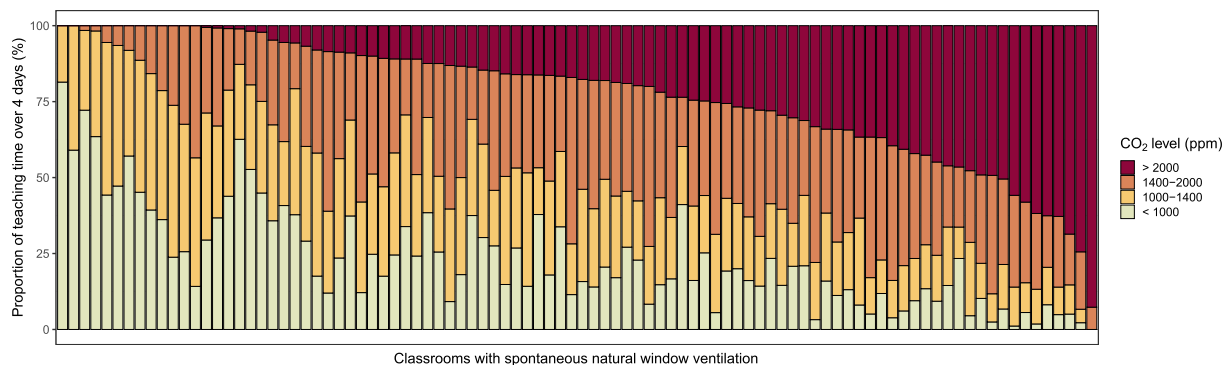[g] Mean of percentage of teaching time for all classrooms.

Fig. 3. Exposure to different CO_2 levels in 94 classrooms with spontaneous natural window ventilation, measured over 4 days in the winter season (cross-sectional study S1).

important for schools to be able to implement improved ventilation practices immediately. Therefore, the impact on CO_2 levels of ventilation behavior guided by instructions and specific ventilation plans was investigated in pilot classes (intervention study).

3.2. Intervention measures as compared with spontaneous natural window ventilation in the same rooms (S2)

3.2.1. CO_2 exposure in the intervention and control groups

Intervention measures took place in 23 classrooms (18 airtight, 5 leaky, including the large Zurich classes) during wintertime, as described in Section 2.3. Although the ventilation objective, involving

compliance with the hygienic limit value of 2000 ppm CO_2, was modest, the intervention was very successful (Fig. 4). Reduction of CO_2 levels and thus exposure to human bioeffluents was highly significant and not restricted to those schools closely following instructions throughout the 4-day measurement period (good compliance); rather, it was observed for the entire intervention group (Wilcoxon signed-rank test for positive differences: p = $3.815e^{-06}$ for all classrooms participating in both the cross-sectional study S1 and the intervention study S2, and p = 0.001953 for the subgroup with good compliance). As shown in Fig. 4, median CO_2 levels dropped from 1600 to 1097 ppm (1068 ppm for the subgroup with good compliance). Moreover, a CO_2 decrease from 1418 to 933 (993) ppm was observed for the 1st quartile, from 2023 to 1346 (1318) ppm for the 3rd quartile, and from 2898 to 1892 (1381) ppm for the median of maxima. In 16 of 19 classrooms, median CO_2 levels decreased by up to 1000 ppm over the 4-day measurement period. In one case, the decline in median CO_2 levels amounted to almost 2000 ppm.

Fig. 5 shows how the intervention and control groups differ in terms of CO_2 categories during teaching time. The average proportion of teaching time at <1000 ppm CO_2 changed from 18% to 42%, at 1000–1400 ppm from 22% to 28%, at 1400–2000 ppm from 29% to 21%, and at >2000 ppm from 31% to 9%. During the intervention, classes spent nearly 70% of time at CO_2 concentrations of 400–1400 ppm and only about 10% at >2000 ppm, as compared to about 40% and 30% in the control group. The intervention goal of improvement in ventilation, with long periods of exposure to low CO_2 levels and compliance with the hygienic limit value during each school day, was thus, on average, achieved. This applies in particular to the classes showing good compliance (Fig. 5).

Consecutive lessons are most challenging, as many lessons start with increased CO_2 levels from the preceding occupancy. In both the control and the intervention group, we used all available 4-lesson sequences over the 4-day measurement period to study the effect of the intervention on consecutive lessons. During the first lessons in the morning, mean CO_2 levels did not differ much between groups, benefiting from low minima of 643 ppm (S1, control), 561 ppm (S2, intervention) and 570 ppm (S2, intervention with good compliance) before the start of lessons. Pronounced differences between the control group and the S2 intervention groups arose in subsequent lessons (Figs. 6 and 7). During lessons two, three and four combined, the proportion of teaching time spent at CO_2 levels below 1400 ppm was 26% in the control group, 60% in the intervention group, and 56% in the intervention group with good compliance (Fig. 7). The 4th lesson – the most challenging – started on average at minima of 1584 ppm (S1), 899 ppm (S2) and 974 ppm (S2 with good compliance) and reached mean maximum values of 2433 ppm (S1), 1960 ppm (S2) and 2131 ppm (S2 with good compliance), with

2000 ppm being exceeded for 43.5% (S1), 17.8% (S2) and 24.1% (S2 with good compliance) of teaching time. Overall, intervention measures counteracted the tendency for CO_2 levels to increase with the number of consecutive lessons, leading to extended times with better IAQ and more balanced CO_2 levels between lessons, which is beneficial to health and performance. However, the exceedances of 2000 ppm accumulated in the 4th lesson. We therefore modified the interface of the SIMARIA tool so that the duration of all school lessons and breaks could be customized individually and independently of each other. Classes can thus improve IAQ during the critical 4th lesson by increasing the ventilation period that precedes it.

3.2.2. Duration and efficacy of natural window ventilation in the intervention and control groups

As shown above, differences in CO_2 levels between the control and intervention groups developed after the first lesson. The mean duration of ventilation events before lessons two, three and four was only about 2 min higher in the intervention groups (S2: 34.7 min, S2 good compliance: 35.0 min) than in the control group with spontaneous ventilation (S1: 32.8 min). Fig. 8 demonstrates, however, that ventilation efficiency was significantly higher. The mean change was −111 ppm CO_2/minute in the intervention group, compared to −72.3 ppm CO_2/minute in the control group.

For the final version of SIMARIA, the rate of change of CO_2 was adjusted, based on the intervention study data (all median data): -110 instead of −100 ppm/min during ventilation, and 1.28 instead of 1.15 ppm/min/person/m³ during lesson, as the intervention study was carried out under controlled conditions and efficient ventilation is a prerequisite for use of the tool.

4. Discussion

4.1. Cross-sectional study (S1)

Two thirds of classrooms did not meet the minimum requirements for CO_2, which is clearly not acceptable from a public health point of view. Most of these classrooms, however, had natural window ventilation. While renovations or the construction of new school buildings provide opportunities to ensure sustained improvements in ventilation by installing mechanical ventilation systems, these opportunities were rarely taken (in only 2 of 10 new buildings and 4 of 63 retrofitted old and airtight buildings) and where this was the case, the standards were not always adequately implemented (deviation from the Swiss Standard SN 546382/1 (2014) for 3/6 mechanical ventilation systems). Clearly, there is a considerable need for action in the implementation of building codes
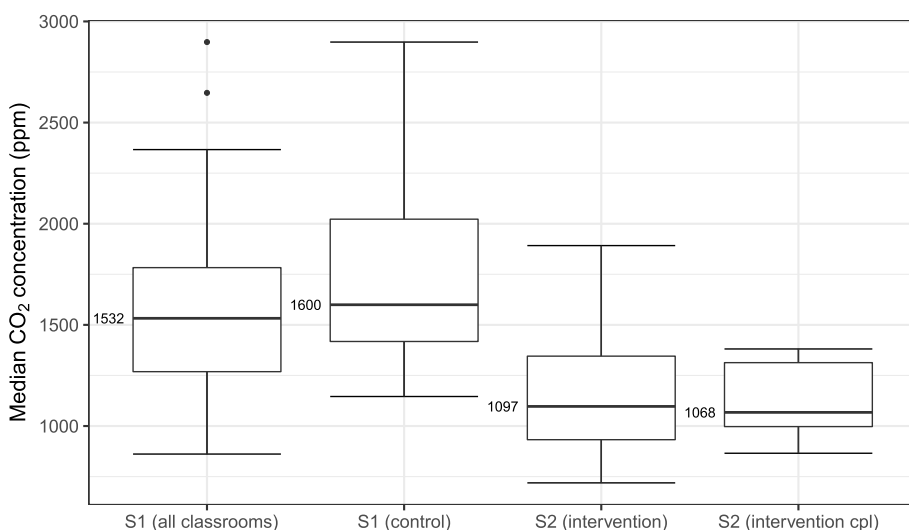

Fig. 4. Median CO_2 levels in classrooms with spontaneous natural window ventilation (S1) and with intervention measures (S2). Measurements for four days in: *S1 (all classrooms)*: entire cross-sectional study group (n = 94); *S1 (control)*: same classrooms as in intervention, measured during cross-sectional study (n = 19); *S2 (intervention)*: intervention group (n = 19); *S2 (intervention cpl)*: subgroup of intervention group, with good compliance (n = 10). Box plots with maximum, 3rd quartile, median, 1st quartile, minimum.
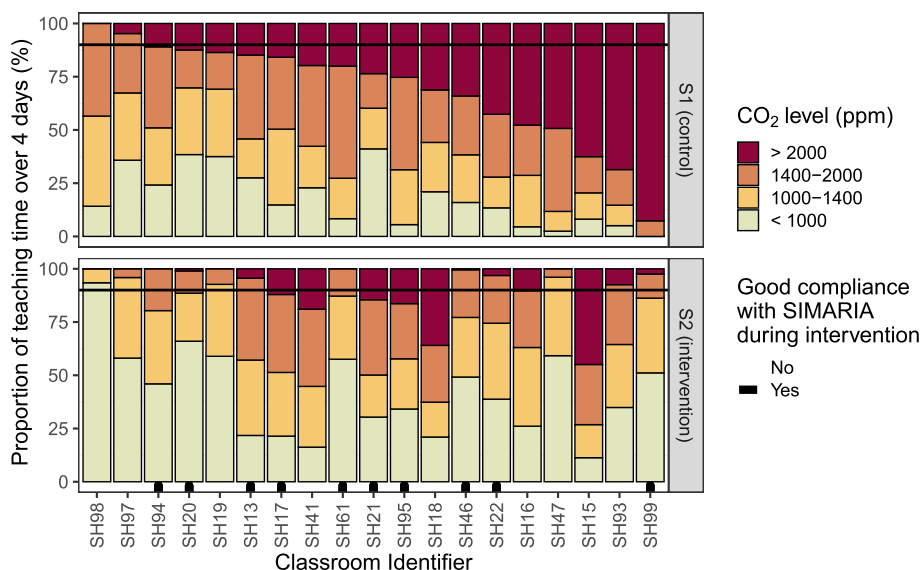
Fig. 5. Exposure to different CO_2 levels in the same classrooms with either spontaneous natural window ventilation (top: *S1, control*) or planned ventilation (bottom: *S2, intervention*), measured over 4 days. The line marks 10% of teaching time, i.e. the proportion specified for compliance with the hygienic limit value of 2000 ppm CO_2.
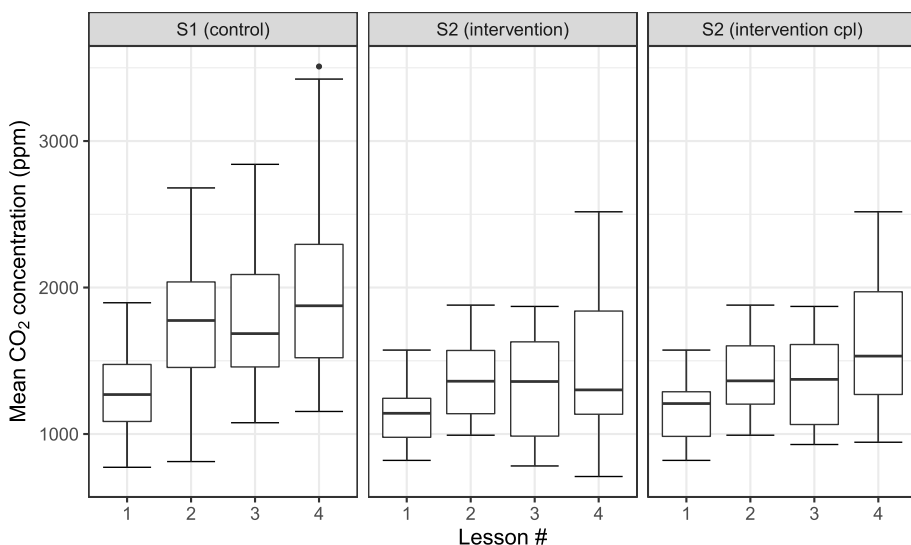

Fig. 6. Effect of intervention on CO_2 levels in consecutive lessons. Mean CO_2 levels are shown for all 4-lesson sequences occurring in the 4-day measurement period in classrooms with natural window ventilation for: *S1 (control)*, rooms with spontaneous window ventilation (30 sequences from 20 classrooms); *S2 (intervention)*, intervention group (20 sequences from 13 classrooms); and *S2 (intervention cpl)*, subgroup of the intervention group with good compliance (11 sequences from 7 classrooms).

and ventilation standards.

In schools with natural window ventilation, CO_2 levels vary widely; overall, values ranged from 401 to 4853 ppm in our study. Key drivers were occupancy and active ventilation behavior during lessons (Fig. 9), as well as the efficiency of window ventilation (Fig. 8). Other factors that influence ventilation efficiency besides fully opening all windows were not studied or appeared to be less relevant: for example, average outdoor temperatures during the school day had only a minor effect on CO_2 levels, because the ventilation behavior was probably adapted to the outside temperatures (Fig. 10, supplementary material). Likewise, background ventilation by natural infiltration through leaky building envelopes – especially unsealed windows and doors – could have influenced results. However, the number of new buildings was small and differences were not significant (Fig. 11, supplementary material)).

In the school setting, additional factors may influence CO_2 levels strongly. For example, as we have shown, consecutive classes make it difficult to ensure long enough ventilation during breaks, so that the load of stale air including CO_2 from preceding occupancy increases (cf. Section 3.2.1). In contrast, frequent teaching in split classes, temporarily

empty classrooms (e.g. when lessons take place in music rooms or sports facilities), and open doors (with classes present or not) favor lower CO_2 values at the start of the following lessons. Discussions with teachers, as well as measurement data and teachers' occupancy records, indicated that CO_2 levels during the cross-sectional study were affected by all of these factors. Such factors could have a great influence on results but are not generally discussed in studies. CO_2 exposure in the cross-sectional study (>1000 ppm during 77% of teaching time in schools with spontaneous window ventilation) is broadly comparable to that reported from studies in Germany (with similarly cold winters) (Ausschuss für Innenraumrichtwerte AIR, 2008; Fromme et al., 2008; Lahrz et al., 2003). Similar median CO_2 concentrations were also reported in other studies (1412–1600 ppm vs. 1533 ppm in our study) (Fromme et al., 2008; Grams et al., 2003; Innenraumlufthygiene-Kommission des Umweltbundesamtes, 2008; Lahrz et al., 2003). However, in a Dutch study, CO_2 exposure was significantly lower than in our cross-sectional study (CO_2 values > 1000 ppm for 64% of teaching time), despite higher occupancy and very high maximum CO_2 values (up to 3500 ppm) in the Dutch study, which could be attributed to the above-mentioned factors
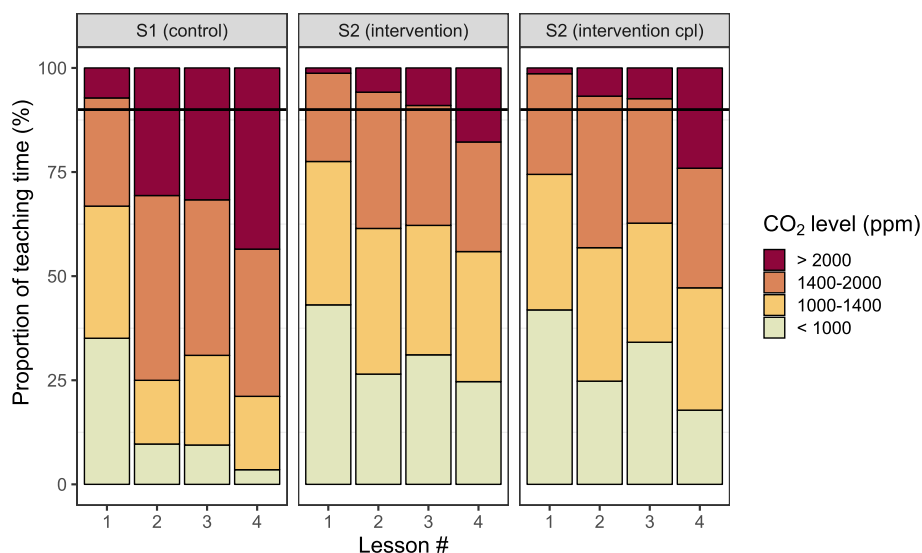
Fig. 7. Effect of intervention on CO_2 exposure in individual lessons. Exposure to different CO_2 levels is shown for all 4-lesson sequences occurring over the 4-day measurement period in classrooms with natural window ventilation for: *S1 (control),* rooms with spontaneous window ventilation (30 sequences from 20 classrooms); *S2 (intervention)*, intervention group (20 sequences from 13 classrooms); and *S2 (intervention cpl)*, subgroup of the intervention group with good compliance (11 sequences from 7 classrooms). The line marks 10% of teaching time, i.e. the proportion specified for compliance with the hygienic limit value of 2000 ppm CO_2.
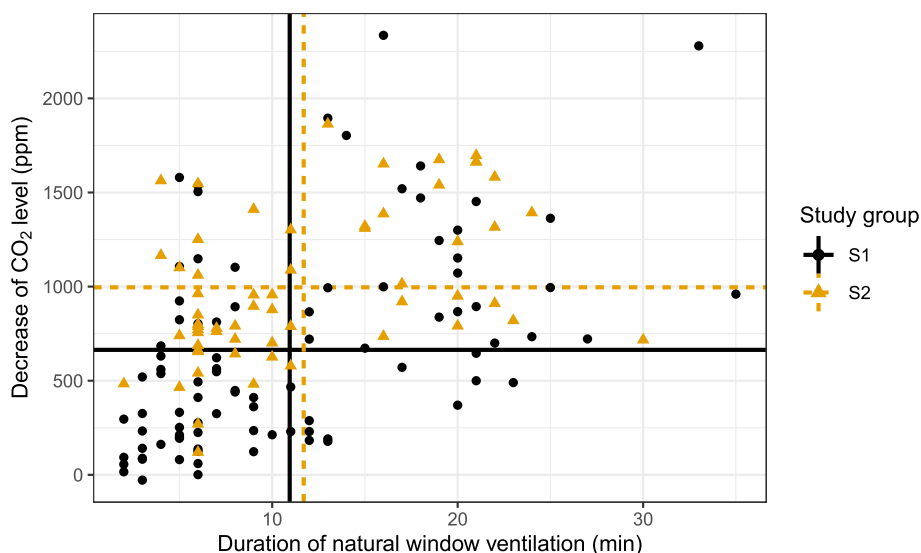
Fig. 8. Decrease in CO_2 levels during window ventilation in the same classrooms: S1: control group with spontaneous window ventilation, S2: intervention group. The average duration and average CO_2 decrease across all ventilation events is shown for the control (solid lines) and the intervention group (dashed lines).

or higher outdoor temperatures (Geelen et al., 2008).

4.2. Intervention study S2

At the core of the intervention study were the following measures: efficient ventilation, low CO_2 levels at the beginning of each morning and afternoon, ventilation performed exclusively during breaks, and compliance with the hygienic limit value of 2000 ppm. As a result of the intervention, the proportion of teaching time that classes spent at low CO_2 levels (400–1400 ppm) increased from 40% to 70%. This improvement was attributable solely to changes in ventilation behavior, with almost no change in the duration of ventilation compared to spontaneous ventilation (Fig. 8). The altered ventilation behavior was thus substantially more effective, with similar heat losses, because the overall duration of ventilation events and room temperatures were similar for the intervention and control groups (n = 19: median 22.4 °C, range 20.1–24.6 °C, versus 22.1 °C, 19.5–23.7 °C), and there was no increase in low room temperatures (10th and 5th percentiles of 20.4 °C and 20.2 °C for the intervention and 20.5 °C and 19.9 °C for the control group). The SIMARIA tool was one of a number of helpful measures,

contributing – together with efficient ventilation due to the opening of all windows – explicitly to resource-conserving ventilation planning, since SIMARIA enables classes to plan the duration of ventilation during breaks based on the resulting overall development of CO_2 levels during the day. This means that, spontaneously, ventilation periods are planned that are no longer than is required for a complete air exchange and to ensure that the defined ventilation objective is achieved (strategic ventilation).

Overall, the question arises as to how accurate such a tool needs to be: in other words, how accurately should it reflect the situation on a particular day in a particular classroom? Precise modeling of changes in CO_2 concentrations with natural ventilation is complex and, for simplification, assumptions need to be made for numerous input parameters. At the same time, parameters such as wind pressure and temperature difference are in fact highly variable and have a considerable influence on the results (Larsen and Heiselberg, 2008). In the highly simplified approach adopted for the SIMARIA tool, we estimated the increase and decrease in CO_2 levels with the aid of average values measured during the heating season. The only remaining input parameters were room volume, occupancy and information on the duration of
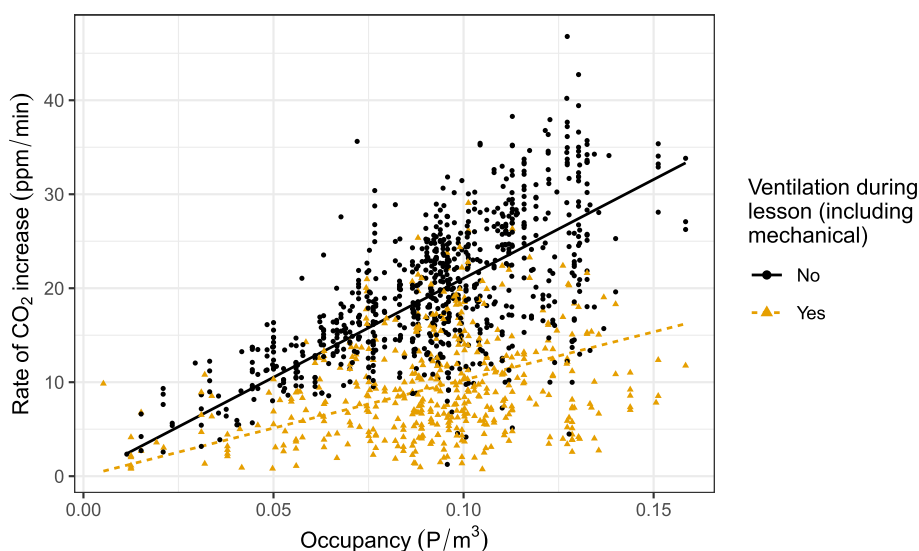
Fig. 9. Rate of change of CO_2 levels versus classroom occupancy, as modified by ventilation during lessons. All lessons are shown for the 100 classrooms over the 4-day measurement period in the cross-sectional study group (S1). Ventilation during lessons includes natural window (n = 94) and mechanical ventilation (n = 6).

lessons and ventilation breaks. Since the target group in this case comprised students from the third grade upwards, we regarded this approach as adequate for awareness-raising and ventilation planning. In particular cases, marked deviations from the actual values measured are to be expected, which seemed acceptable, given the fact that ventilation plans in everyday school life can never be implemented exactly. In fact, the intervention study showed that, on average, the CO_2 levels aimed for with the ventilation plans were achieved, and that consequently, realistic planning of ventilation periods during the heating season in everyday school life is possible with SIMARIA.

One might also ask to what extent the comparisons of S1 and S2 are legitimate. Situations favoring a reduced CO_2 increase during lessons in intervention-group classes, or driving a CO_2 decrease during ventilation, would lead to an overestimation of the effectiveness of intervention measures. Despite a slightly higher number of students in the control group with spontaneous ventilation (mean and median both 21, versus both 20 for the intervention group), the median rate of CO_2 increase during the 4-day measurement period was clearly higher in the intervention group (1.31 versus 0.83 ppm/min/person/m³, same rooms, control), indicating greater difficulty in achieving low CO_2 concentrations. Outliers for the rate of decrease during ventilation, which could have been due to stormy weather, were not identified (Tukey box plot analysis with whiskers of 1.5 times the interquartile range). Besides wind, differences between indoor and outdoor temperatures are the factor most likely to distort results. Again, smaller differences in temperature during ventilation (median difference between indoor and outdoor 16.6 °C for S2 versus 21.6 °C for S1) and correspondingly more difficult conditions were associated with the intervention group S2, supporting the effectiveness of the intervention.

Comparison with the very few other intervention studies is only possible to a limited extent: the authors tested improvement instruments such as awareness-raising and CO_2 sensors with the aim of achieving much lower maximum CO_2 values (e.g. 1200 ppm), in contrast to our study, in which the maximum value of 2000 ppm CO_2 was also the target value during 10% of teaching time (Geelen et al., 2008; Wargocki and Da Silva, 2015). Geelen et al. (2008) extended teaching times at CO_2 levels <1000 ppm, as we did, by about 25%, but starting from a significantly better level (36% versus 18%). Despite our modest ventilation objectives, we achieved a good median value of about 1000 ppm CO_2.

The results of the intervention study demonstrate that most of the

pilot classes were highly motivated to implement the ventilation plans as precisely as possible. This good outcome is probably due to the fact that, in carrying out the study, we attached great importance to awareness-raising and the schools' engagement with the topic (communication of cross-sectional study, individual discussions with teachers, school lesson, flyer, SIMARIA tool), as recommended by Geelen et al. (2008). Maintaining such a high level of compliance over many months is an immense challenge and requires a long-term change in behavior. The prerequisite for this is a high level of acceptance of the measures. It is important that they should be both highly flexible and readily manageable; for this reason, provision is made for the generation of individual ventilation plans and objectives. A more ambitious ventilation objective than that underlying the intervention could also be planned, as could ventilation events during lessons. The latter would need to be considered in the prevention of aerosol transmissions of, for example, SARS-CoV 2 viruses.

In addition to flexibility, comfort also increases acceptance. The school classes must be able to leave the classroom during ventilation in cold weather. This was not always ensured in the intervention study. While we had established the "strong positive intentions or commitment" and the "necessary skills" which are prerequisites for behavioral change, this organizational obstacle would need to be overcome by all schools before the proposed ventilation behavior can be permanently implemented (Geelen et al., 2008; Gielen and Sleet, 2003). Furthermore, behavioral change could be accompanied by occasional control measurements with a CO_2 sensor over longer periods, for two reasons: (i) to estimate how good the prediction for a specific classroom is and whether the duration of ventilation events could be adjusted on very cold days, and (ii) to ensure that the ventilation situation is kept in mind. Grimsrud et al. (2006) showed that displayed, continuous CO_2 monitoring triggered long-lasting improvement. In spite of the promising results achieved, efforts to improve window ventilation will reach their limits in classrooms with high occupancy. This should trigger desperately needed discussions with building owners about the ventilation problem in schools.

5. Conclusions

Our study shows that spontaneous natural window ventilation in countries with cold winters leads to particularly high CO_2 levels. This is the case even though, in this study, good IAQ was important to teaching

staff, and ventilation was generally carried out on a regular basis. With the approach to classroom ventilation presented here, awareness-raising is of central importance and ventilation is performed exclusively during breaks, using flexible ventilation plans. Although the ventilation objective was modest, involving compliance with the hygienic limit value of 2000 ppm CO_2, it was possible to improve CO_2 concentrations markedly in all lessons. This was possible without increasing the overall duration of ventilation compared to spontaneous window ventilation, which speaks for its practicality. A regime with fixed breaks for ventilation events allows students to leave the classroom during ventilation. In countries with cold winters, ventilation will be inadequate unless protection is provided against discomfort from cold and drafts. The intervention described here presumes that the school buildings are in areas with good outdoor air quality. Moreover, it is only suitable as a transitional solution, as it requires sustained efforts and significantly more ambitious goals are difficult to achieve. For long-term solutions, appropriate construction and technical measures are required. Since the ventilation concept can be integrated into everyday school life and is aimed at long stays with low CO_2 levels, it might be helpful during the COVID-19 pandemic to reduce aerosol transmission of SARS-CoV-2 viruses whenever possible in conjunction with intermediate ventilation during class and further measures such as wearing masks.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113746.

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HBM4EU chromates study - Reflection and lessons learnt from designing and undertaking a collaborative European biomonitoring study on occupational exposure to hexavalent chromium

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ABSTRACT

The EU human biomonitoring initiative, HBM4EU, aims to co-ordinate and advance human biomonitoring (HBM) across Europe. As part of HBM4EU, we presented a protocol for a multicentre study to characterize occupational exposure to hexavalent chromium (Cr(VI)) in nine European countries (HBM4EU chromates study). This study intended to collect data on current occupational exposure and to test new indicators for chromium (Cr) biomonitoring (Cr(VI) in exhaled breath condensate and Cr in red blood cells), in addition to traditional urinary total Cr analyses. Also, data from occupational hygiene samples and biomarkers of early biological effects, including genetic and epigenetic effects, was obtained, complementing the biomonitoring information. Data collection and analysis was completed, with the project findings being made separately available. As HBM4EU prepares to embark on further European wide biomonitoring studies, we considered it important to reflect on the experiences gained through our harmonised approach. Several practical aspects are highlighted for improvement in future studies, e.g., more thorough/earlier training on the implementation of standard operating procedures for field researchers, training on the use of the data entry template, as well as improved company communications. The HBM4EU chromates study team considered that the study had successfully demonstrated the feasibility of conducting a harmonised multicentre investigation able to achieve the research aims and objectives. This was largely attributable to the engaged multidisciplinary network, committed to deliver clearly

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understood goals. Such networks take time and investment to develop, but are priceless in terms of their ability to deliver and facilitate knowledge sharing and collaboration.

1. Introduction

By revealing internal exposure and early effects in the human body, human biomonitoring (HBM) can provide an invaluable contribution for public health decision-making based on the risk assessment of chemicals. HBM has been considered as a relevant approach for the health risk management, e.g. under EU REACH regulation (Boogaard et al., 2011) and provides relevant information to support policy development by delivering better evidence of workers' exposure to chemical substances in the scope of different regulatory frameworks (Viegas et al., 2020; Jones, 2020). Both the general population (exposed through environment) and workers (occupationally exposed), can benefit from the use of data generated by HBM studies, but further harmonisation and standardization of methodologies is necessary to improve and refine such assessments (Louro et al., 2019).

The EU human biomonitoring initiative (HBM4EU) is a Joint Programme that aims to standardise and use biomonitoring to understand human exposure to chemicals (via the environment, in occupational settings or through using consumer products) and the related health risks, with the aim to improve chemical risk assessment and management as well as to support policymaking (Ganzleben et al., 2017). HBM4EU is a joint effort of 30 countries, the European Environment Agency and the European Commission, co-funded under Horizon 2020 (www.hbm4eu.eu).

Occupational health has a long tradition of using HBM to control worker's exposure. The use of HBM in occupational settings can serve as a model system to implement and improve HBM approaches that can be expanded to environmental exposure in the general population. More research in best practices and procedures for occupational HBM was to be addressed within the remit of the HBM4EU project as various challenges still present themselves. Typically, a limited number of workers can be, and are, recruited into national studies. Furthermore, the studies performed by different researchers in individual countries are usually not aligned with respect to sampling/analytical methodologies or the collection of contextual data, which complicates the comparison of the findings and use of the data in regulatory risk assessment at a European or international level. Combining national surveys using harmonised study designs, methodologies and protocols can potentially greatly improve the information collected and provide the benefit of harmonised data collected in different countries, as demonstrated in the earlier DEMOCOPHES project (Den Hond et al., 2015).

One of the most important aims of the overall HBM4EU project is the harmonisation of methodologies and standardized collection of the data useful for EU decision making. In line with this aim, we previously presented a multicentre study that intended to characterize occupational exposure to hexavalent chromium (Cr(VI)) in industrial settings across Europe (Santonen et al., 2019). Examples of industrial activities that are sources of workers exposure comprise welding, electroplating, surfaces treatment and leather tanning (Elhosary et al., 2014; Lin et al., 2018; Pan et al., 2018; Wang et al., 2012). The main exposure routes are dermal contact, inhalation of dust and mist or fumes, and ingestion due to hand-mouth contact. Despite its carcinogenicity, the use of Cr(VI) compounds (chromates, chromium trioxide and dichromium tris(chromate)) for specific purposes is still authorized under the European regulation (EC, 1907/2006) concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and thereby still raises concern. The main adverse health outcomes due to chronic Cr(VI) inhalation are lung impairment, including pneumonia, bronchitis, asthma and lung cancer (IARC, 2012). This study also aimed to investigate the relationship between biomarkers of exposure, environmental monitoring and biomarkers of effect and to recognise how all these tools

add to occupational exposure, risk assessment and management processes. Although a number of studies has reported data on human exposure to Cr(VI), comparably fewer have included effect biomarkers analysis. As Cr(VI) is a direct and indirect (through reactive oxygen species production) genotoxic metal (Chen et al., 2019), the effect biomarkers included were associated with oxidative stress and DNA or chromosome damage (Li et al., 2014; Pan et al., 2018). In addition, following innovative methodologies development, omics-based biomarkers were also analysed. Furthermore, it is expected that the data generated may be used to address questions, for example, related to the efficacy of the regulatory measures already implemented, as well as allowing for the identification for further actions. In this contribution, we reflect on the experiences gained from undertaking this study so that lessons can be learnt and shared with the wider scientific community and can be considered when developing future occupational multicentre studies.

2. Materials and methods

The HBM4EU chromates study team participated in a number of activities to design, harmonise and undertake this multicentre study, the key ones of which are summarized in Fig. 1. It is important to highlight that whilst presented as a workflow, the events were not always sequential, with several of the activities occurring in parallel (e.g. company identification and company/worker recruitment and sample and data collection; data entry and cleaning and sample analysis). It is also essential to highlight the several overarching activities that took place across the different elements of the work program. For example, there was extensive quality assurance and quality control round robins for the biological sample analysis aspects. The HBM4EU chromates study core network expanded during the course of the study to include further researchers to collect the field samples and contextual data, additional scientists to process and analyse the collected samples, to analyse cytogenetic and molecular effect biomarkers, etc. Finally, there were extensive communications within the project team via web conferences, email correspondence to discuss the work programme as it progressed and evolved.

It was important for the HBM4EU chromates study project team to reflect on the research and evaluate both the positives and limitations of the harmonised approach that was developed and applied. Following completion of the data gathering and processing elements of the work, members of the project team were asked, via email, to provide feedback on three things that went well with the study and three things that were felt could be improved upon. The responses provided were used to facilitate an open discussion during a session at the 4th HBM4EU training school, May 14, 2020. Here, participants were able to identify any additional points that they wished to raise as well as discuss how the project team could learn from the experiences gained to inform the future HMB4EU occupational multicentre studies, focussed on diisocyanates and electronic waste management sectors. Additional input was provided during the drafting of HBM4EU deliverables, various project team meetings, with further contributions being provided by the (co-) authors during the drafting of this manuscript. The information gathered from these various initiatives is summarized in the following sections, categorized under two broad groups: (i) What were the successes and benefits? (ii) What were the issues encountered and suggested improvements? For the second group, the views are divided into several topics; these being ethics and General Data Protection Regulation (GDPR); standard operating procedures (SOPs); sample collection; sample analysis; data reporting, input and analysis; and communication.

3. Results and discussion

It should be firstly highlighted that undertaking a complex multi-centre study takes a great deal of time and effort and this should not be underestimated. The research plan for the HBM4EU chromates study was published as a HBM4EU Deliverable report in December 2017 (Ndaw et al., 2017), with development of the core chromates study team and discussions to develop the project plan taking place well in advance of this. The development of the SOPs started in February 2018 and continued until the end of 2018, since some of the SOPs were identified as requiring refining/clarification after the first site visits occurred in November 2018. The field measurement campaigns started in most countries at the end of 2018 and progressed through to the later part of 2019. Data analysis and reporting is still ongoing at the time of drafting this manuscript (end 2020) and will continue until at least early 2021.

3.1. What were the successes and benefits of the HBM4EU chromates study?

A number of successes and benefits through adopting the harmonised approach in the HBM4EU chromates study were identified. From a project perspective, that is, in terms of achieving the study aims and objectives, the harmonised approach has provided new information on the usefulness of new biomarkers for the monitoring of Cr(VI) exposure to be generated.

In the research protocol, the target sample numbers for exposed workers were 50 workers per country, aiming to collect 100 urine (two samples per worker), 25 blood and 50 exhaled breath condensate (EBC) samples (from 25 workers, each providing two samples) per country. In addition, 25 controls were to be recruited in each country, each providing one urine, blood and EBC sample (Santonen et al., 2019). In the research protocol eight countries were to participate in the HBM4EU chromates study, however the National Health Laboratory (LNS), together with the Multisectoral Occupational Health Service (STM) of the Ministry of Health in Luxembourg were able to implement the protocol at a later stage, recruiting several welding companies, with their data being included in the HBM4EU chromates study. Thus the country participation increased to nine.

Forty-four companies were recruited for which exposed workers consented to participate in the study. Some of our control population were recruited in, for example, office staff in these companies although three additional companies were also recruited. 399 exposed workers and 203 controls were enrolled into the study, which was very close or in excess of our respective targets of 400 workers and 200 controls (>99.75% of targets achieved).

For the exposed workers, a total of 780 urine samples (397 pre-shift and 383 post-shift), 345 blood samples and 342 EBC samples were collected (167 pre-shift and 175 post-shift). Concerning the control population, a total of 143 urine, 175 blood and 98 EBC samples were obtained. Whilst the numbers of some sample types collected were slightly lower than the overall target, this was due to some countries electing to not collect particular samples (e.g., the UK did not collect blood samples, Poland and Luxembourg did not collect EBC samples, and the UK and Italy did not collect urine samples from the controls). In addition, 25 air and 25 sets of dermal wipe samples were to be collected per country (the aim being to collect these samples from half of the participating workers). 293 inhalable (218 collected outside RPE and 75 inside RPE respectively) and 155 respirable samples were collected, with 270 sets of dermal samples being collected, which exceeded our target.

The number of participants and samples collected from the nine involved countries allowed us to achieve the required statistical power for the study (Santonen et al., 2019) and obtain a more comprehensive and richer dataset to inform regulatory and policy agencies in the EU, as well as further scientific investigation when compared to (smaller) national studies. Field work also allowed us to engage workers, companies and to generate awareness for the need to control exposure to Cr(VI) in the investigated occupational sectors across Europe, as well as for the HBM4EU study at both National and International level.
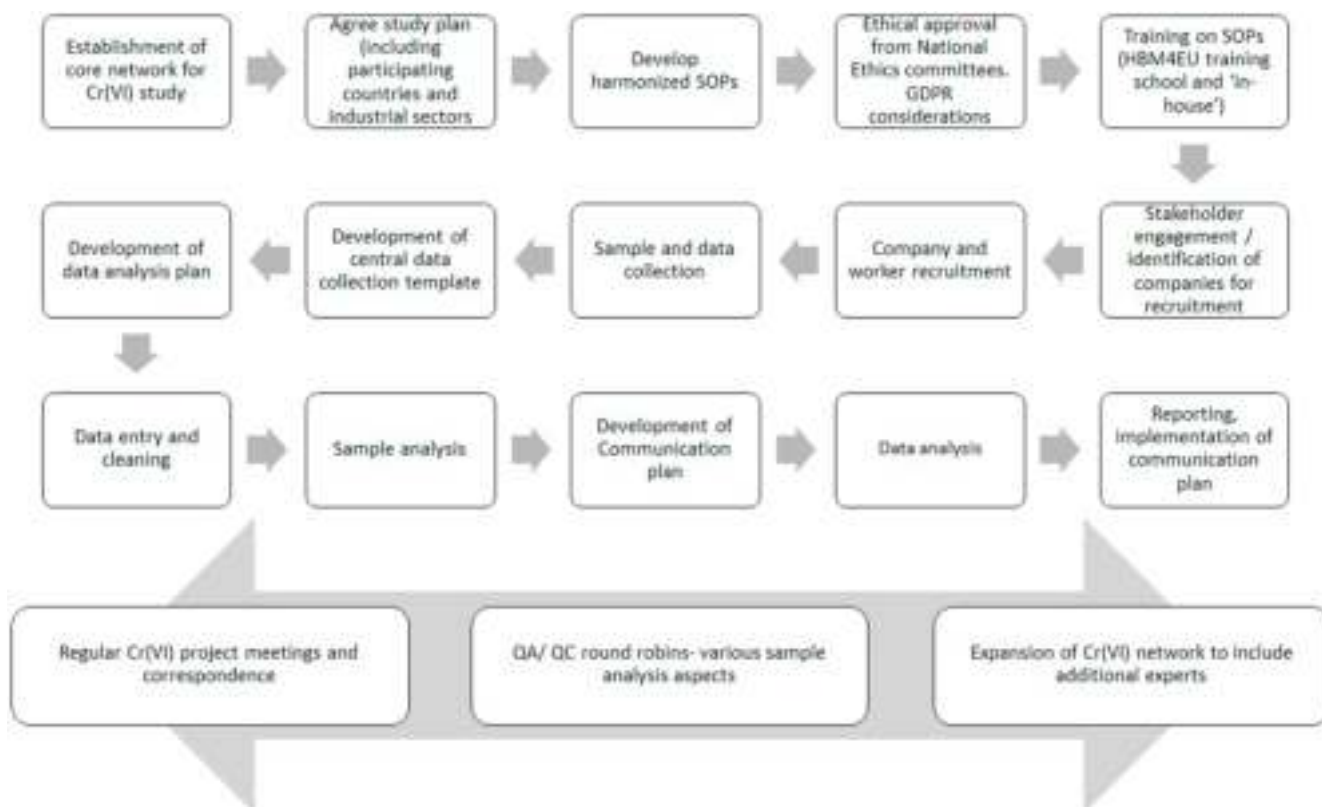

Fig. 1. Key activities in the design and implementation of the HBM4EU chromates study.

The study served also as a valued 'educational tool', where several lessons were learnt concerning the conduct of aligned, multicentre occupational studies (which will be discussed in the following sections). It was also reflected that the effort put into the development of the harmonised process in the HBM4EU chromates study would make the process easier for subsequent occupational monitoring campaigns, for example, in HMB4EU and in future projects, as many of the SOPs, materials and ethical procedures can be reused, updated or adapted if needed.

Other researchers and practitioners in the field of the scientific disciplines involved can also benefit from the harmonised efforts through applying the freely available SOPs (and materials which these contain) (Porras et al., 2019) to their own studies focused on Cr(VI). This will allow the potential of future data to be collected in the same standardized manner, thus allowing opportunities for data pooling, identify exposure trends, comparisons between different risk management measures available etc. to be undertaken. As already mentioned, this opportunity has already been exploited through the inclusion of data collected in accordance with the study protocol in Luxembourg.

It was clear that researchers involved in the study recognised that these successes were achieved through the valued and close co-operation between the participating institutions and researchers from the different countries, who were all focused and working together towards clearly defined aims and objectives. The core project network worked extremely well together and were personally committed and invested in maximizing the successes of the overall project. It was also observed that members of the HBM4EU chromates study network have continued to build and expand their relationships and networks through other biomonitoring networks (e.g., ISES-Europe working group, OECD Working Party on Hazard Assessment (WPHA) and Working Party on Exposure Assessment (WPEA)). These only serve to increase further the benefits to the scientific and regulatory communities, where exposure data and science are of paramount relevance.

3.2. What were the issues encountered and suggested improvements?

Despite adopting a harmonised and consistent approach and the time and effort that was invested into this, issues were encountered and raised. For ease, these have been grouped under several key themes, these being: ethics and GDPR; SOPs; sample collection; sample analysis; data reporting, input and analysis; and communication.

3.3. Ethics and GDPR

The management of a biobank and accompanying transport of biological samples within the context of an international occupational study is regulated by national, European and international regulation (Knudsen and Faber., 2018a). The variation and fragmentation of these biobank regulations and the consequently need for more harmonisation and standardization was the main conclusion of a report (Gottweis et al., 2012) published by the European Commission. One of such regulatory requirements concerning biobank practises is that the transport of biological samples between research partners takes place under material transfer agreements (MTAs) (Cervo et al., 2016). The restrictions and obligations of these MTAs doesn't only apply to primary sample (or parent sample), but also to (post-) interventions made using the primary sample and/or to derivative samples (Bennett et al., 2007). The fragmentation of the regulation in combination with the growing regulatory requirements, makes it more difficult to freely transfer biological samples between research partners. Therefore, international harmonisation and standardization of the biobank practices is required within the context of an international occupational study.

Ethics is a national issue. However to comply with EU regulations, special attention must be given towards data protection with respect to the GDPR regulation (Knudsen and Faber., 2018b). This regulation sets requirements for information to participants about confidentiality measures, as well as requirements for the investigators to secure data. Data handling was organised locally with pseudonymised data forwarded to the central data management. Mutual data transfer agreements were signed and a parallel system was put in place for sample transfers. Prior to submission to National Ethics committees, a standard protocol with information sheets, consent forms etc was developed and implemented. To comply with the GDPR the participants were informed about such transfers and the protocol and information sheets provided explanations and details.

It was evident from the feedback of the various ethics committees that they would often highlight different points for further clarification and for the local project team to respond to, e.g., request to clarify what is meant by 'DNA damage' and addition of a question for the study participant to agree for unexpected results to be shared with their general practitioner. A separate ethics committee also raised the point that workers with 'elevated' results may be worried and request clarification on what is considered as being unsafe, undesirable levels. In the worker participant information sheet, we emphasised that biological monitoring is a measurement of exposure only and not a measurement of ill health and that when participants received their results, we would provide an interpretation on whether these were elevated or not. The above comments led to the ethics committee requesting to see an example results letter outlining the different example sentences for each potential scenario (e.g. within the background reference range, above the reference range but less than the guidance value and above the guidance value). Another ethics committee requested that clarification in the text on what was being studied now; in the future and to what, exactly, participants need to give their consent to. A further Ethics Board requested the information and consent forms to be provided in further languages if appropriate and necessary (e.g. Swedish as well as Finnish).

The lessons learned from an ethics and GDPR perspective are that these are two critical and essential aspects of the study design but that it can be quite time consuming to have everything put in place, submitted, reviewed, responded to and finally agreed, which clearly can then affect timescales for the following phases of the project. Different ethics committees may highlight different attributes of the application and materials for clarification and moving on it is important to synthesize these to establish what the commonalities are so that these can be addressed in subsequent ethics applications at an early stage. The approach adopted for the HBM4EU chromates study was to recruit companies to participate in the campaign and for the team to then invite their workers to participate. One ethics board highlighted that to secure a free choice by the worker to participate, the invitation to participate should be sent out by the researcher to the employees directly (without any intervention of employer) as their decision making may be influenced, either positive or negatively in this manner. This is an important point to consider for future studies, although perhaps very difficult to achieve in practice.

3.4. SOPs

Table 1 lists the SOPs prepared for the HBM4EU chromates study, which are freely available at the online library of the HBM4EU website ((Porras et al., 2019, https://www.hbm4eu.eu/online-library/). The SOPs used in the campaign were developed following an extensive drafting period, with input from many researchers across the project team. The procedures outlined in the SOPs were also delivered as part of a training school, which allowed participants to identify any areas of uncertainty, etc. However, in some cases, it was evident that the field researchers, those who needed to fully understand and apply the contents of the SOPs, had not been involved in these earlier discussions. This resulted in some last-minute modifications on SOPs taking place following the start of the sampling campaigns, which is not desirable.

It was highlighted that SOPs were only available in English, not in the local languages of the other European countries involved in the campaign. Whilst it was reflected that this was not an issue for the

Table 1
List of detailed SOPs prepared for the HBM4EU chromates study (Santonen et al., 2019), with additional information on the laboratory analysis undertaken for the sampling matrices.

SOP No.	Title	Topic/Sampling matrix	Laboratory analysis
1	Standard operating procedure for selection of participants and recruitment, information to the participants, informed consent	Recruitment and consent	–
2	Standard operating procedure for completion of company and worker questionnaires	Company and worker questionnaires	–
3	Standard operating procedure for blood sampling, including sample storage and transfer	Blood	RBC – Cr; Plasma – Cr, Plasma-PFAS; Whole blood - Micronucleus & Comet assays, Epigenetics; Reticulocytes - Micronucleus assay
4	Standard operating procedure for the collection of exhaled breath condensate samples	EBC	Cr(VI) and Cr(III); Ni and Mn
5	Standard operating procedure for urine sampling, including sample storage and transfer	Urine	Total Cr, Ni, Mn, oxidative stress biomarkers (e.g. malondialdehyde, 8-hydroxy-2-deoxyguanosine) and creatinine
6	Standard operating procedure for air sampling of inhalable and respirable dust fraction and (hexavalent) chromium	Air	Total Cr, Cr(VI)
7	Standard operating procedure for obtaining dermal wipe samples	Dermal	Total Cr
8	Procedure for comparing occupational hygiene measurements with exposure estimates generated using exposure models	Contextual exposure determinant information	–

HBM4EU chromates study team it may have been for some of the field researchers, particularly with some of the more technically demanding language used. It was mentioned that in at least one instance the field researchers translated a SOP into their native language themselves so that they were more comfortable with their understanding of what was needed to be done. It was also considered that sample transfer details (list of labs and contract details) should have been included in the SOPs, rather than separately.

The lessons learned from the development of the SOPs is that the time and effort involved in this activity should never be underestimated by researchers, and that, even despite this, last minute, issues do crop up. It is therefore essential that those involved in implementing the SOPs (i.e. the field researchers) are involved in the finalization of these documents. Whilst a training school was held for the HBM4EU chromates study, not all researchers involved in the fieldwork were able to participate and different local training regimes were likely to have been implemented in the various countries. More effort should be made to run centralized training sessions, for example via webinars, well in advance of the monitoring campaigns commencing so that common questions and uncertainties can be promptly addressed. If SOPs are to be translated

into local languages for future studies there would be a need to verify that the translation is indeed correct.

3.5. Sample collection

Field work to collect all the samples and contextual data was highlighted to be more time consuming than anticipated (by both the field researchers and the participating workers). It was considered that completion of the detailed worker questionnaires and collecting all of the necessary samples may have, in some instances, reduced the effective working time during the sampling day. In addition, a significant number of the participating companies were small in size, with low numbers of workers who were actually eligible to participate in each. Therefore, any reduction in working time for the participants may have a significant impact on the company's productivity. Also, due to the fact that companies were smaller in size than expected, more time and effort was spent to identify, visit and recruit additional companies reach the target number of study participants (and obtain their corresponding samples). Exposures in SMEs are important to understand and often overlooked precisely for the reasons just mentioned.

Specific issues were noted with respect to the worker and company questionnaires, with these cited as being too long or short and the collection of the EBC samples were also highlighted as being time consuming. It was highlighted that, where possible, all samplings (excluding pre-shift) should be done as close as possible to the end of the shift (preferably post-shift). However it was also recognised that this may not be possible due to numbers of workers involved and their desire to leave work promptly following completion of their work shift.

Despite the SOPs, there were some variations in procedures that were followed. For example, air sampling pumps were not always switched off during the breaks and there was variability in the sample types collected, with some diversity of air samples being collected depending on the job being undertaken. In one country, only inhalable (total) dust samples were collected, whereas in the other countries both inhalable and respirable dust samples were collected (as per SOP). There was also some variability in the positioning of samples, despite instructions being given in the SOP that samples were to be collected from the breathing zone area, except for those collected from welding operators where these were to be inside the welding helmet. For example, some countries collected samples outside the RPE (in the breathing zone) whereas others collected samples both inside and outside of the RPE. In one country only pre-shift and end shift dermal wipe samples were collected although it was instructed to also collect samples before break periods.

EBC-Cr(VI) is a new biomarker-matrix combination which has been used only once before (Leese et al., 2017) in occupational biomonitoring study. Some EBC results were obtained, which were then excluded from the data analysis (high Cr(III) levels which questioned the reliability of the Cr(VI) results) which was considered to be due to different complexing (EDTA) solutions being used or contaminated glassware.

One of the lessons learned from the sample collection is that it was more time consuming than anticipated. The HBM4EU chromates study was very ambitious, collecting a wealth of biomonitoring, occupational hygiene samples and supporting questionnaire and contextual data so to achieve its aims and objectives. However, consideration must always be given to the impact that such sampling strategies have on company/worker participation and their working practices, numbers of field workers required to ensure efficient sample collection etc. Engagement and recruitment of SMEs into exposure monitoring campaigns is particularly challenging and sufficient time and resources must be allocated to this. Overall, sample and data collection were in adherence with the SOPs focussed on the biological samples, with deviations occurring in some instances with the occupational hygiene samples. Whilst the study was focussed primarily on HBM, the occupational hygiene samples served an important role in the study and so it is important that the same level of robustness and rigour be given to these samples. Ensuring that more structured training of the field researchers

takes place prior to commencing the sampling campaigns may help address this. Concerning effect biomarkers analysis, it is important to ensure that samples reach the laboratory in a short period of time after collection, particularly, if blood cells must be immediately processed upon arrival (e.g., lymphocytes cultures for micronucleus analysis in blood lymphocytes). Although it is trivial to transfer biological samples among laboratories, it must be stressed that adequate packaging and transportation conditions (e.g. temperature) have to be ensured to not compromise the success of the analyses.

3.6. Data reporting, input and analysis

A MS Excel data template was circulated for researchers to input data and results obtained from the chromates study. This data template was very large so to facilitate entry and transfer of all data collected under the HBM4EU chromates study SOPs. Templates of such a size can in advertently lead to data entry errors occurring and so those populating the files needed a great deal of care and diligence.

In most cases, the HBM4EU chromates data template was populated according to given instructions. However, there were many exceptions, and it was evident that some researchers had difficulties with completing the template and there was variation in how this was done. For example, despite requesting that work task and overall work duration be given in hours, it was often given in minutes (sometimes with units included, other times not). On occasions entries asked for the data user to refer to other lines of information or indeed merged cells (e.g., free description of the workplace of all workers in the same company) which added time and effort to the data cleaning process. On a few occasions, it was apparent that details of the weekly work schedule were provided rather than details of the work tasks actually undertaken during the measurement period. Despite the inclusion of drop-down lists to facilitate data entry (e.g. for local exhaust ventilation and respiratory protection equipment), these were not always used and instead were manually removed by those inputting data and replaced with free text data. In response to the question, "If other tasks were done during the measuring day, please specify here (free text)", often an entry of 'yes' was assigned, with no additional information provided. Less commonly, answers were provided which did not relate to the question being responded to. Some of these issues were perhaps due to the fact that the individual SOPs developed for the study did not cover data reporting, and there was no overarching SOP covering this aspect. Whilst instructions were provided separately by email to researchers concerning many of these points (and others) to assist with the data entry process, these were not always considered. This resulted in significantly more time being taken to check, verify and clean the results before data analysis could commence.

Limit of Quantification (LOQs) varied in different laboratories, which may have had an impact on the results of, for example, EBC Cr(VI) measurements since the levels in controls and workers were often low. The verification of LOQs in each country for all sample matrices should have been done before the study began in order to assess the potential impact of possible differences and to consider options for limit the impact of these differences.

There were some deviations in the analysis undertaken for the collected occupational hygiene samples. The overarching focus of the study was on the biomonitoring sampling and analysis with extensive QC and QA procedures being put in place for this. Whilst efforts were made to harmonise the occupational hygiene sample collection, with details of the analytical methods to be used for analyzing the collected samples being included in the SOPs, the sample analysis was not part of the formal QC/QA programme. Despite the SOP stating that all air samples were to be analysed for both total Cr and Cr(VI), only five of the participating countries did so.

Some differences in the biomonitoring analysis did however occur. Although this was a chromates study, blood, urine and EBC samples were also analysed for nickel and manganese. For example, urinary nickel levels were suggested to be analysed for welders and those platers performing nickel plating; urinary manganese levels were suggested to be analysed for stainless steel welders; however, this did not always take place.

An important but still overlooked issue is that of the control group selection. In the majority of occupational studies reporting HBM data the control group consists of administrative workers from the same industry where exposed workers were selected. It is assumed that those workers, while performing their activities in an office, are not exposed to the occupational chemicals under study. Preliminary data from this study showed, however, that some individuals from the so-called "non-exposed group" are indeed exposed to low concentrations of Cr(VI), possibly present in the ambient air or even in surfaces of common spaces like canteens. Therefore, a double lesson is learnt from this knowledge: on one hand, controls outside the industry should be included in occupational studies involving emissions of chemicals that may persist in the air and surfaces and, on the other hand, administrative workers must also be followed by the occupational health services as potentially exposed workers if HBM data is not available for them.

The lessons learned from the data reporting, input and analysis were as follows. It is necessary to have a central tool in which to input data collected from such studies; however, it is essential that this tool is useable and clearly understood by those that need to populate it. It is necessary to hold training sessions with those that will be required to populate the tool so that they are familiarized with this and the data entry process (for both contextual and numerical data) that they must follow. It should be agreed by all involved that the tool will be used as per instructions and that, in the event that the template is unofficially modified or data are not input correctly, it will be returned to the provider to be corrected and amended. This agreement will ensure that the instructions are followed as well as reduce time spent on data checking and data cleaning step. The tool must be accompanied by text in each of the SOPs focused on data reporting for those particular aspects of the study. This text should detail how data entry should be undertaken, how calculations should be undertaken (where necessary), with examples given so to assist those in the tool completion and ensure that this is carried out in a standardised manner. Where possible the tool should be modified to allow for ease of entry and for key aspects to be highlighted to assist data interpretation e.g. flag data below LOQ or possible outliers. It was also apparent that much more instruction with respect to the collection and analysis of the occupational hygiene is warranted and that appropriate QA/QC for these samples should also be included within the programme of work.

3.7. Communication

The HBM4EU chromates study involved numerous companies and participants, with the project team having a wealth of knowledge and experience in undertaking occupational site work. However, challenges still presented themselves when completing the work. Some countries reported difficulties with engagement with companies and workers, with challenges being experienced with receiving clear information on the activities being undertaken from the companies' representative. There were also several instances where field researchers arrived on site to undertake sampling to discover that the workers were not undertaking any work where Cr(VI) exposure may occur, e.g. in some cases welders welded mostly mild steel instead of stainless steel; fewer workers than originally communicated were to be involved, or processes were not operating due to maintenance. These are common problems in industrial hygiene studies and the risks could be minimised by obtaining more accurate communication with the company representatives to ensure that the information they provide has reflects input from site supervisors, foreman, etc., who are more integrally involved in the activities of interest and their daily production regimes.

It was also important that due care and consideration be given to communicating the results of the study to the participating workers,

companies and wider stakeholders. Communication of the company specific data to participating companies was the responsibility of the each participating institute, according to their national/standard practises, with information leaflets also being provided.

The HBM4EU chromates study team however reflected that more consideration needed to be given to the development of a dissemination plan for the wider communications of the project findings.

Whilst the core project team itself communicated extensively via web conferences, email exchanges, training schools etc., it was still evident that communications were perhaps not always reaching those who also needed to receive it (e.g. training for all the field researchers).

The major lesson learned is that good, clear communication networks must be established with those involved in multicentre studies, whether these be the project team itself, potential and actual participants and other stakeholders. For the second European wide occupational measurement campaigns, additional SOPs focused specifically on communication, covering whom, how and when, have been put in place.

4. Conclusion

The HBM4EU chromates study team held honest and frank discussions when reflecting on the experiences gained from undertaking this study and considered it important to communicate these to the wider scientific audience so that others can learn from these too. Occupational studies such as the HBM4EU chromates study are scarce in terms of their breadth of scope and number of countries involved. Overall, even though there is room for improvements, the HMB4EU chromates study showed that it was feasible to conduct a pan-European occupational study in a consistent and concerted way and it's hoped that other research teams can draw upon these experiences. Many of the lessons learned by the HBM4EU chromates team may not be new, or may even be considered as being trivial in nature. For example, Fiddicke et al. (2015) also observed that a biomonitoring survey involving many European countries needs time for preparation and conduct and that extensive communication with potential participants (school-aged children in this instance) was necessary. For example, the importance of the method used to report non-detects was just one of the points highlighted during a workshop regarding biomonitoring study design, interpretation, and communication (Bates et al., 2005). However, this does not negate their importance or the need to reinforce to the wider scientific community that such aspects must be considered and factored into the project planning. Despite the points noted here, the data generated allows for a more robust assessment of exposure in different occupational settings and countries. The high number of data collected in turn will allow for more detailed data analysis that can provide more definitive answers to policy questions and recommendations for future studies aiming to address occupational exposure to Cr(VI). Furthermore, the findings of our study can be useful to define more adequate occupational exposure limits and support regulatory action. It is likely that the study will result in several publications that will prove useful for stakeholders, from the regulatory field to occupational health services, with any relevant limitations to the data set (e.g. due to differences in sample collection, analysis) being highlighted. The true collaborative nature of the HBM4EU chromates study team allowed for open and frank discussion on the issues encountered, has increased our awareness on additional differences in the standard practises used in different countries/laboratories, and may result in further harmonisation of practises between labs/countries. All of the points raised for improvement have been considered and taken forward to improve the SOPs for the future HBM4EU occupational studies focussed on diisocyanates and electronic waste management, which are planned to start sampling in 2021. Furthermore, additional training schools were delivered during Jan 2021 to communicate these to those researchers involved in the fieldwork, which were also recorded so that they can be made available to any additional fieldworkers.

Harmonisation is difficult to achieve and it is inevitable that it can always be improved upon, but it can be facilitated by having a solid network in place to enable this. However, development of such a strong successful network takes effort and engagement from all and it is important to ensure that adequate time, effort, resources is given for such an initiative. It is also a continual process whereby the team learns, shares, evolves from the experiences, and lessons gained. It is very much hoped that the lessons learned highlighted in this manuscript will be of value to researchers planning similar studies.

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 organizations to which they are employed.

Declaration of competing interest

No conflicts of interest are declared.

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Household water insecurity will complicate the ongoing COVID-19 response: Evidence from 29 sites in 23 low- and middle-income countries

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ABSTRACT

In March 2020, the World Health Organization (WHO) issued a set of public guidelines for Coronavirus Disease 2019 (COVID-19) prevention measures that highlighted handwashing, physical distancing, and household cleaning. These health behaviors are severely compromised in parts of the world that lack secure water supplies, particularly in low- and middle-income countries (LMICs). We used empirical data gathered in 2017–2018 from 8,297 households in 29 sites across 23 LMICs to address the potential implications of water insecurity for COVID-19 prevention and response. These data demonstrate how household water insecurity presents many pathways for limiting personal and environmental hygiene, impeding physical distancing and exacerbating existing social and health vulnerabilities that can lead to more severe COVID-19 outcomes. In the four weeks prior to survey implementation, 45.9% of households in our sample either were unable to wash their hands or reported borrowing water from others, which may undermine hygiene and physical distancing. Further, 70.9% of households experienced one or more water-related problems that potentially undermine COVID-19 control strategies or disease treatment, including insufficient water for bathing, laundering, or taking medication; drinking unsafe water; going to sleep thirsty; or having little-to-no drinking water. These findings help identify where water provision is most relevant to managing COVID-19 spread and outcomes.

1. Introduction

The global response to COVID-19 has emphasised basic infection control strategies, such as self-quarantine, handwashing and physical distancing, to "flatten the epidemiological curve" in many contexts (WHO, 2020). Globally, over four billion people experience water scarcity at least one month per year (Mekonnen and Hoekstra, 2016), a reality that may make it difficult to enact COVID-19 control measures with far-reaching consequences across continents (Staddon et al., 2020; Stoler et al., 2020). Water insecurity (and more broadly, inadequate

water, sanitation, and hygiene [WASH]) already contributes to the global disease burden (Prüss-Ustün et al., 2019), and has stood out as a potentially significant multiplier of COVID-19 risk, compounding the paucity of other healthcare resources—especially test kits, personal protective equipment, oxygen and ventilators—that has plagued many nations (Hopman et al., 2020). Identifying the likely location of relevant water insecurity hot spots and clarifying the pathways by which water insecurity adds to the burdens of COVID-19 are potentially vital to ongoing prevention and treatment, especially in low- and middle-income countries (LMICs).

Persistent household water insecurity is defined as the inability to 'access and benefit from affordable, adequate, reliable and safe water' (Jepson et al., 2017). The links between water insecurity and COVID-19 are only beginning to emerge. One early study from Indonesia noted a positive association between household water reuse and COVID-19 transmission (Sihaloho et al., 2020). It also remains unclear whether urban slums, which tend to house many of the most vulnerable, are being sufficiently targeted by national COVID-19 testing and tracing programs (Mollah and Islam, 2020; Monteiro de Oliveira et al., 2020). Overcrowding and lack of basic services in urban slums are believed to contribute to the pandemic in some regions, such as in Latin America (Burki, 2020), while other regions thought to be particularly vulnerable, such as sub-Saharan Africa (Ekumah et al., 2020), have responded well (Makoni, 2020). For example, many African nations have avoided catastrophic COVID-19 outcomes through advance planning and public adherence to preventive measures, and because of younger population structure (Makoni, 2020). But, evolving SARS-CoV-2 variants and recurring waves of infections in late-2020 clarified that many pandemic recovery timelines would be extended.

The World Health Organization's guidance for COVID-19 prevention includes: (1) frequent handwashing; (2) physical distancing; (3) avoiding touching one's face; (4) practicing respiratory hygiene; (5) seeking medical care for cough, fever, and difficulty breathing; and (6) following advice from one's healthcare provider (WHO, 2020). In addition, the United States Centers for Disease Control and Prevention (CDC) also recommends regularly cleaning and disinfecting household surfaces (CDC, 2020). Water is thus critical for stemming the spread of COVID-19 in health facilities and households (USAID Water Leadership Council, 2020).

Many organizations have noted the inherent conflicts between these guidelines and the realities of widespread water insecurity around the world. Among many other examples, WaterAid, one of the largest non-governmental implementers of WASH solutions for the poor and marginalized, has had to change how they work in the midst of this pandemic with increasing focus on water insecurity as a key element of their response (Casey, 2020).

Household-level COVID-19 control strategies are complicated by many factors beyond limits to handwashing and cleaning (Stoler et al., 2020). Physical distancing is less feasible when it impedes access to water, as has been noted with shared sanitation facilities (Caruso and Freeman, 2020). Over a billion people collect water from sources outside of their home (WHO/UNICEF, 2017), which often requires traveling significant distances and queuing for long periods in close proximity with others. Households also frequently cope by sharing water, an underappreciated global phenomenon (Wutich et al., 2018) that typically involves shared physical spaces or physical contact with water containers. Households may also increase water storage to avoid fetching, but improperly stored water increases vulnerability to viral and bacterial illnesses. Informal water vendors who are not officially deemed essential services may cease operation out of fear of illness, resulting in significant supply disruptions to informal water markets. The management of these water-related tradeoffs and disruptions—on top of fears about COVID-19—will likely increase stress, anxiety, and interpersonal violence. All of these factors are exacerbated by the realities of resource-poor settlements that are home to close to one billion people (Corburn et al., 2020).

Our analysis highlights the challenges that many living with household water insecurity may experience in following international COVID-19 control guidelines, many of which hamper their ability to protect themselves, their households, and their communities. Our global data set also allows us to speak with more precision about the magnitude of some of these effects. We use empirical data collected in 2017–2018 from 8,297 households in 29 sites across 23 LMICs to demonstrate how household-level water insecurity not only severely limits handwashing, cleaning, and disinfection of household surfaces, but also greatly complicates physical distancing. We also illustrate how conforming to current COVID-19 control measures may, in some scenarios, lead to cascading health risks beyond COVID-19, including how water insecurity intersects with other resource constraints such as food and sanitation insecurity. Finally, we explore other contextual vulnerabilities that, more broadly, may hinder compliance with WHO guidance, and conclude with considerations for COVID-19 control in resource-limited settings.

2. Material and methods

2.1. Study data

Data were drawn from the Household Water Insecurity Experiences (HWISE) study, which primarily aimed to develop and validate a cross-culturally equivalent household water insecurity scale (S. L. Young et al., 2019b; 2019a). Cross-sectional survey data were collected between 2017 and 2018 from samples of 101–574 households in each of 29 sites across 23 countries (Fig. 1). Sites were selected to maximize heterogeneity by climatic region, predominant water infrastructure, and types of water problems. In most sites, households were selected using simple random sampling, with the exceptions of purposive sampling used in Singida, Tanzania; Kampala, Uganda; and Upolu, Samoa; and parallel assignment in Pune, India. Adults were considered eligible respondents if they "were knowledgeable about their household's water situation" (S. L. Young et al., 2019b). These survey data provided a more detailed view of household water insecurity than other common household surveys such as Demographic and Health Surveys or Multiple Indicator Cluster Surveys.

Enumerators used paper and tablet-based surveys to collect data on sociodemographics and experiences with water availability, accessibility, reliability, and use, which are core components of household water insecurity (Jepson et al., 2017). The survey items elicited the frequency of 34 household experiences related to water in the four weeks prior to survey implementation, and responses were categorized as follows: *never* (0 times), *rarely* (1–2 times), *sometimes* (3–10 times), *often* (11–20 times), or *always* (more than 20 times). This paper presents data from the 34-question survey module to highlight pathways through which different dimensions of water insecurity complicate COVID-19 prevention and control. Data collection occurred in two waves between 2017 and 2018. The total sample size available for this analysis was 8,297 households across 29 sites.

All participants provided verbal or written informed consent in the respective local language. Study activities were reviewed and approved by all relevant ethical review boards (S. L. Young et al., 2019b).

2.2. Statistical analysis

We assessed the frequency of affirmation for each survey item related to handwashing, ability to enact physical distancing, and cleaning and disinfecting household surfaces to demonstrate heterogeneity across sites. We used a descriptive, rather than inferential, approach due to the current lack of reliable COVID-19 prevention or case data in the HWISE study sites that would otherwise help us extrapolate risk to other areas. Our objective was to present the breadth of water insecurity experiences that have implications for COVID-19 prevention and control.

In our analysis of socioeconomic status, we categorized HWISE study

Fig. 1. The 29 HWISE study sites showing the percent of households in each site that experienced one or more water problems in the prior four weeks that potentially undermine COVID-19 control strategies or disease treatment.

participants' responses to the MacArthur Scale of Subjective Social Status, a self-reported measure of one's perceived social standing in the community (Adler et al., 2007), into high, medium, and low tertiles, and examined their relationship with select water insecurity items using chi-square tests and two-sample tests of proportions. All analyses were conducted using Stata 14.0 (StataCorp, College Station, TX, USA).
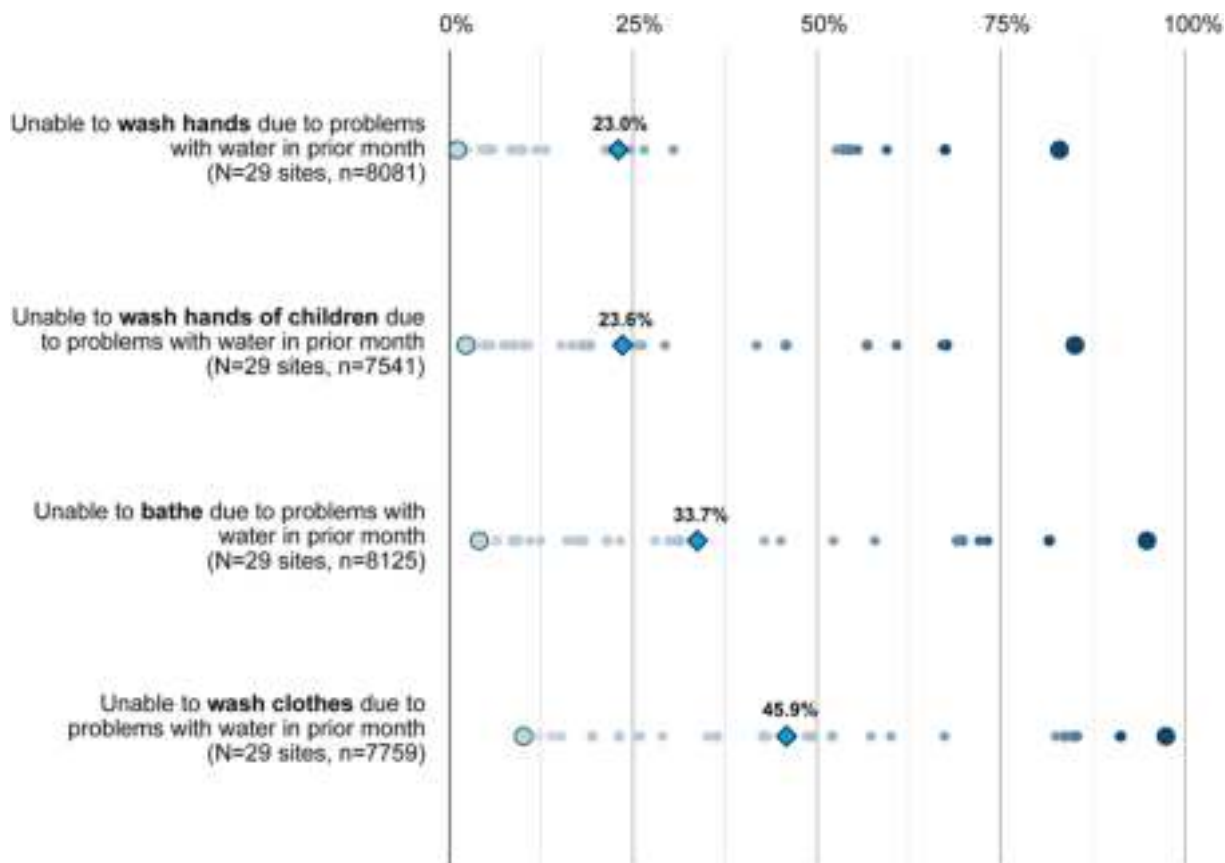

Fig. 2. Distribution by study site of percent of households that experienced interruptions to hygiene behavior in the prior four weeks due to problems with water (with percent across all sites in bold).

3. Results

Frequency statistics for responses to individual water insecurity items among the 8,297 study households are presented in Figs. 2–8. We report these results in sub-groups as they pertain to hygiene, physical distancing, and additional contextual vulnerabilities. Overall, 45.9% of households reported being unable to wash their hands or reported borrowing water in the four weeks prior to completing the survey, which confounds compliance with WHO's first two guidelines for COVID-19 control (frequent handwashing and physical distancing). We also considered households' ability to engage in a broader range of water-related activities that may serve as proximal and distal determinants to COVID-19 outcomes: drinking unsafe water, going to sleep thirsty, having no water or less water for drinking, and lacking water to take medications, bathe, launder clothes, or prepare foods, in addition to handwashing or borrowing water. We found that 70.9% of households experienced at least one of these water-related problems that can undermine COVID-19 control strategies or intensify disease progression.

3.1. Hygiene

3.1.1. Handwashing and bathing

Frequent handwashing with soap and water is critical for reducing the spread of communicable diseases, including COVID-19. Soap destroys the lipid bilayer that envelopes SARS-CoV-2 and water washes away the exposed, inactivated virus (Staddon et al., 2020). Yet nearly one in four households (23.0%) in our study reported that they were "unable to wash their hands in the prior four weeks after dirty activities" (e.g., defecating, changing diapers) due to problems with water (Fig. 2). Of households with children, 23.6% reported being unable to wash their young children's hands and faces because of problems with water. Over a third (33.7%) of households reported being unable to wash their bodies, varying widely from 4.1% of sampled households in Honda, Colombia, to 94.8% of households in Punjab, Pakistan.

Sometimes washing was limited or impossible because water was not available in sufficient quantities. For example, when water was limited due to shutoffs, some households reported that they prioritized water for drinking and cooking over hygiene. Other households reported that there was water available, but that the quality was too low for handwashing or bathing. Although water does not need to meet drinking-water standards for hygienic purposes (WHO/UNICEF, 2020a),

handwashing with severely contaminated water can be unacceptable, irritate the skin and create open sores, or expose individuals to a variety of water-washed diseases (Bartram and Hunter, 2015).

The frequency with which households are unable to wash hands due to problems with water is likely to increase during the pandemic. As hand hygiene messaging spreads, individuals may wish to increase handwashing but may be unable to do so due to competing water demands. For instance, water may become more difficult to access because of physical distancing recommendations or the need to divert greater quantities of water to other household tasks that have increased in frequency due to sheltering in place, such as cooking, cleaning, or caring for the ill. Women and girls typically experience water inadequacy even more acutely (Maxfield, 2020). This can be due to unequal access within households, or specific needs related to menstrual or sexual hygiene. While there is currently no evidence that COVID-19 affects the menstrual cycle or can be spread by blood or semen, the concomitant stress and anxiety are nonetheless important to consider (UNICEF, 2020).

3.1.2. Household cleaning and disinfection

SARS-CoV-2, the virus that causes COVID-19, can travel in droplets and land on common household surfaces, where the virus can remain viable for hours to days, depending on the surface material (e.g., the virus survives longer on plastic and wood compared to copper surfaces) (van Doremalen et al., 2020). Regular disinfection of household surfaces is therefore recommended to reduce the risk of transmission (CDC, 2020). Best practices include laundering soft, porous surfaces (e.g., clothing) with hot water and non-porous surfaces (e.g., tables) with soap and water or bleach diluted with water. These preventative practices are difficult or impossible when a household experiences water problems.

Across all sites, 45.9% of households were unable to wash clothes due to problems with water in the prior four weeks (Fig. 2). Further, 59.1% of households worried that they would not have enough water for all of their needs and 27.9% reported they had no useable water, including for chores such as cleaning surfaces. As the price and availability of water fluctuates throughout the pandemic, households may reduce water-intensive chores and reserve limited water for higher priority activities, such as direct consumption. Indeed, in a previous study in western Kenya, respondents prioritized water for drinking, taking medications, and cooking over other activities such as washing clothes (Collins et al., 2019). The 2018 drought in Cape Town, South Africa, also showed how households can severely reduce water use
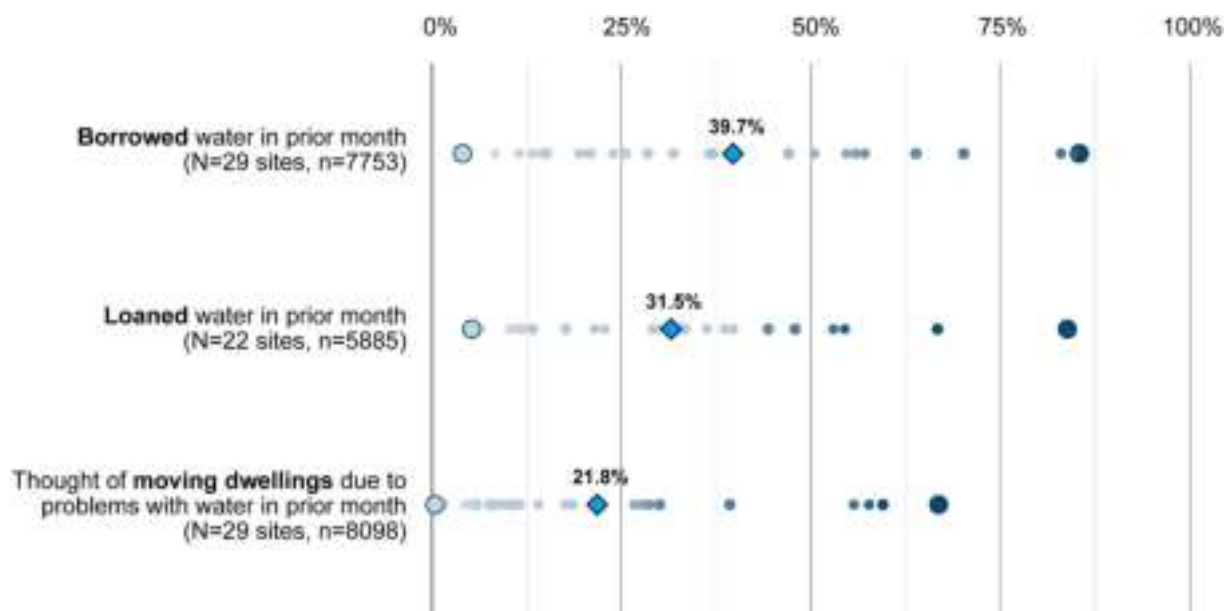

Fig. 3. Distribution by study site of percent of households that borrowed or loaned water in the prior four weeks, as well as those who considered moving due to problems with water (with percent across all sites in bold).
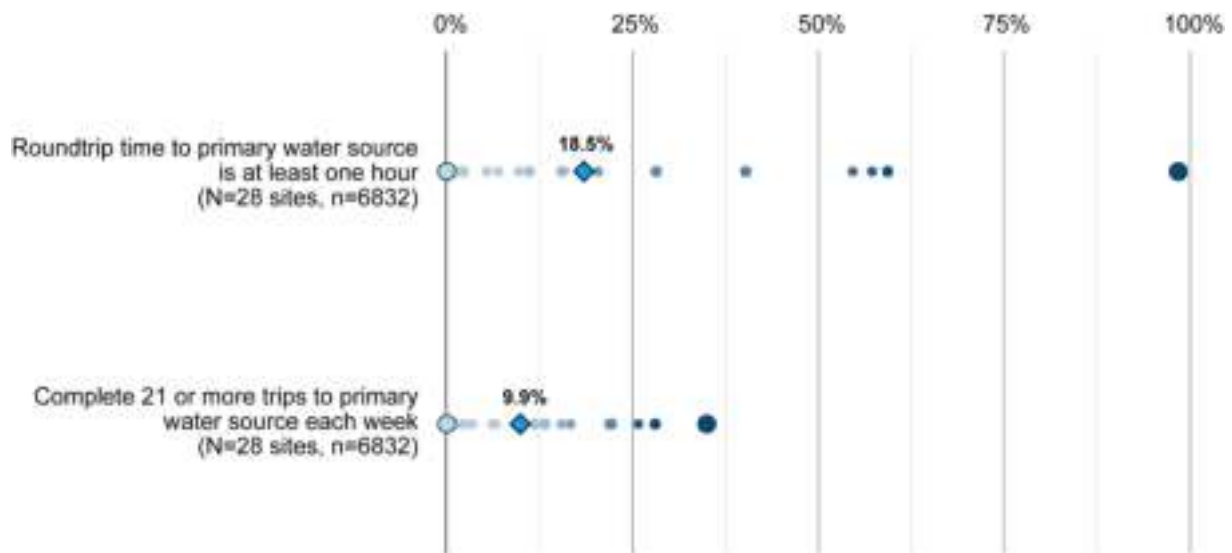
Fig. 4. Distribution by study site of round trip time to primary water source and trips made to water source per week (with percent across all sites in bold).
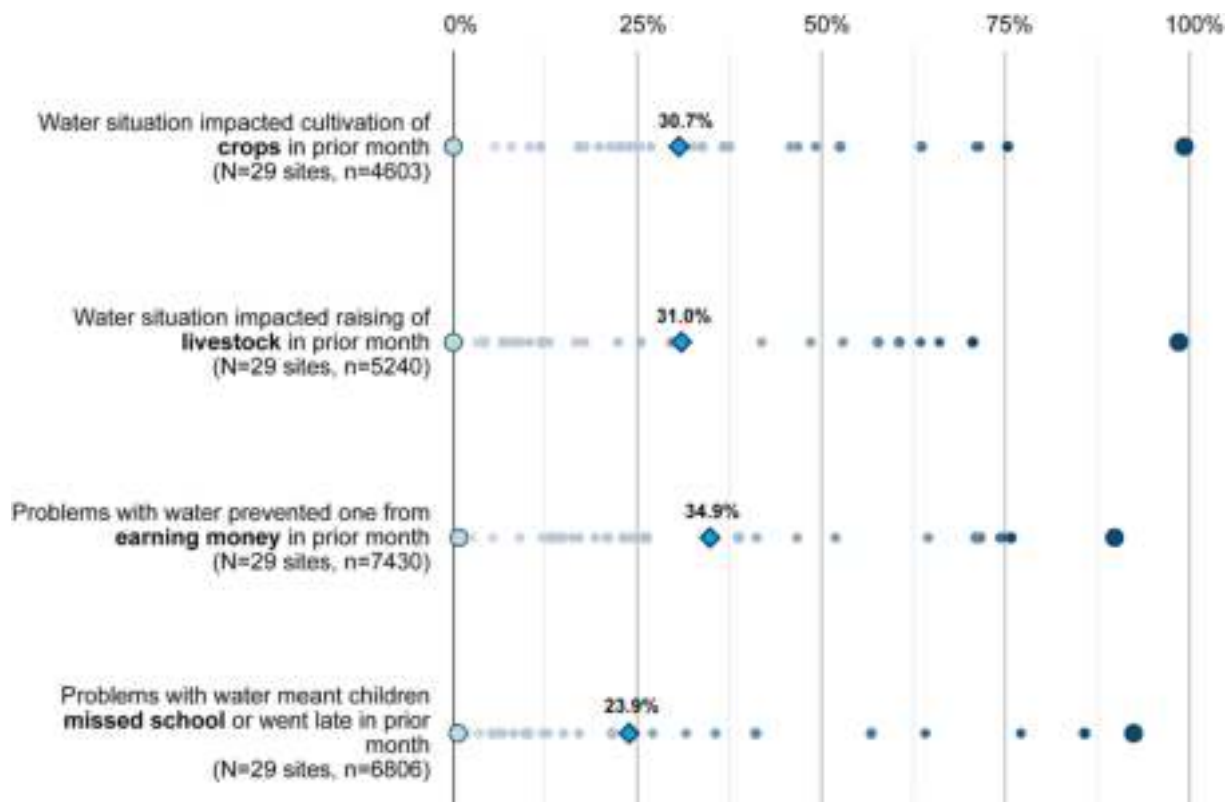

Fig. 5. Distribution by study site of percent of households that experienced livelihood or educational opportunity costs in the prior four weeks due to problems with water (with percent across all sites in bold).

during an emergency—change that was largely driven by policy discourse, social norms, and perceived threats to one's lifestyle (Booysen et al., 2019; Enqvist and Ziervogel, 2019).

3.2. Physical distancing

3.2.1. Water sharing

If people cannot readily access community water supplies when physical distancing, water sharing between neighboring households might increase as a coping strategy (Wutich et al., 2018); this common adaptation has been observed in many LMICs (Rosinger et al., 2020).

Increased sharing potentially brings people into greater direct physical contact with each other. It can also bring people into greater contact with shared objects like water jugs that may then increase transmission risks of infectious disease (Stoler et al., 2019).

Frequency of borrowing water in the prior month ranged between 4.0 and 85.4% of households across all 29 sites, and lending ranged from 5.2 to 83.8% across the 22 sites with lending data (Fig. 3). Of the 3,078 households that borrowed water, 54.2% also reported loaning water. Of the households that loaned water, 66.9% also reported borrowing during the prior four weeks. Taken together, this suggests substantial transactional movement of water (and the containers used to haul it)
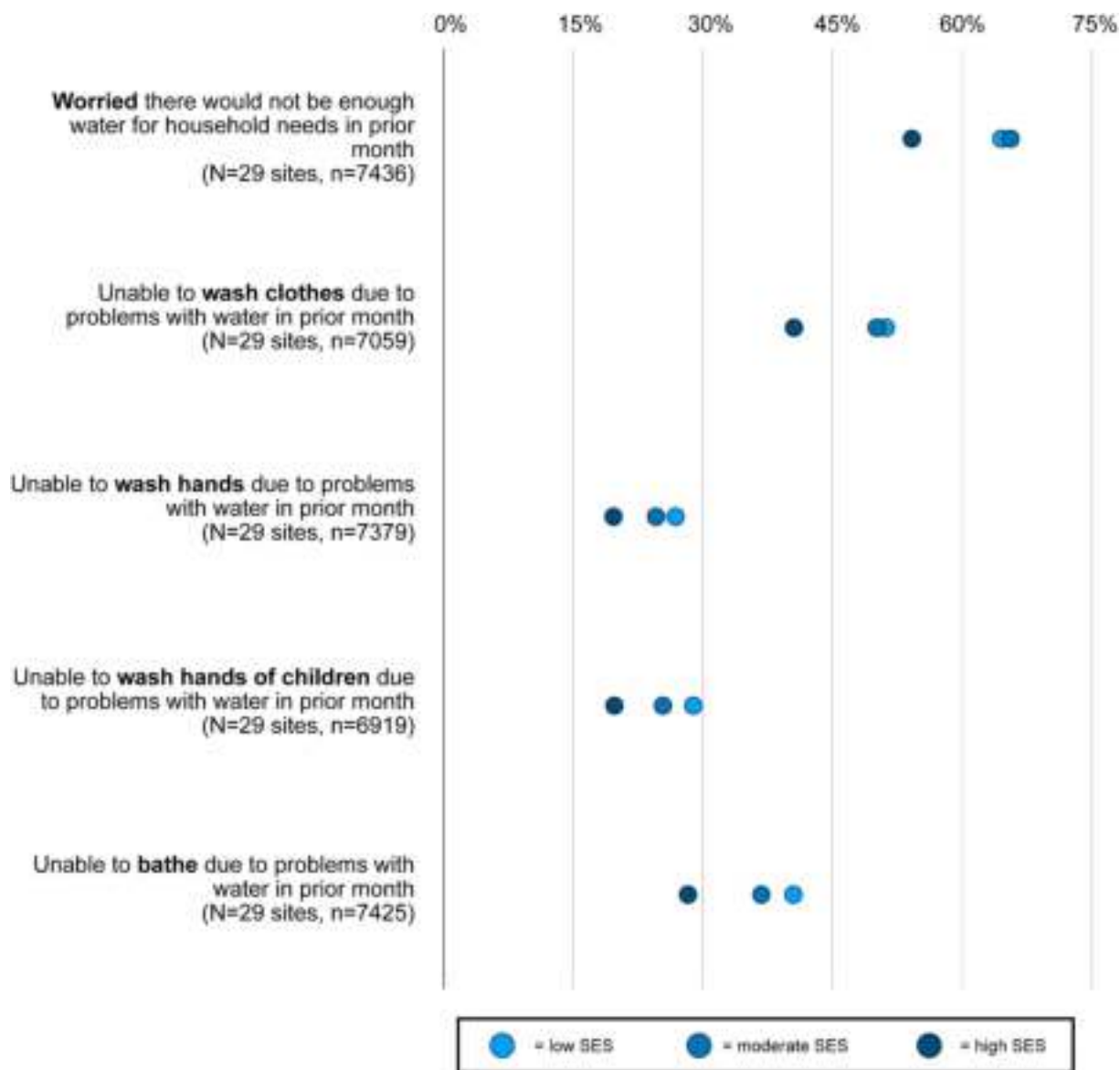
Fig. 6. Percent of households across all study sites that worried about problems with water or experienced interruptions to hygiene in the prior four weeks by tertile of perceived socioeconomic status relative to others in their community.

both in and out of households.

The types of water containers used and the method of exchange may be important risk factors of COVID-19 transmission, given that SARS-CoV-2 survival varies across surfaces. While the HWISE study did not collect data on the types of water containers used during water exchanges, plastic jerrycans are a common water storage container in many water-insecure communities. Jerrycans may pass from one person's hands to another during the filling process, thus increasing transmission risk as the virus can survive on plastic for up to 72 h (van Doremalen et al., 2020). Both individuals involved in the water exchange should ideally wash their hands prior to exchanging water, and the surface of the container should be sanitized afterward. This assumes adequate water for handwashing (see Section 3.1.1), although households often participate in water sharing specifically because of a lack of access to water—including for handwashing.

3.2.2. Water fetching

Water fetching often involves long queues at water kiosks and borehole pumps (Geere and Cortobius, 2017). Spending extended time in close proximity with others is inconsistent with physical distancing guidelines. In the HWISE sample, we obtained data on the frequency of trips to collect water per day. We also collected data on the amount of time spent obtaining water per day (which includes queue time). We found that 18.5% of households had a roundtrip water collection time equal to or over 1 h (Fig. 4). Also, 9.9% of households made more than 21 trips to their water source each week and 23.4% reported spending at least 7 h/week fetching water. It is also well documented that there are frequently gender differences with respect to water fetching (Geere and Cortobius, 2017), potentially leading to differential disease exposure.

When households are unable to fetch water, or if fetching presents too large of an opportunity cost in terms of time spent, individuals may be forced to resort to higher-priced or lower-quality water from tankers, carts, and other forms of water vending, or use nearby surface water (Smiley and Stoler, 2020). For lower-income households, higher water costs may present tradeoffs for other household priorities, particularly health care for ill family members or COVID-19 preventive gear such as masks, gloves, disinfectant, soap, detergent and hand sanitizer.

Physical distancing guidelines would also seemingly discourage water fetching in groups, which eliminates a way of maintaining social capital and an important safety buffer for women and children, who
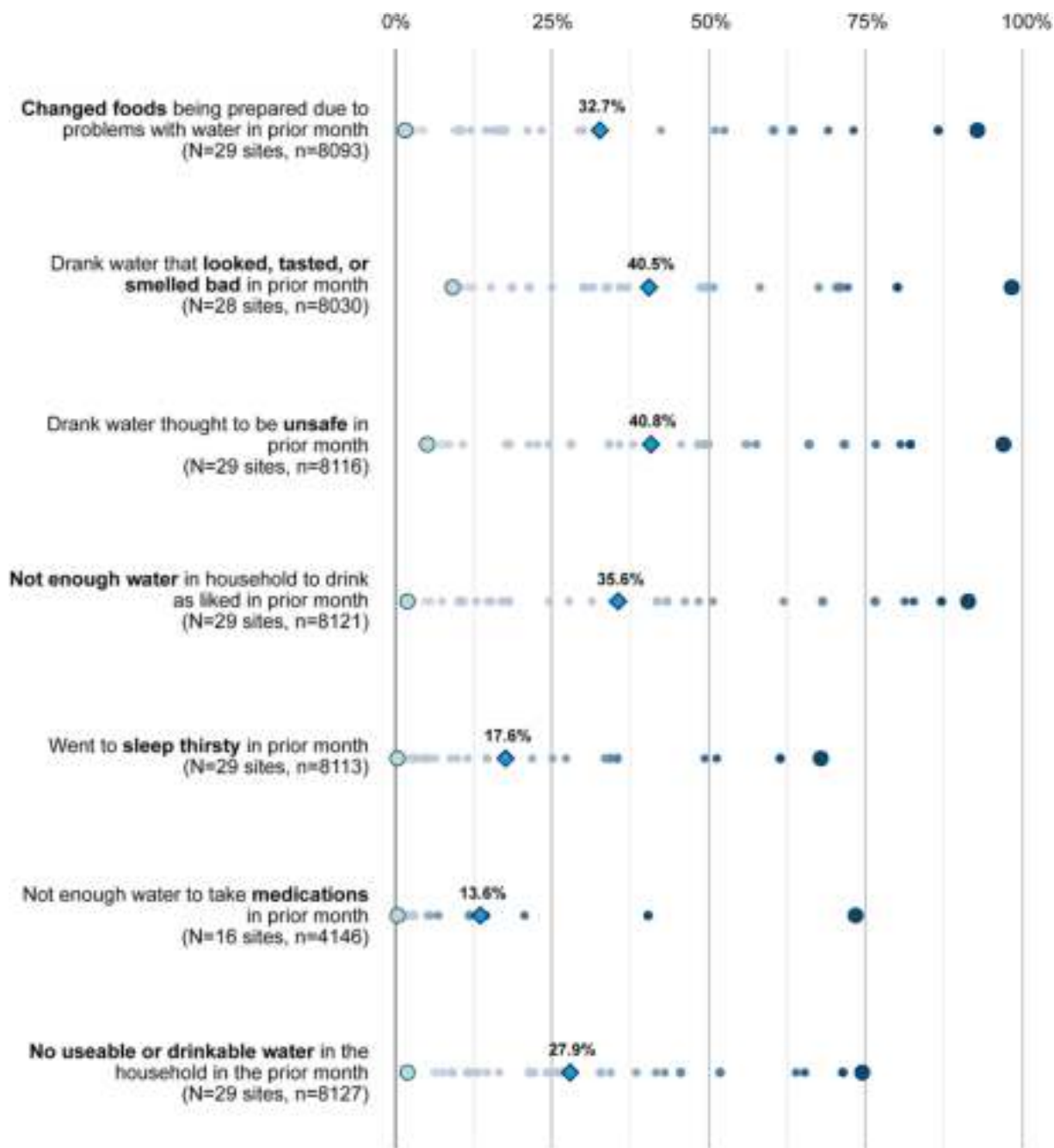
Fig. 7. Distribution by study site of percent of households that experienced water problems in the prior four weeks that could undermine physical or nutritional health (with percent across all sites in bold).

frequently suffer harassment, assault, and accidents while fetching water (Collins et al., 2019; Venkataramanan et al., 2020). This reality can shape water-related decision making and cause women to take risks, such as using alternative inferior water sources or navigating physically dangerous terrain to obtain water elsewhere (Smiley and Stoler, 2020).

3.2.3. Mobility

Because human mobility facilitated rapid transmission of COVID-19 from the start of the outbreak (Kraemer et al., 2020), many nations and municipalities have enforced self-quarantine in order to reduce spread and flatten the epidemiological curve. Some lockdowns have been vigorously enforced by police and even military forces (Human Rights Watch, 2020). In many regions, lockdown restrictions have had the unintended consequence of triggering mobility, as many struggled to return home in the absence of work opportunities and public transport.

The HWISE study asked how frequently households had considered moving their dwelling due to their water situation in the prior four weeks. We found that for most sites, at least some households had considered moving in the past four weeks, with an average of 21.8% across all sites (Fig. 3). In San Borja, Bolivia, 66.8% of households considered moving due to problems with water.

When water insecurity drives relocation, the introduction of COVID-19 into new locations is possible. Further, households often seek out more desirable water sources that can be chained to their commute and may therefore increase the risk of transmission to new neighborhoods if this source is outside their daily activity space (Smiley and Stoler, 2020). Such dynamics may be further complicated if these destinations are ill-equipped to support new arrivals safely. Common forms of circular migration, such as commuting or agricultural migrant labor travel patterns, also present opportunities to spread COVID-19 to origin
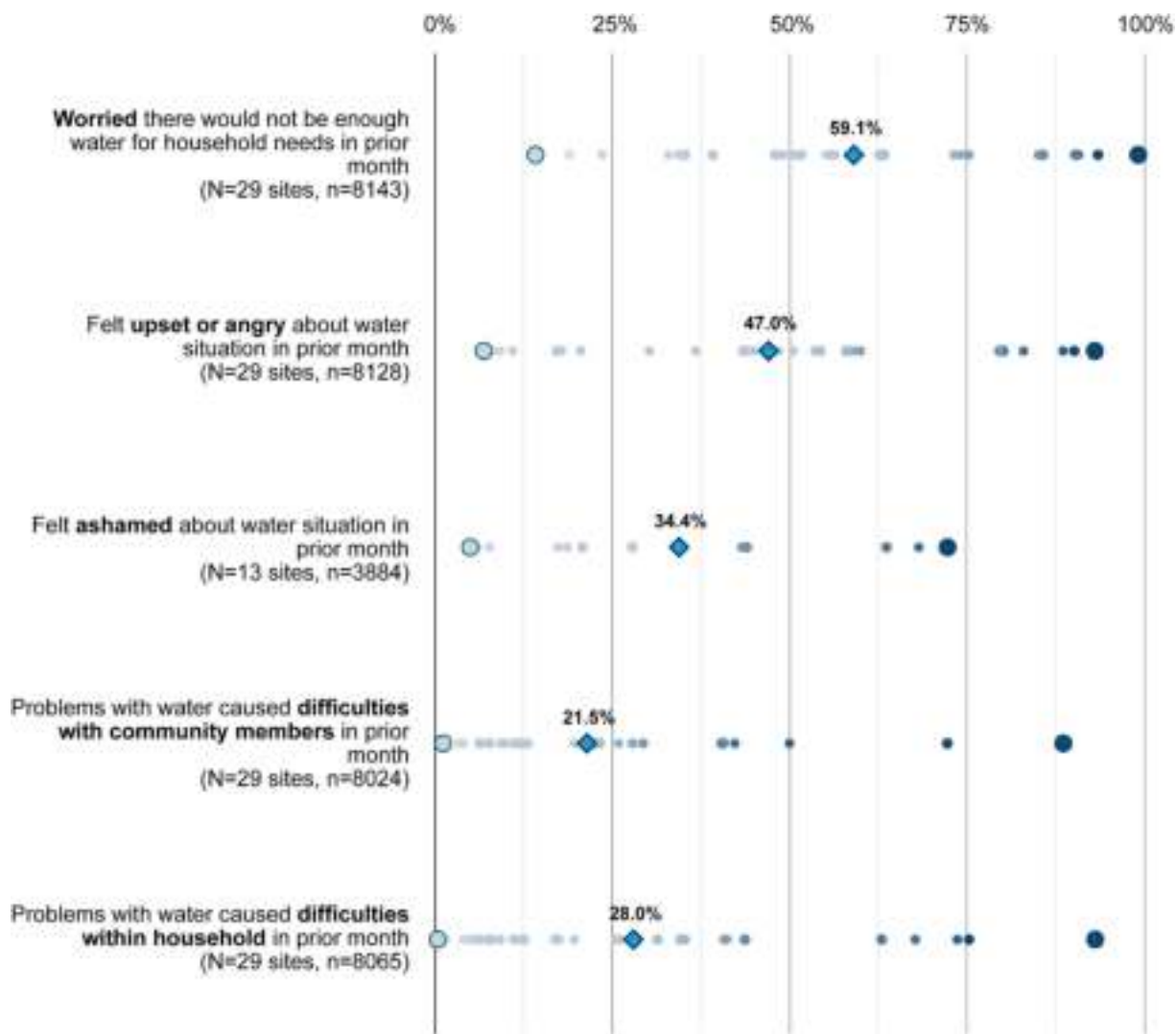
Fig. 8. Distribution by study site of percent of households that experienced perceived stress or interpersonal conflict in the prior four weeks due to problems with water (with percent across all sites in bold).

communities. If movement induced by water insecurity is migratory and COVID-19 is introduced, there is even greater potential for hostility in host destinations.

3.3. Contextual vulnerabilities

Many underlying biocultural and socio-economic vulnerabilities render water-insecure households even more susceptible to COVID-19 infection and at higher risk of severe disease. Context matters and can magnify vulnerabilities in communities with high population density and poverty, particularly places where self-isolation is impractical, such as informal settlements (Corburn et al., 2020) and refugee camps (Raju and Ayeb-Karlsson, 2020). For instance, in Beirut, Lebanon, we found that households inside urban refugee camps had higher water insecurity scores than nearby households outside the camps (mean $\pm$ sd, 10.2 ± 6.9 vs. 5.5 ± 6.5, $P < 0.0001$).

Water insecurity is shaped by water systems and the use and availability of different water sources. Water insecurity also intersects with livelihoods, socioeconomic status, and gender, as well as the sociomedical realities of food insecurity, thirst and dehydration, and mental health. We summarize the effects of each of these contextual factors in turn.

3.3.1. Water systems

The HWISE study evaluated the myriad configurations of how households collect, convey, and access water for domestic needs. We used these data to examine the variation in primary drinking water sources—one of the key metrics of the WHO Joint Monitoring Programme for Water Supply, Sanitation, and Hygiene—and assess how piped, collected, or delivered water may contribute to COVID-19 vulnerability.

Almost a quarter of surveyed households (24.3%) accessed water through a community water system (e.g., on-premises piped water). While the HWISE study did not collect data on shared connections across or among homesteads, overall these households are expected to face fewer risks of COVID-19 exposure unless they are selling water to others. Other important primary drinking water sources included dug wells (protected or unprotected) and boreholes, used by 17.9% of households. Only 1.8% of households engaged in rainwater collection as a primary drinking water source, though many sites were interviewed in the dry season. Taken together, these water sources—community water systems, wells, and rainwater collection, used by 44% of households—provide mechanisms of obtaining needed water for basic needs and hygiene that are generally compatible with physical distancing. Although community water systems depend on a labor force that may or may not be able to maintain systems during the height of quarantine, public systems and their workforce are still generally supported in ways that limit water service intermittency.

On the other hand, over half of our study households accessed water

in ways that potentially place people at an increased risk of COVID-19 transmission. Water provisioning is a profoundly social activity that requires considerable physical labor and social relations to fetch or collect water (Geere and Cortobius, 2017). Water fetching, as noted earlier, frequently involves queuing in close proximity and spending significant time waiting for one's turn or for water to be available. In the HWISE study, 27.4% of households reported that their primary drinking water source was a spring (3.5%), surface water (6.6%), or public standpipe (17.4%). COVID-19 exposure may occur while collecting or fetching water at any one of these sources through person-to-person contact. At springs or surface water sources, increased risk may result from proximity to others when there are limited safe fetching locations. At public standpipes, increased risk may occur with contact of common water points that are not disinfected.

The HWISE study also reported that almost 11% of surveyed households depended on drinking water delivery services. Over 7% of households reported that their primary drinking water source was delivered by tanker truck, and another 3.9% received water from a small-scale vendor. Similarly, over 15% of households depended on bottled or sachet water. Although not all modes of water delivery necessarily come directly to households, nearly all involve person-to-person conveyance and payments. Water delivery services have varying practices of contact with water containers that do not guarantee disinfection. During a public health emergency, dependence on informal water vendors carries additional risk of service disruption, as the informal workforce may cease operations in the name of physical distancing or due to other pandemic-driven government mandates.

3.3.2. Livelihoods

Livelihood impacts can be divided into two basic categories: non-monetary and monetary (Scoones, 2009). Non-monetary impacts involve aspects of the domestic economy that are not directly part of the cash economy, such as household tasks linked to the social reproduction of labour including child-rearing, domestic maintenance, and subsistence food production. Monetary economic impacts are those that are directly linked to the earning or spending of cash income, such as day labouring, trading or commodity agriculture. In practice, households are likely to be involved in a complex range of monetary and non-monetary economic activities, all of which could be impacted by the COVID-19 pandemic. We first present data about non-monetary livelihood impacts before turning to impacts on cash dimensions of livelihood strategies.

The HWISE study revealed that, prior to COVID-19, a significant number of households borrowed (39.7%) and loaned (31.5%) water as part of their regular livelihoods (Fig. 3). The variability in these indicators across sites was high, with respondents in sites like Arua, Uganda, suggesting that they almost never borrow/loan water, while those in other sites such as Cartagena, Colombia, very frequently do. Depending on specific lockdown policies, such non-monetised exchanges of water could be significantly affected. In Uganda, for example, the military was used to police the nationwide lockdown, and research partners reported feeling like "prisoners" in their homes, often unable to go out even to secure basic necessities (Human Rights Watch, 2020).

More indirect consequences could ensue if any of the borrowed/ loaned water is used for other sorts of productive activities, such as crop or livestock raising. In the HWISE study, 30.7% and 31.0% of households reported a lack of sufficient water for raising crops or livestock, respectively (Fig. 5). Moreover, 34.9% of respondents reported that problems with water prevented them from earning money, and 23.9% reported that water problems prevented children from attending school. It is plausible that suppression of the loaning/borrowing of water between vulnerable households could have damaging effects on income-generating activities (Wutich et al., 2018).

With respect to the monetary economy, there was considerable variability among the 29 sites; in Kisumu, Kenya, 74.7% of households experienced water problems that impacted their ability to earn money

whereas only 0.8% of households in Ceará, Brazil, reported this. Many who reported significant impact were day laborers, and the escalation of water-related obstacles to income generation may produce catastrophic effects on households' ability to earn needed cash. As we have seen in Peru, India, and elsewhere, lockdown policies triggered an exodus of poorer people from the cities, presumably since returning to home towns and villages offered more immediate access to non-commodity subsistence production (Staddon et al., 2020). Even in smaller towns, as earning potential decreased, households decamped to even smaller villages and rural areas in order to be closer to their agricultural fields (World Bank, 2020). The sudden severe curtailment of informal markets has disproportionately hindered poorer households' ability to satisfy even basic needs.

Across all sites, there was evidence that these impacts are experienced differently across socio-demographic groups. For example, in southwestern Uganda, minority Batwa communities reported more restrictive lockdown policing than in majority Bufimbira communities (Staddon et al., 2020). Indeed, as with many communicable disease outbreaks, socioeconomic status (SES) and gender disparities have considerable implications for COVID-19 risk and outcomes.

3.3.3. Socioeconomic status

The uneven distribution of resources, power, and voice by historical and current social and economic systems affects household water security (Jepson et al., 2017). The U.S. burden of COVID-19 has disproportionately fallen upon those of lower SES and communities of colour, intersecting with existing structural vulnerabilities, health comorbidities, and differential capacities for everyday mitigation efforts (Dorn et al., 2020) that has led to utility shutoffs and evictions (Meehan et al., 2020). These patterns are likely to be further magnified in LMICs (Hopman et al., 2020). We analysed the relationship between household SES tertile (i.e., relative low = 1, medium = 2, and high = 3) and the affirmation of several water insecurity experiences related to interruptions in household hygiene: (1) being worried that there would not be enough water for their needs, (2) unable to wash clothes, (3) unable to bathe due to water-related problems, (4) unable to wash their hands, and (5) unable to wash their children's hands. A higher percentage of low- and medium-SES households affirmed these water insecurity experiences than high-SES households (Fig. 6).

First, SES tertile was negatively associated with whether a household affirmed being worried that there would not be sufficient water for their needs ($\chi2$: 93.20, $P < 0.01$). Using a two-sample test of proportions (adjusted for multiple comparisons), we found that 54.2% of high-SES households affirmed water worry, a lower percentage than low-SES (64.5%; $P < 0.01$) and middle-SES (65.6%; $P < 0.01$) households.

Second, SES tertile was negatively associated with whether a household affirmed the inability to wash clothes ($\chi2$: 72.48, $P < 0.01$) and bathe ($\chi2$: 90.69, $P < 0.01$) in the prior four weeks. High-SES households reported being unable to wash their clothes (40.5%) and body (28.4%), at lower frequencies than low-SES (51.2% and 40.5%; both $P < 0.01$) and medium-SES (50.1% and 36.8%; both $P < 0.01$) households.

Third, SES tertile was negatively associated with whether a household affirmed the inability to wash their hands ($\chi2$: 39.90, $P < 0.01$) and wash the hands of their children ($\chi2$: 56.14, $P < 0.01$). High-SES households affirmed being unable to wash their hands (19.7%) and children's hands (19.8%) at lower frequencies than low-SES (26.8% and 28.9%; both $P < 0.01$) and medium-SES (24.6% and 25.4%; both $P < 0.01$) households.

Overall, a higher percentage of medium- and low-SES households affirmed water insecurity experiences compared with high-SES households, but the overall percentages across tertiles were high, ranging from approximately 20-66%. This underscores the need for efforts at all scales to improve household water security across multiple dimensions of human well-being, particularly minimizing risks associated with COVID-19 transmission, morbidity, and mortality. Such efforts will require

improved water service provision as well as efforts to reduce potential socioeconomic barriers to sufficient water access through government subsidies or other policies.

The HWISE study also recorded the sex of the household head, and we found that female-headed low-SES households most commonly affirmed water insecurity experiences related to aspects where higher quantities of water are required, such as not being able to bathe (43.3%) or wash their clothes (53.6%) in the prior four weeks. COVID-19 clearly presents specific risks for women, including challenges for menstrual hygiene (UNICEF, 2020), and serious economic impacts given potential for lower earnings and savings, higher exposure to COVID-19 through occupational work (women comprise 70% of the global healthcare workforce), additional unpaid work such as caring for sick children or elders, and gender-based violence resulting from policy measures designed to reduce the transmission of COVID-19 (United Nations, 2020).

3.3.4. Food insecurity

Household food insecurity often co-occurs with water insecurity. In the HWISE study, the overall correlation between food and water insecurity scores was 0.37 ($P < 0.001$), with correlations among study sites ranging from −0.02 to 0.74. Brewis et al. (2020) also tested multiple sub-dimensions of water insecurity (need, quality, water worry) using the same HWISE dataset, and found all to be associated with greater household food insecurity. In site-specific regressions, greater water need was positively associated with food insecurity for 24 of 27 sites. The only exceptions were Acatenango, Guatemala (the site with the smallest sample), Punjab, Pakistan (the most water-insecure site), and Pune, India (the most water-secure and also one of the most food-secure sites) (Brewis et al., 2020).

Theoretically, there is reason to expect that water insecurity is a driver of food insecurity rather than the other way around, particularly because water is needed to grow crops, feed livestock, and prepare and cook adequate safe foods but not the converse (Brewis et al., 2020; Wutich and Brewis, 2014). For example, water is needed to irrigate household gardens and maintain animals, prepare food safely, and many of the most readily available cereals, tubers, and grains (e.g., rice, cassava) specifically require treatment with water to make them digestible and safe. Note that many sites reported high levels of "having to change foods being prepared" (Fig. 7) and "water situation impacted livestock" and "water situation impacted crops" (Fig. 5).

The observation of a strong and consistent association between food and water insecurity across many of the global sites, with water insecurity as a potential driver, has implications for COVID-19 in several dimensions based on elevation of risk of food insecurity. There are many mechanisms by which household food insecurity might potentially impact COVID-19 risk to individuals, households, and communities, and we would then expect water insecurity to elevate these. First, it is well-established that acute and chronic undernutrition creates additional risks for infection (Calder and Jackson, 2000). Undernutrition undermines barrier function, allowing easier entry of infectious agents, and it also weakens immune function, making it harder to recover from infections. Also, water security appears to be important for mothers' capacity and decisions about how they (breast)feed their children (Collins et al., 2019; Schuster et al., 2020), an additional way in which water may be hypothesized to affect children's immune function. Third, under low-resource conditions, money, assets, or social capital deployed to deal with water (or food) insecurities can undermine household budgets or other means to cope with health challenges, including healthcare and other spending relevant to the prevention and treatment of infectious disease (Brewis et al., 2020).

3.3.5. Thirst and dehydration

One of the most common recommendations that patients with respiratory infections receive from doctors, is to "drink plenty of fluids" (Guppy et al., 2004). This recommendation is given to reduce risk of dehydration through insensible water loss and account for potential

lower water intake, as well as reduce mucus thickness (Guppy et al., 2004). Poor water accessibility and reliability may make it difficult to meet this recommendation. Lacking access to water while in a mildly dehydrated condition increases anxiety and can change decision-making practices (H. A. Young et al., 2019), which may increase transmission risk of COVID-19.

Across all HWISE study sites, 17.6% of households reported going to sleep thirsty at least once in the last four weeks. At every site, some households reported water problems so severe that they reported at least one night of going to sleep thirsty, ranging from 0.4% in Ceara, Brazil, to 67.9% in Kahemba, DRC (Fig. 7). In addition, 27.9% of all households had no useable or drinking water at least once in the prior month; 40.5% drank water that looked, tasted, or smelled bad; and 40.8% drank water thought to be unsafe. We would not expect any of these experiences to be associated with optimum hydration.

Our results indicate that a large proportion of households would not be able to meet the treatment recommendation of "drink plenty of fluids" if they were to contract COVID-19. While dehydration was not explicitly measured in the HWISE study, from a physiological perspective, thirst can be indicative of dehydration (Weinberg et al., 1995). It is likely that some proportion of the individuals in these households are also dehydrated, which can negatively affect cognitive performance and lead to confusion, particularly among older adults, as well as lead to higher risk of kidney stones (Popkin et al., 2010; Weinberg et al., 1995). Therefore, prevention and at-home treatment of COVID-19 may be more challenging under these conditions.

3.3.6. Mental health

Water insecurity is strongly linked to mental ill-health. Early exploratory research found high rates of worry, anger, shame, and conflict among people experiencing water insecurity (Sultana, 2011; Wutich and Ragsdale, 2008). Recent research finds that water insecurity is associated with symptoms of anxiety and depression (Mushavi et al., 2020). Seven key mechanisms appear to produce water-related mental ill-health: (1) worry about illness and health problems, (2) conflict in households and communities, (3) status loss or social failure, (4) loss of connection to meaningful places, (5) reduced autonomy or opportunities, (6) poverty, deprivation and uncertainty, and (7) unjust or unfair treatment (Wutich et al., 2020). It is likely that COVID-19 will intensify most, if not all, of these pathways. We highlight three likely dynamics: worry about illness and health problems, conflict in households and communities, and status loss or social failure.

Water insecurity puts people at higher risk of contracting COVID-19, and thus is distressing. Past research shows that people feel significant worry over the risks of drinking contaminated water, the physical risks of water fetching, and thirst and hunger borne of water insecurity (Wutich et al., 2020). Our data indicate that 59.1% of households reported that at least one member experienced worry that they would not have enough water in the last four weeks (Fig. 8). This is likely to intensify as people worry about contracting COVID-19 at water-fetching points, contamination of stored water, and the necessity of drinking less-safe water if cash becomes short and water-points become inaccessible.

Water insecurity precipitates significant conflicts both within households and across communities. Within households, conflicts tend to center on financial worries and water shortages, with women often bearing the brunt of blame and experiencing higher risk of intimate partner violence (Choudhary et al., 2020). Within communities, water insecurity produces conflict as people argue over access to water sources (Sultana, 2011). In the HWISE study, 28.0% of households reported intra-household difficulties over water, and 21.5% reported extra-household difficulties over water (Fig. 8). As the stakes of water insecurity increase during a COVID-19 outbreak, we would predict such conflict would also increase—for example, if people are unable to meet physical distancing expectations while queuing for water.

Water insecurity can create status loss, especially for households that

are impoverished, unable to maintain hygiene norms, or are perceived as too dependent on others (Wutich and Brewis, 2014). This can be particularly true for households that lean more heavily on water sharing arrangements when water becomes scarce (Wutich et al., 2018). Shame and anger are common emotional responses to the threat of status loss (Sznycer et al., 2016). In our sample, 47.0% of households reported anger/upset and 34.4% reported shame over water (Fig. 8). As communities experience economic and social disruptions related to COVID-19, people will likely find it increasingly difficult to conform to social norms and expectations (such as hygiene norms) and may experience anger and shame over status loss as a result.

3.3.7. Household sanitation

Inadequate sanitation and water insecurity are mutually reinforcing, as the inability to effectively separate human waste more readily introduces pathogens into the domestic environment. Recent evidence indicates gut involvement of SARS-CoV-2, with prolonged viral shedding found in both symptomatic and non-symptomatic patients (Wu et al., 2020). Although the HWISE study did not ask about water use for sanitation, the gut involvement of SARS-CoV-2 poses several implications for WASH-insecure households.

Gastrointestinal symptoms require increased attention to fecal management when sick individuals increase use of bathrooms, latrines, or chamber pots. Evidence is also emerging for fecal-oral transmission of the virus (Yeo et al., 2020), with implications for WASH-insecure areas. Even if fecal-oral transmission proves uncommon, sanitation security nonetheless is critical. Shared sanitation facilities, such as latrines, are often small enclosed spaces that experience high usage and thus present additional COVID-19 transmission risk (Caruso and Freeman, 2020). We may see increases in droplet and fomite transmission of COVID-19 if facilities are inadequately cleaned. Effective management of diarrhea requires sufficient quantities of safe water for cleaning as well as for hydration, hand hygiene, and caregiving.

Second, inadequate sanitation is a key driver of environmentally mediated transmission of enteric infections. As COVID-19 spreads to countries with high burdens of enteric infections, enteric infections may emerge as important comorbidities that shape epidemic trajectories given the noted effects of infection with SARS-CoV-2 in the gastrointestinal tract.

4. Conclusion

Water is a critical component of human well-being and development (Jepson et al., 2017), and household water insecurity presents an array of complications for COVID-19 prevention and control. Although the pandemic is exacerbating socio-medical inequalities in high-income nations (Dorn et al., 2020), water insecurity is experienced everywhere, and its effects are especially salient in LMICs. Our analysis of empirical data from 8,297 households across 29 LMIC sites illustrate how the WHO guidelines for COVID-19 control are difficult to meet given the everyday realities faced by billions of people. For example, 71% of households had recently experienced a water-related problem that has the potential to undermine COVID-19 control strategies or intensify disease progression, and 46% faced specific challenges with handwashing and physical distancing, both core elements of WHO's guidelines for COVID-19 prevention and control. In light of new, more infectious strains of COVID-19 like B.1.1.7 (Galloway et al., 2021), prevention strategies such as masking and physical distancing are even more important given initial delays to vaccine deployment. Although our study sample is not representative of all LMICs or communities in the countries sampled, the water problems documented here clearly exemplify the *range* of substantial issues endured by water-insecure households across diverse rural and urban contexts. While water issues alone cannot forecast COVID-19 intensity, these data suggest some practical means to identify where particular and layered risk for COVID-19 are located within LMICs: those communities with particularly high levels of water insecurity.

Our analysis has limitations. The data do not include information beyond households, and non-household water sources could be an important and effective means for households to obtain water. Nearly two billion people depend on healthcare facilities without basic water services, which increases COVID-19 risks for patients and health care workers (WHO/UNICEF, 2020b). Additionally, over 30% of schools globally lack access to an onsite water point and 36% have no hygiene facilities. These institutions are more likely to be found in communities with poor household water security, multiplying the risks to people living there, especially during the pandemic.

This pandemic necessitated legislation of economic stimulus and relief packages in high- and middle-income nations. Although the cost of achieving Sustainable Development Goal (SDG) 6 would require a significant increase over our current global investment, the cost of achieving universal basic WASH services has been estimated at US$28.4 billion per year from 2015 to 2030 (i.e., US$426 billion, non-discounted), or 0.10% of global GDP (Hutton and Varughese, 2016). In March and April 2020 alone, the United States committed US$3 trillion in a series of four economic relief packages, and the European Union committed €540 billion, with significant additional relief legislation pending on both continents. Other countries have followed suit, such as Japan's US$992 billion stimulus package, China's US$500 billion fiscal stimulus, and India's US$260 billion COVID-19 relief package. Compared to what the world will ultimately end up spending on emergency economic measures, the cost of universal basic WASH looks like a bargain, something we have known for years (Hutton et al., 2007). We emphasize that while these issues are most acute in situations such as the current pandemic, basic WASH services have implications for many other key considerations ranging from mitigating the global burden of disease to improving education outcomes and improving gender equity. WASH service coverage has long been a pressing global concern; COVID-19 has made its importance more visible and urgent.

In the short term, the COVID-19 crisis should trigger increased investments and attention to WASH issues by LMICs and the development community as seen after the 2014-16 Ebola outbreak in West Africa (Cooper, 2020a). Eleven African nations announced different forms of free water for the urban poor and other groups, for example paying users bills in some areas in Ghana (Amankwaa and Ampratwum, 2020; Smiley et al., 2020), providing water for vulnerable communities and informal settlements in Kenya, and the World Bank supporting 20 new water points for poor communities in Democratic Republic of the Congo supplied by utility-owned water tankers (Cooper, 2020b). Government stimulus programs may also need to directly address public-private partnerships (PPP) that have historically played a crucial role in WASH service provision. These partnerships may have the physical and social infrastructure to immediately improve water access for many vulnerable communities. But the pandemic has created financial stress for PPPs across industries globally and threatens widespread project failure from logistical disruption and new budget constraints, including the loss of revenue from regional lockdowns (Baxter and Casady, 2020; Casady and Baxter, 2020). PPP engagement requires caution, as goals and accountability are not always well-aligned between partners, and profit imperatives can clash with equity and other priorities.

As policymakers and program implementers scale-up the immediate distribution of emergency WASH supplies, we encourage deeper consideration of how these activities can be transitioned into targeted, sustainable, long-term WASH investments. High-resolution geospatial mapping of water and sanitation access, like those produced by the Local Burden of Disease WaSH Collaborators in 2020, can provide subnational guidance on priority regions (Deshpande et al., 2020). Although solutions should be tailored to local needs and constraints, it is clear that most communities will benefit from improvements in both water infrastructure and water governance, and also the way water managers distribute water and remain responsive to the needs of diverse users. Ultimately, such strategies have the potential to greatly improve the overall health of communities and increase their resilience to future

shocks, including another pandemic. Housing and utility policies may help deter COVID-19 transmission as well: in the US and Iran, moratoria on eviction and electricity and water shutoffs have helped contain disease spread, although the economic impacts on landlords and utilities remain unclear (Jowers et al., 2020; Seddighi et al., 2020).

Strengthening household water security is critical for preventing and mitigating the effects of future pandemics in underserved communities around the world (Cooper, 2020b). The world was already off track to achieve SDG 6 before the COVID-19 pandemic, and innovations in water economics, engineering, and management will be critical to accelerate the pace of progress (Sadoff et al., 2020). In the meantime, adequate and affordable WASH services can mitigate the potentially compounding effects of COVID-19 and future pandemics with the traditional burdens of water insecurity, especially in the most historically marginalized households and communities.

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Declaration of competing interest

This research does not contain any conflict of interests.

Acknowledgments

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Impact of short- and long-term exposure to air pollution on blood pressure: A two-decade population-based study in Tehran

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ABSTRACT

Plenty of recent studies on the impact of air pollution on blood pressure (BP) exist; however, there is a lack of data for the highly polluted Eastern Mediterranean region. We evaluated the associations of short-term exposure to air pollutants with systolic BP (SBP) and diastolic BP (DBP) and the long-term impact of air pollutants on incident hypertension, among Tehranian adults. In the Tehran Lipid and Glucose Study, 4580 nonhypertensive participants aged 20–69 years (41.6% male) were followed from 2001 to 2018 through 3-year follow-ups and 4–5 examinations of them were recorded. The air pollutants included particulate matter with a diameter ≤ 10 μm (PM_{10}), carbon monoxide (CO), ozone (O_3), nitrogen dioxide (NO_2), and sulfur dioxide (SO_2). The mixed-effects transition model estimated the air pollution impact on BP. The proportional hazards Weibull model measured the long-term effects of air pollutants on the multivariate hazard of incident hypertension. The air pollutants were put in the models in the form of mean annual level, applying three versions of 1, 2, and 3 years before the follow-ups. During a median follow-up of 12.3 years, 1618 cases of hypertension were found. In the short-term, increase in CO did not affect SBP but decreased DBP with a delay effect lasting for 14 days; increase in NO_2 raised SBP with a 14-day lag, however did not change DBP; increase in O_3 reduced SBP with a 14-day lag but made slight non-significant increase in DBP; rise in PM_{10} concentrations led to increased SBP (lag 0–3 days) and DBP with lags of 0–3 days and 12–14 days and increase in SO_2 made the largest increases in DBP with lags lasting for 14 days, but did not affect SBP. Regarding incident hypertension in the long-term, the increase in CO had no significant effect; increase in NO_2 decreased the risk over the 2- and 3-year time spans; rise in O_3, PM_{10}, and SO_2 levels increased the risk in all time spans; the largest hazard ratio [1.96 (95% CI: 1.48, 2.62)] for incident hypertension was attributable to PM_{10} in 3 years. Considering the major effects of air pollutants including O_3, SO_2, and especially PM_{10} on incident hypertension, urgent public health policies should be implemented to reduce the burden of air pollution in metropolitan city of Tehran.

1. Introduction

Hypertension plays causal role in a broad spectrum of cardiovascular and renal diseases and is one of the leading causes of the global burden of diseases (Lackland and Weber, 2015; Lim et al., 2012). According to the recent studies, hypertension is no longer restricted to developed countries. Nowadays, many individuals throughout the world including a high proportion of population living in the Eastern Mediterranean region (EMR) suffer from hypertension and its consequences (Bromfield and Muntner, 2013; Turk-Adawi et al., 2018). Among Tehranian adults, the prevalence of hypertension is 47.1% and 20.4% according to American College of Cardiology/American Heart Association

(ACC/AHA) and JNC7 guidelines' definitions, respectively (Asgari et al., 2019). Moreover, we demonstrated that hypertension has a population attributable fraction of approximately 21% and 17% for cardiovascular diseases and all-cause mortality, respectively (Sardarinia et al., 2016). Hence, there is a mounting imperative to identify, control, and if possible mitigate predisposing factors for hypertension.

Numerous studies have confirmed the key role of sodium intake, obesity, sex, aging, lack of activity, diabetes and smoking in the hypertension incidence (Sun et al., 2017). Environmental factors particularly air pollution, have been at the center of focus of several recent studies (Brook et al., 2011). A growing number of studies over the past decade have found out that exposure to several air pollutants can affect blood pressure (BP). A comprehensive systematic review and meta-analysis conducted by Yang et al. demonstrated that long-term exposure to particulate matter ≤ 2.5 μm in diameter ($PM_{2.5}$) is associated with hypertension and exposures to particulate matter ≤ 10 μm in diameter (PM_{10}), and nitrogen dioxide (NO_2) are associated with diastolic blood pressure (DBP). Moreover, similar associations were found between short-term exposure to PM_{10}, $PM_{2.5}$, NO_2, and sulfur dioxide (SO_2) with hypertension, $PM_{2.5}$ and SO_2 with systolic blood pressure (SBP), and PM_{10}, $PM_{2.5}$, NO_2, and SO_2 with DBP. However, the included studies were mostly conducted in European countries and China, an issue reported as a significant source of heterogeneity. There remains a paucity of data from other heavily-polluted and highly populated regions such as countries located in the EMR (Yang et al., 2018).

Although the level of air pollution and burden of high BP are greater in low- and middle-income countries (Balakrishnan et al., 2019), most prior studies have been conducted in high-income ones (Szyszkowicz et al., 2012; Wellenius et al., 2012). More importantly, as a result of differences in ethnicity (e.g., genetics), lifestyle, diet, and environmental exposures, the findings of studies from high-income countries cannot be necessarily extrapolated to low- and middle-income countries (Su et al., 2011; Yang et al., 2018). Therefore, conducting epidemiological studies in these regions is beneficial to clarify the effects of higher concentrations of air pollution on BP and to inform about the entire global burden of disease of air pollution.

Tehran, the capital of Iran, is the 19th largest city in the world and like other large cities is confronting heavy burden of air pollution mainly originating from motor vehicles. The major pollutants are PM_{10}, SO_2, NO_2, ozone (O_3), hydrocarbon, and carbon monoxide (CO) (Hosseinpoor et al., 2005; Naddafi et al., 2012). As far as we are aware, no prior study has evaluated the effects of air pollution on BP in the EMR region. Therefore, we designed a study with the aim of examining the associations of short-term exposure to several air pollutants including PM_{10}, SO_2, NO_2, CO, and O_3 with BP (including SBP and DBP) as well as their long-term exposure with incident hypertension in Tehranian adults during 2001–2018.

2. Materials and methods

2.1. Study design and population

The Tehran Lipid and Glucose Study (TLGS) is a prospective cohort study initiated in 1999, designed to investigate cardiovascular diseases (CVD) risk factors among a representative sample of Tehran (Azizi et al., 2009; Khalili et al., 2018). TLGS recruitment contained two stages, the first one from Jan 1999 until Jul 2001, and the second recruitment stage in the period of Oct 2001–Sep 2005. Upon entering the study (phase 1), five more examinations (phases 2 to 6), approximately every 3 years, have been conducted until April 2018.

Due to the scarcity of the air pollution data over the TLGS recruitment period, the TLGS second phase was chosen as the baseline in the present study with 7698 participants aged 20–69 years. Exclusions included those with prevalent hypertension at the baseline (n = 1327) and those having body mass indexes (BMI) <18.5 or >50 kg/m^2 (n = 513), leaving 5858 participants. Besides, 1278 individuals were

excluded due to having <4 out of five possible examinations, remaining 4580 individuals for data analysis. Comparison of the baseline characteristics of these two groups (1278 vs. 4580) is presented in Supplementary Table 1. All participants signed written informed consent. The Ethics Committee of Shahid Beheshti University of Medical Sciences studied and confirmed this investigation (IR.SBMU.RETECH. REC.1399.230).

2.2. Clinical and laboratory measurements

A skilled interviewer obtained information concerning demographic characteristics, family history of cardiovascular disease, history of medications, and smoking habits using a standard questionnaire. Detailed information regarding anthropometric measurements including BMI, waist circumference (WC), BP measurements as well as laboratory measurements were reported elsewhere. Specifically, for BP measurement, after 15 min of rest in sitting position, SBP and DBP were measured using a standardize mercury sphygmomanometer on the right arm. The first and fifth Korotkoff sounds were considered as SBP and DBP, respectively (Azizi et al., 2002).

2.3. Definition of terms

Hypertension was defined as SBP ≥ 140 and/or DBP ≥ 90 mmHg or using anti-hypertensive medication. BMI was defined as weight (kg)/ height (m^2). Current and past smokers were defined as ever-smokers. A person was considered to have type 2 diabetes if declared to be on glucose lowering medications or having fasting plasma glucose (FPG) ≥ 126 mg/dL.

2.4. Exposures

The air pollution indices constituted the covariates of interest. The data were obtained from the Tehran air quality control website (TehranAQI), a data source which reports the daily measurements of six chief urban air pollutants: PM_{10}, $PM_{2.5}$, SO_2, CO, NO_2, and O_3. These items are measured hourly by 23 stations, located in various parts of the Tehran municipality, using the Beta Attenuation Monitoring technique, ultraviolet (UV) fluorescence, infrared absorption, chemiluminescent reaction, and UV-spectrophotometry methods, respectively; their daily averages were utilized in the analyses. The measurement unit was mg/m^3 for CO, and μg/m^3 for the other five indices. The Tehran's air pollution has been monitored since 1999; thus, mentioned air pollutants have been measured through the TLGS follow-up years except for the $PM_{2.5}$ which was not measured before 2010. These air pollution data have been validated independently in two studies (Shamsipour et al., 2014; Yousefian et al., 2020).

Likewise, during the study follow-up years (i.e. 2001-2018), daily temperatures of Tehran were measured by four stations situated in Tehran metropolitan area and the stations' averages were used in the analyses. These data were provided from the national oceanic and atmospheric administration (United States department of commerce) website (NOAA).

2.5. Statistical analysis

First, in the short-term analysis, the impact of air pollution on two response variables (SBP and DBP) was assessed. These longitudinal measures contained two distinct sources of diffusion including between individual differences and the impact of previous responses on the proceeding ones. The analytical model capable of considering both of these sources is known as the mixed-effects transition (MET) model (Funatogawa and Funatogawa, 2012). The model could be displayed as follows:

$$y_{it} = \beta_0 + \beta_1 \, Air \; pollution + b_0 + \gamma y_{i,t-1} + \varepsilon_{it}$$

In the model, y represents either of the two responses, the i and t subscripts correspond to individuals and measurement times, respectively, b_0 is the random intercept capturing the inter-individual heterogeneity, γ measures the effect of the lagged response, and ε is the random error. The study participants had at least four separate BP levels measured every 3 years during follow-up and the exposures were related to BP at each time point.

The air pollutants, CO, O_3, PM_{10}, NO_2, and SO_2 were each put in the model, separately; the limited measurement of $PM_{2.5}$ prevented it from being used in the longitudinal analysis. The air pollutants effects were assessed on the same-day and going back for 14-day lags. Besides fitting the model in this crude form, the potential confounders including temperature ($\circ$C), age, sex (male/female), BMI (kg/m^2), WC (cm), diabetes (yes/no), anti-hypertensive drug use (yes/no), and being ever-smoker (yes/no) (Sun et al., 2017) were considered in the adjusted model. To facilitate the model fitting process, all right-hand side terms were put in the model in a centered form.

Fitting the MET model using any of the standard mixed-effects packages would produce inconsistent regression coefficients because of ignoring the correlation between the lagged response and the random intercept. This difficulty is known as the initial conditions problem (Crouchley and Davies, 2001). A few solutions are proposed for this problem; we used the instrumental-variable estimation approach suggested by Arellano and Bond (1991) (Arellano and Bond, 1991) to obtain valid estimates.

The second analysis measured the long-term effects of air pollutants on the hazard of incident hypertension through fitting the proportional hazards Weibull models. Hazard ratios (HRs) were computed for every 10-unit increment in each of the air pollutants excluding CO considering its relatively narrow range, in which the risk was estimated for every 1-unit increment. The participants were followed until the hypertension incidence, death or the end of follow-up. As the measurements were done in almost 3-year time spans, the exact time of incidence was unknown; hence, the interval censoring was reflected in the modeling to capture this phenomenon and the parametric model of Weibull was used. The models were fitted both crudely and adjusted for temperature ($\circ$C), age, sex (male/female), BMI (kg/m^2), WC (cm), diabetes (yes/no), and being ever-smoker (yes/no), just as the first analysis. The air pollutants and temperature were put in the models in the form of mean annual level, applying three versions of 1, 2, and 3 years before the follow-ups.

The analyses were performed using the Stata software version 15. The significance level is chosen to be 0.05.

3. Results

The study sample consisted of 4580 nonhypertensive participants (male = 1904) aged 20–69 years at baseline. Table 1 presents a description of the response variables and the assessed confounders at the baseline; Table 2 shows the exposures distribution during the study period. The Spearman correlations between the air pollution indices are

Table 1
Baseline characteristics of the study population (Tehran Lipid and Glucose Study).

Variable	Mean (SD) or number (%)
Age (years)	40.8 (12.3)
Sex (male)	1904 (41.6%)
Ever-smoker (yes)	912 (19.9%)
Diabetes (yes)	265 (5.8%)
Body mass index (kg/m^2)	27.3 (4.4)
Waist circumference (cm)	90.4 (11.8)
Systolic blood pressure (mmHg)	110.6 (12.0)
Diastolic blood pressure (mmHg)	72.4 (8.4)

Values are presented as mean (SD) or number (%).
SD, standard deviation.

described in Table 3. Among the five pollutants, negative associations of NO_2 with CO and SO_2 were the highest.

3.1. The short-term analysis

The amounts of changes in SBP and DBP for each 10-unit increase of the air pollutants (1-unit for CO) in the adjusted model and in terms of the lag effects of 0–14 days are shown in Fig. 1; supplementary Tables 2 to 11 present the corresponding results for both crude and adjusted models.

According to Fig. 1, increase in CO did not affect SBP but decreased DBP with delay effect lasting for 14 days; increase in NO_2 was accompanied by raised SBP with a lag of 0–14 days and no change in DBP; increase in O_3 reduced SBP with the lag of 0–14 days but made slight non-significant increase in DBP; rise in PM_{10} concentrations led to increase of SBP (lag 0–3 days) and DBP with lags of 0–3 and 12–14 days and most interestingly, increase in SO_2 was associated with the largest increases in DBP with lags lasting for 14 days and no change in SBP. Among the adjusted covariates, age and BMI were always associated with significantly increased levels of BP (data not shown).

3.2. The long-term analysis

During a median follow-up of 12.3 (interquartile range of 10.9–13.3) years, 1618 cases of incident hypertension were found. The adjusted HRs of hypertension for each 10-unit increase of the air pollutants (1-unit for CO) in three different time windows of 1, 2, and 3 years are shown in Fig. 2. Supplementary Table 12 details the equivalent results for both crude and adjusted models.

As Fig. 2 shows, for incident hypertension, the increase in CO had no significant effect; increase in NO_2 decreased the risk over the 2- and 3-year time spans and rise in O_3, PM_{10}, and SO_2 levels increased the risk over the 1-, 2-, and 3-year time spans. The largest HR [1.96 (95% CI: 1.48–2.62) for incident hypertension was attributable to PM_{10} in 3 years. Among the adjusted covariates, age, BMI, WC, and diabetes were associated with significantly higher risk of incident hypertension (data not shown).

4. Discussion

In this population-based study from the EMR region over nearly two decades of follow-up, we investigated the short- and long-term effects of five major air pollutants on BP among residents of the Tehran metropolitan area considering well-known hypertension confounders. In the short-term, throughout the 14-day lag, NO_2 and O_3 had positive and negative significant associations with SBP, respectively, and SO_2 and CO had positive and negative associations with DBP, respectively. Moreover, the positive associations between PM_{10} and SBP and DBP were observed in several lag days. Likewise, long-term exposure to O_3, PM_{10}, and SO_2 increased the risk of hypertension in all three years; 2-year and 3-year exposures to NO_2 decreased the hypertension risk significantly.

4.1. PM$_{10}$

In the current study, generally, in the short-term, increase in PM_{10} levels had no significant association with SBP but positive associations with DBP in 8 out of 14 lag days, findings in accordance with a recent meta-analysis showing no association between the short-term exposure of PM_{10} and the level of SBP and a positive association with DBP. It is of importance to note that the association between PM_{10} and DBP was highly heterogeneous (Yang et al., 2018). Exposure to PM_{10} significantly increased the risk of hypertension in all three years. In the Yang et al. study, although the overall association between PM_{10} and hypertension was not significant, a significant relationship was found in studies with high air pollution values (Yang et al., 2018), an issue justifying significant associations which we found among residents of Tehran, a highly

Table 2

The distribution of exposures in Tehran during the study period 2001–2018.

	Descriptive statistics					Percentiles						
	Mean	SD	Min	Max	IQR	1	5	25	50	75	95	99
PM_{10} (µg/m³)	62.9	19.2	5	278	22	29	37	51	61	73	95	120
SO_2 (µg/m³)	34.4	12.5	5	108	17	10	16	25	33	42	56	72
NO_2 (µg/m³)	41.0	21.4	6	110	36	11	14	22	35	58	78	92
CO (mg/m³)	3.5	1.5	1	30	1.5	1.7	1.9	2.5	3	4	6	8
O_3 (µg/m³)	35.2	18.4	2	130	22	7	12	22	32	44	69	99

Table 3

Spearman correlation between PM_{10}, SO_2, NO_2, CO, and.O_3

	PM_{10}	SO_2	NO_2	CO	O_3
PM_{10}	1				
SO_2	0.19*	1			
NO_2	0.21*	−0.27*	1		
CO	0.15*	0.34*	−0.42*	1	
O_3	0.13*	−0.02	0.02	−0.21*	1

* P-value<0.05.

polluted city (Hosseinpoor et al., 2005).

4.2. CO

CO is produced from incomplete combustion of fossil fuels. In our study, short-term exposure to CO was associated with non-significant increase in SBP. A panel study has reported similar positive association which was significant only in lags 0 and 4 (de Paula Santos et al., 2005). Moreover, we demonstrated short-term CO exposure was associated with lower DPB in all lag days. A randomized crossover study showed non-significant positive and negative associations between CO and DBP in lags 0 and 1, respectively (Liu et al., 2014).

In the long-term, we found a signal for the lower risk of hypertension
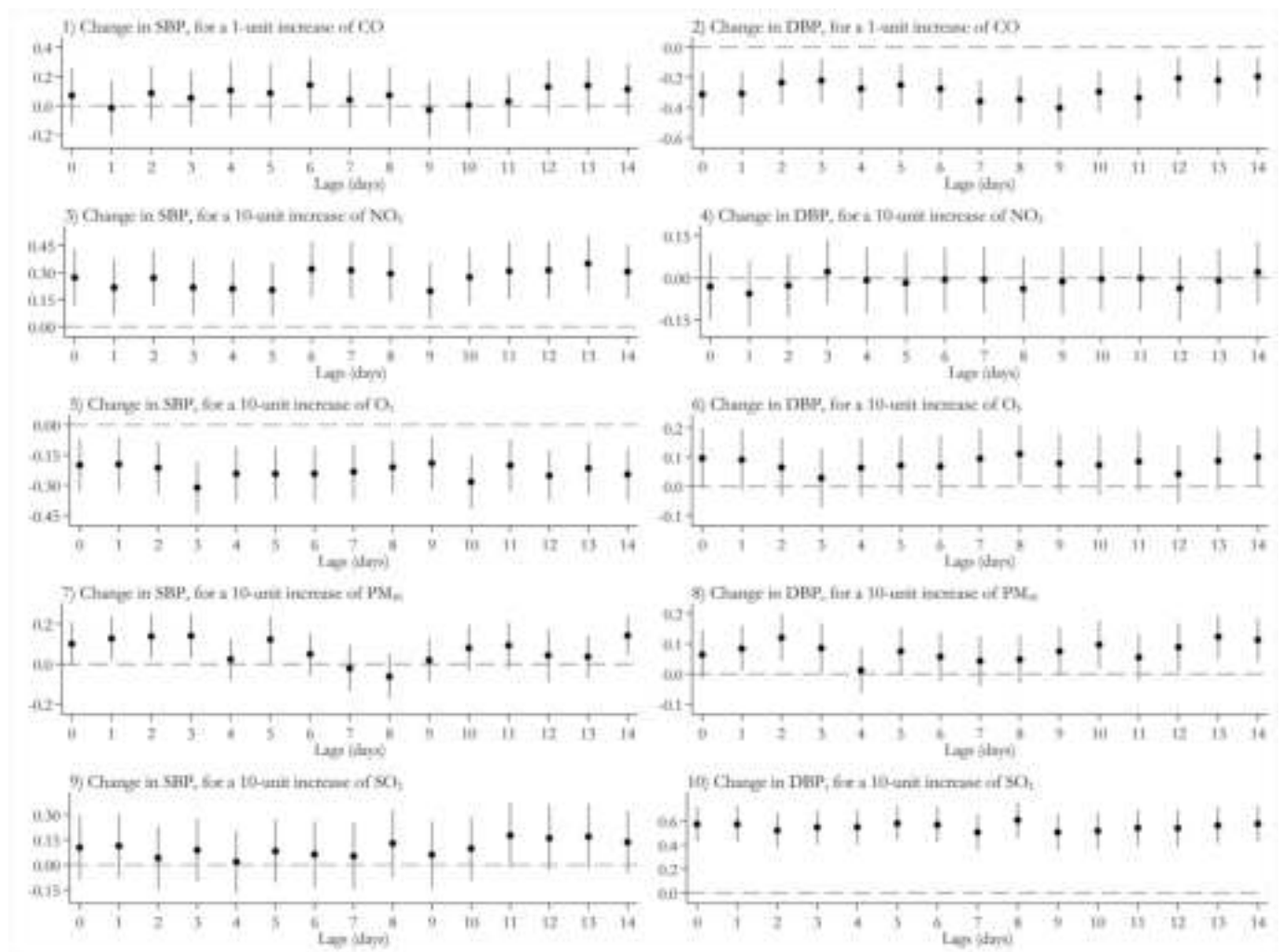

Fig. 1. Changes in diastolic blood pressure (DBP) and systolic blood pressure (SBP) as a function of a 1-mg/m³ increase of CO and 10-µg/m³ increase of NO_2, O_3, SO_2, and PM_{10}, along with their 14-day lag effects, adjusted for temperature, age, sex, body mass index, waist circumference, diabetes, anti-hypertensive drug use and being ever-smoker.
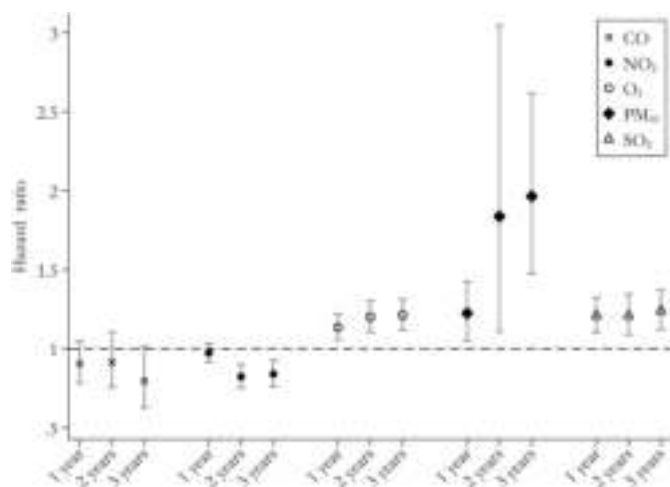
Fig. 2. The incident hypertension hazard ratios for 1-mg/m^3 increase of CO and 10-μg/m^3 increase of NO$_2$, O$_3$, SO$_2$, and PM$_{10}$ in three time spans of 1, 2, and 3 years, adjusted for temperature, age, sex, body mass index, waist circumference, diabetes, and being ever-smoker.

with CO exposure, being accompanied by the marginally significant 20% lower risk in three years. The effect of CO on BP is a matter of debate and there are firm experimental evidences supporting its negative relationship with BP (Stec et al., 2008). Two clinical studies with inconsistent results have assessed the long-term impact of CO on BP. Dong et al. claimed that CO is able to significantly increase the risk of hypertension (Dong et al., 2014); nonetheless, the other study did not find any significant association between CO and BP (Chuang et al., 2011).

4.3. SO₂

Burning fossil fuels by industrial facilities is known as the main source of SO$_2$ (Choi et al., 2019). In the short-term analysis of the present study, a non-statistically significant positive association between SO2 exposure and SBP and significant positive association between SO2 and DBP in all lag days were detected. Kim et al. study in 547 elderly participants showed that increase in the level of SO$_2$ would result in rise in BP (Kim et al., 2016). Likewise, positive associations between SO2 and SBP and DBP at different lag times have been reported in 9354 Chinese children (Zeng et al., 2017).

We demonstrated significantly higher risk of long-term exposure to SO$_2$ for hypertension in all three years. This finding has an important public health implication considering tremendous increase in fuel consumption, the main source of SO$_2$ in Iran through recent decades (Lotfalipour et al., 2010). To our best, no cohort study has yet examined the impact of long-term exposure of SO2 on BP. Chuang et al. did not find any significant association between SO$_2$ and SBP and DBP (Chuang et al., 2011); however, in a cross-sectional study, long-term exposure to SO$_2$ had significant association with the prevalence of hypertension with the odds of 1.33 (95% CI: 1.21–1.47) (Dong et al., 2014).

4.4. NO₂

In the short-term, increased NO$_2$ exposure was consistently associated with increasing values of SBP, but not DBP. Cakmak et al. analyzed data of 5604 participants aged 6–79 years and found that higher level of NO$_2$ is associated with increasing levels of SBP/DBP (Cakmak et al., 2011); a finding in contrast to the results of the Chen et al. study showing that increased exposure to NO$_2$ leads to a decreased SBP/DBP (Chen et al., 2012).

In the present study, 2-year and 3-year exposures to NO$_2$ were significantly associated with lower risk of hypertension. Similarly, in a

cohort of African American women, it was shown that long-term exposure to NO$_2$ is inversely associated with hypertension with HR of 0.92 (95% CI:0.86–0.98) (Coogan et al., 2017). In the sister study, NO$_2$ had a negative association with DPB, whereas no association was found for hypertension prevalence (Chan et al., 2015).

4.5. O₃

The process of organic compounds oxidation produces O$_3$ as a by-product (Walcek et al., 1997). Based on our results, O$_3$ was inversely associated with SBP in all lag days. The association between O$_3$ and DBP was slightly positive but only significant in the 8-day lag. Previous studies have found heterogeneous results about BP alternations in exposure to O$_3$. According to the Lie et al. study, SBP and DBP of patients with sleep apnea were negatively and positively associated with O$_3$, respectively (Liu et al., 2016). Another study showed that elevated O$_3$ is associated with rise in DBP, as well as apolipoprotein B and hemoglobin A$_1$C (Chuang et al., 2010).

In the long-term, in all three years, increase in O$_3$ values was significantly associated with increased incidence of hypertension. In residents of Los Angeles, rise in O$_3$ concentrations increased the risk of hypertension in the multivariate model [HR 1.09 (95% CI: 1,00, 1,18)] (Coogan et al., 2017). This finding was previously replicated by several cross-sectional studies (Chuang et al., 2010; Dong et al., 2013).

4.6. Potential mechanisms

A number of studies are dedicated to clarify the exact mechanisms behind relationships of air pollutants and BP; nonetheless, they have not yet fully understood. First, air pollutants are capable of provoking oxidative stress and systematic inflammation, which in turn prompt pro-hypertensive responses such as vascular dysfunction including impaired vasorelaxation and heightened contractility (Brook and Rajagopalan, 2009). Second, the interaction between inhaled particles and neural receptors located throughout the airways, may perturb autonomic nervous system balance and result in vasoconstriction (Widdicombe and Lee, 2001). Third, short-term exposure to air pollutants is associated with increase in endothelin-1 bioactivity which can play a pivotal role in hypertension (Peretz et al., 2008).

4.7. Implications

It has been postulated that small changes of BP even in individuals with normal BP contribute to a higher risk of cardiovascular diseases and mortality rate (Lim et al., 2012; Prospective, 2002). Besides, the whole EMR region is being affected by air pollution (Organization, 2016). Taken together with findings of the present study, air pollution, apart from potential risk factors, can remarkably enhance the rate of cardiovascular events through BP rise in Tehran.

Our study has some notable strengths. Firstly, to the best of our knowledge, the current study is the first comprehensive longitudinal study from EMR region assessing the influences of different components of air pollution on BP. Secondly, we examined the effects of five various air pollutants on BP, while very few (only 5) of the 66 studies reviewed by Yang et al. evaluated all five pollutants in the same population with respect to BP or hypertension risk (Yang et al., 2018). However, due to lack of data, we could not assess the relationship between PM$_{2.5}$ and BP. Thirdly, for the first time, we estimated the short- and long-term effects of air pollution on BP in adults. While the level of adjustment for potential risk factors are varying among relevant studies, we adjusted for several well-known traditional risk factors. Finally, our study investigated the association between air pollution and BP during nearly two decades.

As a limitation, we gathered air pollution data from central monitoring sites, which cannot reflect the exact level of personal exposure; hence, there is a chance that the level of personal exposure be different

from central measurements mainly because of the difference in any individual's behaviors. Additionally, this study was conducted in Tehran metropolitan area and its results might not be generalized to the rural zones of the country.

5. Conclusions

In this Middle-Eastern cohort, we examined the short- and long-term impact of exposures to air pollutants on BP during a two-decade follow-up. Considering the major effects of air pollutants including O_3, SO_2, and especially PM_{10} on incident hypertension, urgent public health policies should be implemented to reduce the burden of air pollution in the metropolitan city of Tehran.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113719.

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Interlaboratory comparison investigations (ICI) and external quality assurance schemes (EQUAS) for cadmium in urine and blood: Results from the HBM4EU project

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ABSTRACT

Human biomonitoring (HBM) of cadmium is essential to assess and prevent toxic exposure. Generally, low cadmium levels in urine and blood of the general population place particularly high demands on quality assurance and control measures (QA/QC) for cadmium determination. One of the aims of the HBM4EU project is to harmonize and advance HBM in Europe. Cadmium is one of the chemicals selected as a priority substance for HBM implementation in the 30 European countries under HBM4EU. For this purpose, analytical comparability and accuracy of the analytical laboratories of participating countries was investigated in a QA/QC programme comprising interlaboratory comparison investigations (ICI) and external quality assurance schemes (EQUAS). This paper presents the evaluation process and discusses the results of four ICI/EQUAS rounds for the determination of cadmium in urine and blood. The majority of the 43 participating laboratories achieved satisfactory results, although low limits of quantification were required to quantify Cd concentrations at general population exposure levels. The relative standard deviation of the participants' results obtained from all ICI and EQUAS runs ranged from 8 to 36% for cadmium in urine and 8–28% for cadmium in blood. Applying inductively-coupled plasma mass spectrometry (ICP-MS), using an internal standard, and eliminating molybdenum oxide interferences was favourable for the accurate determination of cadmium in urine and blood. Furthermore, the analysis of cadmium in urine was found to have a critical point at approximately 0.05 µg/l, below which variability increased and laboratory proficiency decreased. This QA/QC programme succeeded in establishing a network of laboratories with high analytical comparability and accuracy for the analysis of cadmium across 20 European countries.

1. Introduction

Cadmium is a heavy metal which has been used in many industrial products and processes, such as in the production of pigments and more recently in the manufacture of cadmium telluride solar panels (Hetherington et al., 2008; IARC, 2012; Nordberg et al., 2015). Industrial emissions, massive use of fertilizers, leaching processes and inadequate recycling strategies, but also geological sources have led to a ubiquitous presence of cadmium in the environment (Thornton, 1992; Işikli et al., 2006; Akram et al., 2019; Hou et al., 2019). Dietary intake and smoking are the main determinants of cadmium exposure of the general population in industrialised countries (Mezynska and Brzóska, 2018; EFSA, 2009), whereas in developing countries other additional relevant exposure routes exist, for example from electronic waste recycling (Motawei and Gouda, 2016; Kim et al., 2019; Adam et al., 2021). Cadmium can be stored and accumulated in various organs, especially in the liver and kidneys (Järup and Akesson, 2009). Biochemical, morphological and functional disorders of renal function are the primary toxic effects of chronic cadmium exposure (Järup, 2003; Satarug et al., 2010). In addition, cadmium and cadmium compounds are classified as carcinogenic to humans (IARC, 2012). Due to the low but prevalent exposure of the general population and the toxic effects following chronic low dose exposure, the assessment and prevention of cadmium exposure is a major public health issue (Järup and Akesson, 2009; Satarug et al., 2003).

For human biomonitoring (HBM) of cadmium exposure, primarily the determination of cadmium in urine, but also of cadmium in blood is applied (Vacchi-Suzzi et al., 2016; Klotz et al., 2013; Fransson et al., 2014; Aoki et al., 2017; Garner et al., 2017). The available HBM data have revealed generally low cadmium levels in both matrices in the general population of industrialised countries (Ruiz et al., 2010; Berglund et al., 2015; Bonberg et al., 2017; Nisse et al., 2017; Saravanabhavan et al., 2017), requiring limits of quantification (LOQ) of 0.1 µg/l or below. This LOQ was met in almost all recent HBM studies by using inductively-coupled plasma mass spectrometry (ICP-MS). However, this technique involves the challenge of controlling the impact of interfering element clusters, particularly of molybdenum oxide, which is generated in the ICP from background molybdenum content (Jarrett et al., 2008; Akerstrom et al., 2013; Schindler et al., 2014; Cañas et al., 2013). Thus, a high level of quality assurance is crucial for the determination of cadmium in biological matrices of the general population.

HBM4EU is a European project which represents a joint effort of 30 countries and European Commission authorities, co-funded under Horizon 2020 (https://www.hbm4eu.eu). The main aim of this initiative is the harmonization and advancement of HBM in Europe. HBM4EU targets the exposure of EU citizens to a variety of chemicals and their possible health effects to support policy making (Ganzleben et al., 2017). Cadmium was included in the first priority substance list of HBM4EU and in the first joint HBM studies of the project (Louro et al., 2019; Vorkamp et al., 2021).

One of the objectives within the HBM4EU project is the establishment of a network of analytical laboratories across Europe (Esteban López et al., 2021) for the HBM of environmental pollutants, generating high-quality and comparable HBM data for the prioritized substances. Thus, HBM4EU has implemented a quality assurance/quality control (QA/QC) scheme to verify analytical comparability between candidate laboratories for the analysis of samples within HBM4EU. An essential component of this QA/QC scheme is the design and implementation of interlaboratory comparison investigations (ICI) and external quality assurance schemes (EQUAS). The ICIs included candidate laboratories from the HBM4EU consortium and investigated the results with a view to comparability between these laboratories, whereas the EQUAS involved additional external expert laboratories that have experience with HBM population studies in other regions of the world and that applied comprehensively validated analytical procedures (Esteban López et al., 2021).

This paper presents the ICI/EQUAS programme for cadmium developed in HBM4EU, including the evaluation process, difficulties encountered and the results obtained.

2. Materials and methods

2.1. Design of the HBM4EU ICI/EQUAS programme for cadmium in urine and blood

In the QA/QC programme, four rounds of tailor-made ICI and EQUAS exercises were conducted from February 2018 to November 2019 to assess the proficiency of laboratories for cadmium in urine (Cd (U)) and cadmium in blood (Cd (B)). The organisational processes and conditions of ICI and EQUAS exercises for all substance groups in the HBM4EU project are described in detail in Esteban López et al. (2021).

Successful participation in the ICI/EQUAS for Cd (U) and Cd (B) was mandatory for laboratories to analyse the respective HBM4EU project samples. Candidate laboratories were requested to apply the same procedure in the ICI/EQUAS as they would use for analysis of samples in the frame of the HBM4EU project. A total of four ICI/EQUAS rounds for Cd (U) and Cd (B) were organized. The first round was conducted as an ICI and the following three rounds were conducted as EQUAS to be consistent with the overall HBM4EU QA/QC programme (Esteban López et al., 2021). Each round comprised the analysis of control materials (CMs) with two concentrations of Cd (U) and Cd (B), respectively. The results were reported to the laboratories before the next round. After the 1st round, a web conference was held for the participants to solve possible difficulties and improve future results. A web conference was also offered after each of the following rounds, but was not deemed necessary by the participants as no major difficulties had been encountered.

2.2. Invitation of participants

The process for selecting the candidate laboratories that participated in the Cd ICI/EQUAS has been described elsewhere (Esteban López et al., 2021; short description in the Supplemental Material). In brief, two calls to identify candidate laboratories to perform Cd analysis in HBM4EU were carried out, resulting in a list of 38 candidate laboratories from 22 countries after the first call. This number increased to 58 laboratories from 25 countries after the second call.

All laboratories that had previously registered as candidate laboratories for the analysis of cadmium in HBM4EU samples were invited to the ICI/EQUAS programme for Cd (U) and Cd (B). Candidate laboratories could participate in the ICI/EQUAS for either Cd (U) or Cd (B) or both. The 38 candidate laboratories established after the first call were invited to participate in the 1st round of the programme. The participants were asked to report LOQs of the analysis and the details of the applied methods in addition to the measured concentrations. The candidate laboratories were also informed that LOQs of 0.05 µg/l for Cd (U) and 0.15 µg/l for Cd (B) were advisable for successful participation. The setting of these LOQ target values was aligned according to the existing HBM data on cadmium in population studies (Castaño et al., 2012; Järup and Åkesson, 2009).

After the 1st round, the revised candidate list was used to invite 58 laboratories to participate in the 2nd, 3rd and 4th round for Cd (U) and/ or Cd (B).

2.3. Selection of expert laboratories

For the rounds organized as EQUAS (2nd, 3rd and 4th), six expert laboratories for Cd (U) and Cd (B) were selected by the HBM4EU Quality Assurance Unit (QAU). Experts were laboratories with experience in the analysis of Cd (U) and Cd (B), having documented their expertise in peer-reviewed publications. In addition, the following selection criteria were considered, although none of them was mandatory: number of

years of experience in the analysis of Cd (U) and Cd (B), application of highly sensitive and selective analytical techniques with sufficiently low limit of detection (LOD) and LOQ, application of isotope-labelled standards for quantification, availability of in-house validation reports, data on on-going intra-laboratory performance (e.g. control charts), ISO 17025 accreditation for the biomarker of interest and success rate in ICI/EQUAS or comparative results in HBM studies. For Cd (U) and Cd (B), two expert laboratories were from outside Europe. Four expert laboratories were from Europe and three of them also participated as candidates in the ICI/EQUAS.

2.4. Preparation and testing of control materials

The control materials were freshly prepared and tested for homogeneity before each round of the ICI/EQUAS programme. The native control material consisted of human urine (Cd $(U)_{native}$) or bovine blood (Fiebig-Nährstofftechnik, Idstein-Niederauroff, Germany) in EDTA solution (Cd $(B)_{native}$), both with the addition of sodium azide. For the animal materials, health conditions were certified. Stock solutions (Cadmium ICP standard, $Cd(NO_3)_2$ in HNO_3 2–3%, 1000 mg/l, Merck) were diluted to two different concentrations to obtain the spiking solutions. The addition of these spiking solutions to Cd $(U)_{native}$ and Cd $(B)_{native}$ yielded the target concentrations for the CM (Cd $(U)_{low}$, Cd $(U)_{high}$, Cd $(B)_{low}$, Cd $(B)_{high}$) (Suppl. Table. 1).

Five millilitres of the CMs for Cd (U) were filled into tubes with caps (82 × 13 mm, polypropylene, Sarstedt). Three millilitres of the CMs for Cd (B) were filled into tubes with caps (57 × 15.3 mm, polypropylene, Sarstedt). Previous investigations did not show any Cd contamination of the tubes used in the programme. CMs were prepared for each round, stored at ≤ -18 °C until shipment and then shipped under ambient conditions (Suppl. Fig. 1).

Details of the analytical method for the determination of homogeneity and stability of Cd (U) and Cd (B) are given in Table 1. A more detailed method description can be found in the Supplementary material. For the determination of homogeneity, ten randomly selected tubes of each CM of each round were taken from storage, thawed, re-homogenised by vortex shaking and simultaneously analysed in duplicate.

Homogeneity testing: Homogeneity was evaluated according to ISO 13528:2015 (Fearn and Thompson, 2001; Thompson, 2000) using ICP-MS (see Supplementary material).

Stability testing: The stability of the CM was tested in accordance with ISO 13528 (Statistical methods for use in proficiency testing by inter-laboratory comparison, 2015) and the International Harmonised Protocol for the Proficiency Testing of Analytical Laboratories (Thompson et al., 2006). For stability assessment, samples were stored under conditions representative of storage at the participants' laboratories (−18 °C) and at −80 °C (considered the maximum stability). Stability was determined by simultaneous ICP-MS analysis of six randomly selected samples from each concentration and after storage at both −18 °C and −80 °C for a time interval covering the time between shipment and the deadline for result submission. Stability was assessed by comparing the means of the six samples at −18 °C and −80 °C using the T-test.

2.5. Distribution of control materials

The control materials were dispatched to the participants under ambient conditions (Suppl. Fig. 1). Each participant received samples for Cd $(U)_{low}$, Cd $(U)_{high}$ and/or Cd $(B)_{low}$, Cd $(B)_{high}$, according to their registration. In the 1st ICI/EQUAS round, three samples of Cd_{low} and three samples of Cd_{high} were sent to the participants. Additionally, the participants received three samples of Cd_{native}, with the purpose of determining potential background levels. As no background was found, no further Cd_{native} samples were sent to the participants in subsequent rounds. From the 3rd round onwards, the participants received only one sample of each concentration (Cd_{low}, Cd_{high}) in order to mimic best the real analysis situation of the HBM4EU project samples.

At the time of shipment, a letter with instructions on sample handling, a sample receipt form, a result submission form and a method information form were sent to the participants. Participants were asked to perform a single analysis of each sample using the same procedure as used for analysis of samples in the frame of HMB4EU and to submit their results within four weeks after sample shipment.

In the 2nd, 3rd and 4th round, the selected expert laboratories received three samples of each CM (Cd_{low} and Cd_{high}) and were asked to provide a single or duplicate analysis so that they should submit at least six results per material (Cd_{low} and Cd_{high}, both for Cd(U) and Cd(B)).

2.6. Assessment of laboratory performance

In brief, for an ICI, a minimum of seven quantitative results from participating candidate laboratories was required for regular evaluation. For Cd $(U)_{low}$, Cd $(U)_{high}$, Cd $(B)_{low}$ and Cd $(B)_{high}$, the following values were calculated using robust statistics so that outliers were not excluded, but only had a minor impact on the performance parameters (Thompson et al., 2006; Analytical Methods Committee, 1989a, b; ISO 13528:2015): robust mean of the participants' results taken as consensus value (C), uncertainty of the consensus value (u_{ICI}), robust ICI standard deviation of the consensus value (σ_{ICI}) and the Z-scores for each participant.

The uncertainty of the consensus value was calculated as follows:

$$u_{ICI} = 1.25 \frac{\sigma_{ICI}}{\sqrt{n}} \tag{1}$$

with: n = number of results used for calculation of the consensus value with n $\geq$ 7.

In the EQUAS, the evaluation of the candidate results was based on the data generated by a minimum of three and a maximum of six designated expert laboratories. The mean-of-means of the individual expert laboratories was used as the assigned value. The uncertainty (u_{EQUAS}) was defined as the relative standard deviation of the expert means ($RSD_{mean-of-means}$) divided by the square root of the number of expert laboratories:

$$u_{EQUAS} = \frac{RSD_{mean-of-means}}{\sqrt{n}} \tag{2}$$

The mean-of-means was considered suitable for use as assigned value (A) in EQUAS studies if u_{EQUAS} did not exceed a value of 17.5% derived from the following equation:

$$u_{EQUAS} \leq 0.7 \times \sigma_T \tag{3}$$

Table 1
Analytical method parameters for determination of homogeneity and stability of Cd (U), Cd (B) and Mo background levels.

Quantitated ion and matrix	Instrument	Reagent gas	Sample volume	Dilution	Internal standard	Calibration	LOD Cd/Mo (µg/l)	LOQ Cd/Mo (µg/l)
[114]Cd or [98]Mo in urine	ICP-triple quadrupole-MS	Argon	0.4 ml	1:10	Rhodium	external, matrix-based, multi-level	0.023/0.040	0.050/0.127
[114]Cd or [98]Mo in blood	ICP-triple quadrupole-MS	Argon	0.2 ml	1:20	Rhodium	external, matrix-based, multi-level	0.040/0.050	0.050/0.270

with σ_T = a pre-set relative target standard deviation for proficiency of 25%

This target relative standard deviation (σ_T) reflected the maximum variability that was considered acceptable for the candidate results and was also used for the Z-score calculation in the ICI/EQUAS. The value of σ_T (25%) was set based on expert opinion, taking into account what was technically feasible and realistic in current routine practice.

As measure of proficiency, Z-scores were calculated using the consensus value derived from the participants' results (ICI) or the mean of the expert laboratories (EQUAS) as the assigned value, and the σ_T of 25% (Equation (4)).

In the first round, conducted as an ICI, the value of u_{ICI} was negligible for Cd (U)$_{low}$, Cd (U)$_{high}$, Cd (B)$_{low}$ and Cd (B)$_{high}$ so that the Z-scores (Z) of the results submitted by the participants (x) were calculated according to the equation:

$$Z = \frac{x - C}{\sigma_T * C} \quad (4)$$

In the 2nd, 3rd and 4th round, conducted as EQUAS, the Z-scores of the participants' results were calculated according to:

$$Z = \frac{x - A}{\sigma_T * A} \quad (5)$$

In ICI/EQUAS, Z-scores were classified in three categories:

- $|Z| \leq 2 \Rightarrow$ satisfactory
- $2 < |Z| < 3 \Rightarrow$ questionable
- $|Z| \geq 3 \Rightarrow$ unsatisfactory

The results of the participating laboratories were evaluated on an individual biomarker/matrix/concentration basis.

If no numerical value for a CM was reported by a participant, the specified LOQ that was reported by the participant was used for the Z-score calculation according to equation (5). These LOQ-Z-scores (LOQ-Z) were not included in the final evaluation, but were only used to assess the laboratory's performance with respect to its reported LOQ.

Statistical analyses were conducted using IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.

3. Results and discussion

3.1. Spiking concentrations, homogeneity and stability testing

Spiking concentrations in the CM (Cd (U)$_{low}$, Cd (U)$_{high}$, Cd (B)$_{low}$, Cd (B)$_{high}$), as shown in Suppl. Table 1, were selected in accordance with the expected exposure levels of the general population, which was the target population in HBM4EU aligned studies. DEMOCOPHES, a previous pan-European HBM project in mother-child pairs, used urinary CM with ranges of 0.2–1.0 μg/l for Cd on the basis of data from the German Environmental Survey 1998 and the German Environmental Survey for Children (Schindler et al., 2014). A more recent study that analysed Cd (U) in the general population of northern France reported a geometric mean (GM) of 0.39 μg/l and 0.37 μg/l Cd (U) for women and men, respectively (Nisse et al., 2017). For Cd (B), low levels starting from 0.02 μg/l, high levels up to 4.4 μg/l and mean values ranging from 0.3 μg/l to 1.53 μg/l have been documented for smoking and non-smoking men and women in the general population of several European countries (Mezynska and Brzóska, 2018). Nisse et al. (2017) reported a GM of 0.375 μg/l for Cd (B). The spiking concentrations chosen for the ICI/E-QUAS of 0.000 (native urine unspiked) to 0.350 μg/l Cd (U) and 0.120–0.720 μg/l Cd (B) were therefore considered adequate to test the accuracy of the participating laboratories for quantitative determinations within the environmental exposure range.

Homogeneity and stability testing of the CMs was conducted separately for each of the four ICI/EQUAS rounds by the same laboratory

(IPASUM). The results of the homogeneity testing for Cd (U) and Cd (B) in the four ICI/EQUAS rounds are shown in Table 2. No outliers were detected in any ICI/EQUAS round for Cd (U) and Cd (B), the homogeneity was adequate and the method was considered suitable. The Mo background levels determined by single analysis for each CM were below 10 μg/l and below 5 μg/l in urine and blood, respectively (Table 2).

The results of the stability testing for Cd (U) and Cd (B) in the four ICI/EQUAS rounds are shown in Suppl. Table 2. No statistically significant instability was detected in any ICI/EQUAS round, minor deviations were caused by the day-to-day imprecision of the applied method.

3.2. Participation and range of reported LOQs

In the first round of the ICI/EQUAS programme, 21 and 19 laboratories (55% and 50%) out of 38 candidate laboratories participated (Table 3) for urine and blood, respectively. 58 laboratories were invited to the following ICI/EQUAS rounds.

The range of LOQs reported by the participants in the four ICI/EQUAS rounds is shown in Suppl. Table 6. The recommended LOQs were ≤0.05 μg/l for Cd (U) and ≤0.15 μg/l for Cd (B). Two candidates did not provide their LOQs. In the 1st round, 14 candidates met the LOQ requirements for Cd (U) and Cd (B), representing 67% and 74% of all reported LOQs, respectively. In the following ICI/EQUAS rounds, the proportion of candidates meeting the required LOQs increased for Cd (U) and remained fairly constant for Cd (B). The expert laboratories also reported their LOQs and met the respective requirements except for one laboratory in the 2nd round for Cd (B) (Suppl. Table 6).

3.3. Establishment of assigned values derived from expert laboratories (EQUAS)

Each expert laboratory analysed either three samples of each CM (Cd$_{low}$ and Cd$_{high}$) in duplicate (round 2) or six samples of each CM in single or duplicate analysis (round 3 and 4). In the 2nd and 3rd round, all six selected expert laboratories submitted results, while in the fourth round one expert (Exp4) was missing. The details of the expert analyses for Cd (U) and Cd (B) are shown in Suppl. Fig. 2 and Suppl. Fig. 3, the corresponding assigned values and uncertainties can be found in Table 4 and Table 5. The RSD of the assigned values derived from the expert laboratories (RSD$_{expert\ labs}$) decreased from the 2nd to the 4th round for all CMs except for Cd (B)$_{high}$. Overall, the RSD$_{expert\ labs}$ for the high CM was lower than for the low CM in each round. The precision of the mean values ($\pm 2*SD$) varied considerably among the expert laboratories from round to round and for the different CMs.

3.4. Method characteristics

Details of the methods used by candidates and experts to analyse Cd (U) and/or Cd (B) are shown in Suppl. Table 3 and Suppl. Table 4. All experts and most candidates applied ICP-MS to analyse Cd in urine and blood. The use of atomic absorption spectrometry (AAS) was very

Table 2
Results of the homogeneity testing for Cd (n = 10) and Mo (n = 1) background levels in urine (U$_{low}$ and U$_{high}$) and blood materials (B$_{low}$ and B$_{high}$) of the four ICI/EQUAS rounds (mean ± SD in μg/l).

	Round 1	Round 2	Round 3	Round 4
Cd (U)$_{low}$	0.198 ± 0.010	0.063 ± 0.007	0.055 ± 0.007	0.076 ± 0.005
Mo (U)$_{low}$	9.47	8.61	9.59	7.38
Cd (U)$_{high}$	0.451 ± 0.013	0.213 ± 0.022	0.156 ± 0.009	0.156 ± 0.008
Mo (U)$_{high}$	9.51	8.50	9.61	7.31
Cd (B)$_{low}$	0.238 ± 0.016	0.105 ± 0.013	0.168 ± 0.017	0.174 ± 0.023
Mo (B)$_{low}$	4.51	1.19	1.29	3.56
Cd (B)$_{high}$	0.508 ± 0.017	0.749 ± 0.083	0.300 ± 0.025	0.407 ± 0.051
Mo (B)$_{high}$	4.56	1.18	1.35	3.58

Table 3

Number of registered laboratories and of laboratories submitting results for Cd (U) and Cd (B).

		Round 1	Round 2	Round 3	Round 4
Cd candidate laboratories		38	58	58	58
Cd (U)	Registration	21	39	42	22
	Participation	21	37	42	20
Cd (B)	Registration	19	35	37	21
	Participation	19	33	36	20

limited among the participants of the ICI/EQUAS and ranged between 5% and 11% over all rounds.

For Cd (U), an internal standard and the respective normalisation were used by 71% of all participants in the first ICI/EQUAS round. In the following rounds, the percentage of laboratories using the response normalised to an internal standard increased to 89% in the 4th round. The percentage of candidates using an internal standard without normalising the response declined from 10% in the first round to 3%–5% in the following rounds. In the first round, 19% of the participants did not use an internal standard, while this percentage decreased to 5% in the last round. Measures to suppress molybdenum oxide interferences were applied by around half of the participants. More details on the suppression measures are given in Suppl. Table 8.

For the analysis of Cd (B), around 80% of the participants used an internal standard with normalised response in the first three ICI/EQUAS rounds (Suppl. Table 4). In the last round, the proportion of laboratories that normalised their response to an internal standard was highest, reaching 94%. Molybdenum interferences were eliminated by a variable percentage of candidates across the four ICI/EQUAS rounds. While in the 1st round only 27% of the participants reported the elimination of molybdenum oxides by effective reaction/collision cell application, half of all laboratories applied such a procedure in the last round for Cd (B).

Among the six experts, the methods to determine Cd in urine and blood were quite uniform as all of them used ICP-MS and normalised their results to an internal standard (Suppl. Table 3; Suppl. Table 4). The percentage of expert laboratories that eliminated molybdenum oxide interferences was 60% for Cd (U) and 40% for Cd (B) in the 2nd and in the 3rd round. In the last round, one expert could no longer participate so that 50% of the experts applied elimination of molybdenum oxides.

3.5. Assessment of laboratory performance

When comparing the overall performance of all participating laboratories in the different rounds, various aspects of the test design, such as organisation as ICI or EQUAS, target concentrations of the CMs and applied methods, should be taken into account.

One interesting aspect of the EQUAS exercises is the comparison of the assigned values for Cd calculated from the results of five to six selected expert laboratories with the consensus values achieved by the

participating candidates in the three EQUAS rounds. The switch from ICI to EQUAS in the 2nd round was not due to problems with the ICI evaluation, but aimed to harmonize the exercises for all substance groups in the HBM4EU QA/QC programme targeting information on the accuracy (Esteban López et al., 2021). For all CMs of Cd (U) and Cd (B), the difference between the assigned and the subsequently calculated consensus values was within the range of the standard deviation of the assigned value (except for Cd(B) $_{low}$ in 4th round) and of the consensus value (Table 4). This indicates that the different evaluation schemes of ICI and EQUAS generated comparable Z-score results for the participating laboratories.

For the appraisal of the participant results, Z-score values were determined based on a pre-set relative target standard deviation for proficiency (σ_T) of 25%, which was extracted from previous experience for these parameters (Esteban López et al., 2021). The Z-scores of the participants who submitted quantitative results in the ICI/EQUAS rounds are shown in Suppl. Fig. 4. The performance of the participants who did not provide quantitative data but indicated '<LOQ' as a result was assessed using LOQ-Z-scores, but was not included in the final evaluation of the candidates. The respective interpretations of the LOQ-Z-scores are shown in Suppl. Table 5. The LOQ-Z-scores achieved by the candidates are shown in Suppl. Table 6.

The effect of the Cd target concentrations on candidate performance is reflected in Table 5, which shows details of the outcome of the four ICI/EQUAS rounds for Cd (U) and Cd (B). The number of participating laboratories that were unable to detect the biomarker in the CM and thus indicated '<LOQ' in their report was highest for Cd (U)$_{low}$ in the 2nd round, where the sample was not spiked and the expert-assigned value was the lowest (0.041 µg/l). Accordingly, the lowest number of satisfactory results for this CM was recorded in the 2nd round, as satisfactory Z-scores were only obtained by 22 participants (69%) for Cd (U)$_{low}$. The best performance of all rounds was obtained for Cd (U)$_{high}$ in the 3rd round with 40 candidates (98%) achieving a satisfactory evaluation. The Z-scores of the participants who submitted quantitative results for the respective CMs in the respective rounds are shown in Suppl. Fig. 5.

The effect of Cd concentration on study RSDR and Z-scores was also apparent for the blood samples. For Cd (B)$_{low}$, the average study RSDR over all rounds was 19.5%, while it was 11.0% for Cd (B)$_{high}$. The interlaboratory variability of the results was highest for the 2nd round of Cd (B)$_{low}$. This CM contained the lowest cadmium concentration detected in the homogeneity testing (0.105 µg/l; Table 2) and eight of the 33 participants could not detect it. Furthermore, the analysis of Cd (B)$_{low}$ in the 2nd round resulted in the lowest percentage of satisfactory results (i.e. 76%) of all blood samples. The best performance of all rounds was achieved for Cd (B)$_{high}$ in the 4th round, where all participants obtained a satisfactory evaluation.

The analysis of CMs with higher concentrations generally resulted in lower study RSDR values compared to CMs with lower concentrations, not only for all laboratories reporting results (Table 5), but also for the

Table 4

Comparison of assigned values and consensus values for Cd (U) and Cd (B) in rounds 2-4.

	Round	CM	Results from 5 to 6 experts		Results from all participants		
			Assigned value (A) (µg/l)	SD (µg/l)	Consensus value (C) (µg/l)	SD (µg/l)	difference of C from A (µg/l)
Cd (U)	2	low	0.041	0.012	0.053	0.019	0.012
		high	0.215	0.046	0.236	0.032	0.021
	3	low	0.087	0.007	0.091	0.011	0.004
		high	0.190	0.010	0.186	0.024	−0.004
	4	low	0.060	0.004	0.064	0.011	0.004
		high	0.146	0.007	0.150	0.012	0.004
Cd (B)	2	low	0.133	0.042	0.163	0.046	0.030
		high	0.767	0.036	0.759	0.079	−0.008
	3	low	0.210	0.116	0.214	0.048	0.004
		high	0.354	0.048	0.362	0.056	0.008
	4	low	0.135	0.008	0.148	0.026	0.013
		high	0.405	0.019	0.422	0.038	0.017

Table 5
ICI/EQUAS results obtained for Cd (U) and Cd (B).

	round	CM	Consensus (ICI)/Assigned value (EQUAS) in µg/l	RSDexpert labs	Uncertainty of ICI (uICI)/ EQUAS (uEQUAS)	Study RSDR	No. of labs reporting results	Performance (Classified by Z-score results)		
								% satis	% quest	% unsat
Cd (U)	1	low	0.238	n.a.	5%	21%	20 (1[a])	90%	5%	5%
		high	0.448	n.a.	3%	13%	20 (1[a])	90%	5%	5%
	2	low	0.041	30%	12%	36%	32 (5[a])	69%	3%	28%
		high	0.215	21%	9%	14%	37	92%	0%	8%
	3	low	0.087	8%	3%	12%	40 (2[a])	95%	0%	5%
		high	0.190	5%	2%	13%	41 (1[a])	98%	0%	2%
	4	low	0.060	7%	3%	17%	19 (1[a])	90%	5%	5%
		high	0.146	4%	2%	8%	20	95%	0%	5%
Cd (B)	1	low	0.251	n.a.	3%	11%	18 (1[a])	89%	0%	11%
		high	0.536	n.a.	2%	10%	18 (1[a])	89%	0%	11%
	2	low	0.133	31%	13%	28%	25 (8[a])	76%	16%	8%
		high	0.767	5%	2%	10%	32 (1[a])	91%	6%	3%
	3	low	0.210	28%	11%	22%	31 (5[a])	87%	6%	6%
		high	0.354	14%	6%	15%	34 (2[a])	91%	3%	6%
	4	low	0.135	6%	3%	17%	16 (2[a])	88%	13%	0%
		high	0.405	5%	2%	9%	17 (1[a])	100%	0%	0%

[a] Number of laboratories reporting ,<LOQ'; n.a: not applicable; satis: satisfactory results; quest: questionable results; unsat: unsatisfactory results.

successful laboratories (Suppl. Table 7). The study RSDR values were comparable to the participant RSD found in the DEMOCOPHES ICI/ EQUAS programme (Schindler et al., 2014). In the former study, the participant RSD ranged between 6.2% and 32% for concentrations between 0.21 and 0.99 µg/l.

The average performance of the candidates over all ICI/EQUAS rounds for Cd (U) and Cd (B) regarding all low and all high CMs was quite similar. The best results were obtained for Cd (U)$_{high}$ and Cd (B)$_{high}$ with an average of 94% and 93% satisfactory Z-scores, while only 86% of the participants achieved satisfactory Z-scores for Cd (U)$_{low}$ and 85% for Cd (B)$_{low}$.

A comparison of the Z-scores obtained for CM$_{low}$ over all rounds with Z-scores achieved for CM$_{high}$ showed a statistically significant difference only among the results of the candidates for Cd (U). The mean Z-score for Cd (U)$_{low}$ was significantly ($p < 0.01$) higher than the mean Z-score for Cd (U)$_{high}$ (Suppl. Fig. 6 A). No significant differences were found between the Z-scores of the candidates for Cd(B)$_{low}$ and Cd (B)$_{high}$ (Suppl. Fig. 6 C) and for Cd analyses performed by the expert laboratories (Suppl. Fig. 6 B,D). Overall, the mean Z-scores for candidates were slightly higher than the Z-scores for experts, but there were no statistically significant differences between these groups (Suppl. Fig. 7). The Z-scores obtained for all analyses of Cd (U) compared to all analyses of Cd (B) did not differ significantly either (Suppl. Fig. 8).

Regarding the rounds with similar target concentrations, a decreasing study RSD$_R$ for Cd(U)$_{low}$ from round 2 to round 4 combined with an improved candidate laboratory performance could be observed. The percentage of satisfactory results increased from round 2 to round 4. These tendencies may be due to a training effect, which however was not observed when comparing the study RSD$_R$ and the Z-scores between the similarly spiked rounds 3 and 4 for Cd (U)$_{low}$. Another possibility could be that the determination of Cd (U) has a critical point between the low CM of round 2 (A = 0.041 µg/l) and round 4 (A = 0.060 µg/l). This assumption is supported by the fact that the reported LOQ of several laboratories was 0.050 µg/l, which was also the recommended LOQ for Cd (U). Furthermore, the mean LOQ of all participants was 0.053 µg/l in round 2, i.e. above this hypothetical critical point of approximately 0.050 µg/l, while the mean LOQ of round 4 (0.049 µg/l) was just below 0.050 µg/l.

For Cd (U)$_{high}$, however, a training effect might become visible when comparing the study RSD$_R$ values and the performance of the candidates between all rounds (Table 5). Although the highest concentration was applied in the 1st round, the percentage of satisfactory results was higher in the following rounds, while the interlaboratory variability remained constant and even decreased in the 4th round, which could be indicative of a training effect. In addition, performance improved from rounds 2 and 3, which were both conducted as EQUAS with a similar number of participants. A further reduction of the concentration in round 4 only led to an increase in unsatisfactory Z-scores, which would be consistent with a tipping point near the LOQ.

For Cd (B), the participants' results for CMs with low consensus/ assigned values showed a higher study RSDR than the results for CMs with higher consensus/assigned values (Fig. 1 B). This hyperbolic dependency is consistent with the known association between precision and concentration. However, such a clear reciprocal effect could not be confirmed for Cd (U) (Fig. 1 A).

The dependency of the study RSD$_R$ in blood on the Cd concentrations (Fig. 1 B) is consistent with the general association between precision and concentration (Thompson, 1988). An exponential increase in the coefficient of variation with decreasing Cd concentration in urine has also been shown for urine in the German External Quality Assessment Scheme (G-EQUAS) (Göen et al., 2012). In the present study, such clear dependency was not found for urine. However, the examined concentration range in the ICI/EQUASs for Cd (U) was limited to very low levels from 0.041 µg/l to 0.448 µg/l (Fig. 1 A) compared to the observed Cd concentrations between about 0.1 and 3.5 µg/l in G-EQUAS (Göen et al., 2012).
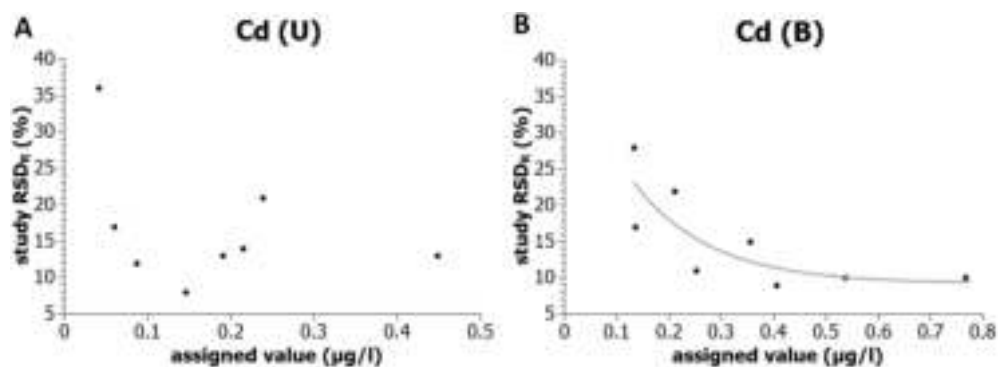
Fig. 1. Relationship between the study RSD$_R$ and consensus/assigned value for Cd (U) and Cd (B).

Although many European laboratories can provide high-quality data for Cd (U) and Cd (B), challenges remain with regard to method sensitivity, in particular for blood samples. LOQs for Cd (B) were generally higher than for Cd (U), resulting in a higher number of results reported as '< LOQ' (Suppl. Table 6). The biological matrix can have a strong impact on the analysis, especially for the determination of Cd (B) (Trzcinka-Ochocka et al., 2016). However, the obtained Z-scores showed no significant differences between Cd (U) and Cd (B) over all rounds at either level (Suppl. Fig. 8).

With regard to Cd (B)$_{low}$, the comparison of rounds 2 and 4 with the same spiking concentrations also pointed to a training effect of the participating laboratories in terms of a higher percentage of satisfactory Z-scores and a lower interlaboratory variability. The highest percentage of unsatisfactory Z-scores was observed in the 1st round, which also
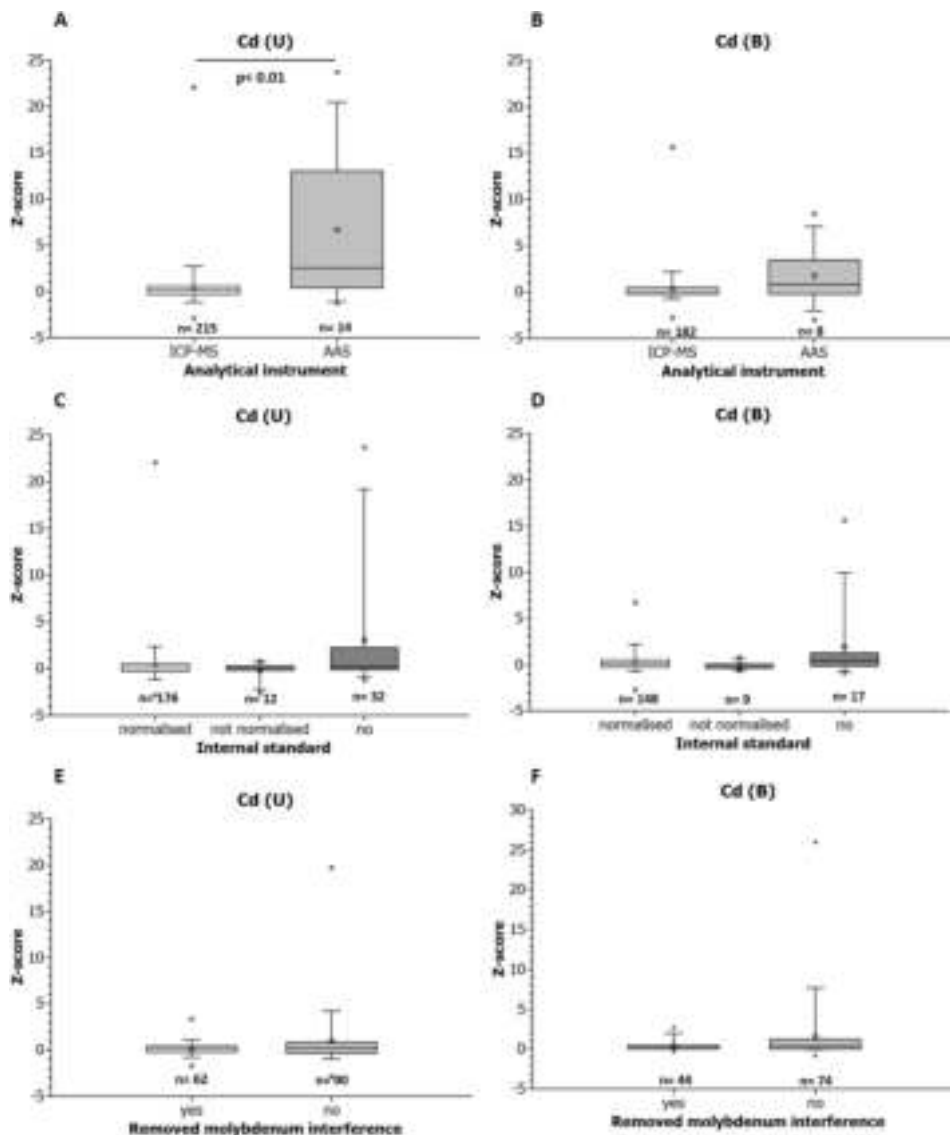

Fig. 2. Boxplots of the unsigned Z-scores obtained with different methods for the analysis of Cd (U) and Cd (B) by all participants in rounds 1–4. The box of the boxplots ranged from the 25th to the 75th percentile with the horizontal line showing the mean, the whiskers showing the 5–95th percentiles and the crosses the minimum and maximum values. Groups of Z-scores for all CMs over all rounds were compared using Mann–Whitney U tests: Z-scores obtained by using ICP-MS and AAS for determination of Cd (U) and Cd (B) (A, B), Z-scores obtained by using or not using an internal standard for the measurement of Cd (U) and Cd (B) (C, D), Z-scores achieved when molybdenum interferences were eliminated during the analysis of Cd (U) and Cd (B) (E, F).

accounts for a certain training effect of the participating laboratories; however, no clear improvement was observed for Cd $(B)_{low}$ between rounds 1 and 3.

The Z-scores obtained for the analysis of Cd were also compared with regard to the different methods used by the participants. Quantitative data of all rounds for both CM_{low} and CM_{high} were combined and divided into two to three groups according to methodological differences. Each group is presented in Fig. 2. The unsigned Z-scores were lower when the results were obtained by ICP-MS rather than by AAS (Fig. 2 A, B). Statistical analysis using the non-parametric Mann–Whitney *U* test showed significant differences ($p < 0.01$) between the application of different instruments only for the determination of Cd (U), but not for Cd (B). Furthermore, there was a tendency for participants who used an internal standard to quantify Cd (U) or Cd (B) to achieve better Z-scores than participants who did not use an internal standard (Fig. 2 C, D). In tendency, lower Z-scores were also achieved when molybdenum interferences were eliminated during the analysis of Cd (U) or Cd (B) (Fig. 2 E, F). However, these differences were not statistically significant.

The possible influences of the different analytical methods were investigated mainly with regard to the instrument used (ICP-MS or AAS), the use of an internal standard and the elimination of molybdenum oxide interferences. In general, the AAS technique as a mono-elemental method of metal determination has a high sensitivity and selectivity for specific elements such as Cd (Trzcinka-Ochocka et al., 2016). Nevertheless, the most powerful technique presently applied for Cd measurements in biological matrices is ICP-MS as it shows less spectral interference, a wider dynamic range and a lower LOD than AAS (Trzcinka-Ochocka et al., 2016; Vorkamp et al., 2021). The better outcome associated with the use of ICP-MS instead of AAS to determine Cd (U) and Cd (B) in the four ICI/EQUAS rounds might, however, also be influenced by the fact that laboratories using AAS mainly did not use an internal standard (IS) or did not communicate it if they used one. Furthermore, the number of candidates working with AAS represented only 6% of all participants for Cd (U) and 4% for Cd (B), so that deductions from this comparison have to be interpreted with caution.

Brodzka et al. (2013) investigated the effectiveness of internal standardization of ICP-MS for trace element determination in urine and came to the conclusion that this method requires the use of internal standards. Thus, it is not surprising that the Z-scores obtained by Cd analyses without application of an IS (15% for urine, 10% for blood) were higher compared to data generated with an IS. Interestingly, the lack of a subsequent normalisation to the IS showed no clear change in the Z-scores. However, the statistical power was poor due to the low number of non-normalised values.

The present background levels of Mo in the CMs for Cd were on average 8.75 µg/l in urine and 2.65 µg/l in blood (Table 2). These Mo concentrations were only at a moderate level considering a biological reference value (BAR) of 150 µg/l Mo in urine, which represents the background exposure in a reference population without occupational exposure to Mo (Michalke et al., 2020). Despite these relatively low Mo concentrations in the analysed CMs, the achieved Z-scores for the Cd determination without compensation for Mo tended to be higher than the Z-scores of laboratories that eliminated Mo interferences (Fig. 2 E, F) (Schindler et al., 2014). This effect was not yet statistically significant, but it was stronger for Cd (U) than for Cd (B), which might be mainly due to the higher Mo concentrations in the urinary materials. Comparing the Z-scores of all participants over all rounds showed that the best results were obtained when using ICP-MS, an internal standard and a method that excludes interfering molybdenum clusters (see Fig. 2).

Finally, laboratories from 20 European countries were approved for the analyses of Cd in the HBM4EU project according to the criterion of having to achieve satisfactory Z-scores in both CMs from at least two rounds. For Cd (U), 37 of the 43 participants (86%) and for Cd (B) 29 of the 37 participants (78%) successfully participated in the ICI/EQUAS programme of the HBM4EU project. The study RSD_R for the laboratories

with satisfactory results ranged from 35% (CM_{low} in round 2) to 8% (CM_{high} in round 4) for Cd (U) and from 21% (CM_{low} in round 2) to 7% (CM_{high} in round 2) for Cd (B) (see Suppl. Table 7). This demonstrated not only a good existing capacity for the analysis of Cd (U) and Cd (B) across Europe, but also a high analytical comparability and accuracy of the generated data among the successful participants of the ICI/EQUAS programme. Hereby, a quality-assured network of laboratories meeting the requirements of the HBM4EU programme has been established.

4. Conclusions

The QA/QC programme of the HBM4EU project for Cd (U) and Cd (B) provided insights into the European interlaboratory comparability of these parameters at the concentration level of the general population. The results of the quality assurance programme demonstrated that high interlaboratory comparability could be achieved at this exposure level, if the laboratories used state-of-the-art techniques, e.g. ICP-MS with collision/reaction cell, effective procedures for interference suppression/elimination and compensation measures against imprecision (e.g. by using an internal standard). Moreover, trends of increasing comparability during the programme for both parameters indicated a training effect of recurrent proficiency tests and encourage the implementation of continuous interlaboratory comparison investigations at international level. Altogether, 37 and 29 European laboratories achieved satisfactory results in the determination of Cd (U) and Cd (B), respectively, in at least two rounds, indicating a high capacity of high-quality Cd analysis in Europe, although not all EU countries were represented. The data indicated that a high interlaboratory comparability with a mean study RSD_R of 17% for Cd (U) and 15% for Cd (B) among the participating laboratories could be ensured for joint population studies within this pan-European human biomonitoring project. The current network for cadmium analysis within 20 European countries should be maintained and, if possible, extended in follow-up projects aiming to support public authorities in risk assessment.

Declaration of competing interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113711.

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Reducing dermal exposure to agrochemical carcinogens using a fluorescent dye-based intervention among subsistence farmers in rural Honduras

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ABSTRACT

Background: Occupational exposure to agrochemicals, some of which are known or suspected carcinogens, is a major health hazard for subsistence agricultural workers and their families. These impacts are more prevalent in low-and-middle income countries (LMIC) due to weak regulations, lack of awareness of the risks of contamination, predominant use of handheld backpack style spraying equipment, general lack of personal protective equipment (PPE), and low literacy about proper agrochemical application techniques. Reducing exposure to agrochemicals was identified as a paramount concern by rural Hondurans working with a community-engaged research initiative. Fluorescent tracer dyes have been described as a means of visualizing and quantifying dermal exposure to agricultural chemicals, and exposure models adapted for LMIC have been developed previously. Tracer dyes have also been used in educational simulations to promote pesticide safety. However, studies evaluating the effectiveness of these educational dye interventions in reducing future exposure have been lacking.
Aim: To evaluate whether observing one's own chemical contamination after applying agrochemicals changed the amount of occupational dermal exposure during a subsequent chemical application.
Methods: We employed a multi-modal community intervention in a rural village in Honduras that incorporated chemical safety education and use of a fluorescent tracer dye during pesticide application on two consecutive occasions, and compared dermal exposure between the intervention group (previous dye experience and safety education, $n = 6$) and the control group (safety education only, $n = 7$).
Results: Mean total visual score (TVS) of the tracer dye, which accounts for both extent and intensity of whole-body contamination, was lower among those who had previously experienced the dye intervention (mean TVS = 41.3) than among participants who were dye-naïve (mean TVS = 78.4), with a difference between means of −37.10 (95% CI [-66.26, −7.95], $p = 0.02$). Stratifying by body part, contamination was significantly lower for the anterior left lower extremity and bilateral feet for the dye-experienced group vs. dye-naïve, with most other segments showing a trend toward decreased contamination as well.
Conclusion: Participants who had previously experienced the dye intervention were significantly less contaminated than the dye-naïve control group during a subsequent spraying event. The findings of this small pilot study suggest that a multi-modal, community-based approach that utilizes fluorescence-augmented contamination for individualized learning (FACIL) may be effective in reducing dermal exposure to carcinogenic agrochemicals among subsistence farmers in Honduras and other LMIC.

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1. Introduction

Occupational exposure to agrochemicals is a major health hazard for subsistence agricultural workers and their families. The adverse health

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Abbreviations

BSS	body segment score
CBA	contaminated body area
CLARO	Community-led Action Research in Oncology
EPA	Environmental Protection Agency
FACIL	Fluorescence-Augmented Contamination for Individualized Learning
LMIC	low and middle-income countries
MES	mean extent score
MIS	mean intensity score
NOAEL	no observed adverse effects level
PPE	personal protective equipment
TVS	total visual score
WES	weighted extent score

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effects of agrochemicals are more prevalent in low and middle-income countries (LMIC) for various regulatory, social, cultural, and occupational reasons, including weak regulations, lack of awareness of the risks of contamination, predominant use of handheld backpack style spraying equipment prone to leaking, lack of personal protective equipment (PPE) and washing facilities, and low literacy about proper agrochemical application, storage, and exposure reduction techniques (Blanco et al., 2005; Corriols et al., 2009; Schreinemachers and Tipraqsa, 2012).

Acute and chronic agrochemical exposure is associated with a range of neoplastic, neurological, respiratory, endocrine, and metabolic adverse health effects (Gangemi et al., 2016b). Because agrochemicals can alter the immune system, lead to chronic inflammation, and cause potentially harmful mutations and epigenetic modifications, the role of pesticide exposure in cancer development is of great concern (Collotta et al., 2013; Corsini et al., 2013).

Agrochemical exposure is associated with increased risk of lung cancer, multiple myeloma, non-Hodgkin's lymphoma, ovarian cancer, prostate cancer, rectal cancer, testicular and skin cancer (Gangemi et al, 2016a, 2016b; Lewis-Mikhael et al., 2016; Schinasi and Leon, 2014). Chronic low-dose exposure to pesticides during gestation and early postnatal life among children of pesticide applicators adversely affects neurobehavioral development and may lead to infant and pediatric leukemia (Hernández and Menéndez, 2016; Rodríguez Altamirano, 2012).

In rural Honduras, where most people rely on subsistence agriculture of maize (*Zea mays*), beans (*Phaseolus* spp.), rice (*Oryza sativa*), bananas and plantains (*Musa* spp.), avocado (*Persea americana*), pineapple (*Ananas comosus*), mango (*Mangifera indica*), and coffee (*Coffea arabica*), multiple agrochemicals are widely sprayed with little to no use of PPE, post-spraying clean-up, or even dedicated spraying clothing. Highly hazardous products are often available to consumers despite national and international regulations banning their use (Jansen, 2008). Further, chemicals are often stored in the home alongside other household items, placing workers and their families at increased risk of adverse health outcomes associated with agrochemical exposures.

Reducing exposure to agrochemicals was identified as a paramount concern by rural Hondurans working with a community-engaged research initiative known as CLARO (Community-Led Action Research in Oncology), a joint venture between the community, the Honduran anti-cancer league (*La Liga contra el Cáncer*), and Dartmouth's Norris Cotton Cancer Center. To address this concern, the CLARO team

designed and evaluated strategies that could be useful for reducing agrochemical exposures among LMIC agricultural workers and acceptable to this rural Honduran community.

While a comprehensive hazard reduction plan ideally emphasizes reducing, substituting, or even eliminating the use of hazardous substances, which in this context could be achieved through preferential use of less-toxic products as well as agroecological approaches such as integrated pest management, various cultural and socioeconomic factors have made, and continue to make, these goals difficult to achieve in the Honduran subsistence agriculture setting (Murray, 1991; Wyckhuys and O'Neil, 2010, 2007).

Though community members were aware of the risks from exposure to agrochemicals in a general sense, consistently achieving adequate crop yields is crucial to their livelihood. Agrochemicals are largely seen as necessary to protect vulnerable crops, and some even conceived of the products as "medicine for the beans." Therefore, an initial goal of harm reduction via behavioral, administrative, engineering, and personal protective equipment strategies was chosen by stakeholders as a more timely and achievable means of addressing the problem of exposure within the context of current agrochemical use practices. Specifically, this was to include a multi-modal chemical safety educational campaign, construction of a community decontamination and chemical storage facility to help foster a culture of safety, and the testing of a fluorescent dye-based intervention.

Previous observational studies have validated the use of fluorescent tracer dye visualized under ultraviolet (UV) light, together with an anatomic model that divides the body into segments and quantifies dermal agrochemical exposure. These studies were conducted in the neighboring country of Nicaragua, and the methods were adapted for feasibility in rural Central America (Aragón et al, 2004, 2006; Blanco et al., 2005; Fenske et al., 1985). This approach was found to be sensitive and specific enough to identify distinct application behaviors and other factors that are associated with greater contamination (Blanco et al., 2005). This metric of exposure is also biologically relevant, as fluorescently measured dermal exposure has been shown to correlate well with directly measured dermal pesticide residues, and with levels of urinary pesticide metabolites (Cherrie et al., 2000; Fenske, 1988) However, these exposure studies have been criticized as mostly academic and of unclear ongoing benefit to the communities under study (Brisbois, 2014).

Fluorescent tracer dye has also been widely used in pesticide safety demonstrations and provides a visually impactful means of communicating the insidious ways in which exposure can occur (Bierman et al., 1998). Although the use of fluorescent dye to enhance safety demonstrations has been shown to result in greater self-report of behavior change than other educational techniques (Foss et al., 2002), it is not known whether these dye interventions actually lead to reductions in objectively measured dermal exposure to pesticides.

In this small feasibility study, we employed a fluorescent tracer dye intervention with rural Honduran agrochemical users on two consecutive occasions, with the goal of not just providing an educational intervention, but also quantifying changes in dermal exposure over time in order to assess the effectiveness of the dye intervention in reducing exposure to agrochemicals, and thereby potentially reducing the risk of adverse health effects.

2. Materials and methods

2.1. Study population

The study was conducted in the rural village of El Rosario, Department of Yoro, Honduras. A total of 51 subsistence agricultural workers, 18 years of age and older, enrolled in the study. All participants provided written informed consent prior to participation in study activities. All protocols were reviewed and approved by the Dartmouth Committee for the Protection of Human Subjects (Study # 00030149) and Universidad

Católica de Honduras. All interactions with subjects throughout the study were conducted in Spanish by native and fluent non-native speakers.

2.2. Inventory of agrochemicals in use in the study community

Information was collected from local agricultural workers about pesticides in use throughout the growing season in El Rosario. Local trade names were matched with chemical names and a toxicological literature review was performed. Weight-of-evidence classifications regarding carcinogenicity were compiled from the International Agency for Research on Cancer (IARC, 2020) and the United States Environmental Protection Agency (USEPA, 2019), as were acute hazard classifications from the World Health Organization (WHO, 2020).

2.3. Chemical safety education campaign

The agrochemical safety education campaign was developed by the local community leaders and presented in March 2017 on the study registration day by local teenagers as a skit open to all community members including agrochemical workers and their families. Content was targeted to a third-grade education level. Topics included high-risk exposure behaviors, best practices for spraying, exposure risk reduction, and the recognition and early treatment of acute pesticide poisoning; 44 study participants attended this event.

A decontamination facility was built for the agricultural workers in the community with showers for decontaminating after spraying, locked storage for pesticides and work clothes, and wash basins for cleaning equipment. A log was kept in the facility for the community to track usage of the facility. In October 2017, the decontamination and pesticide storage facility opened to the community.

2.4. Fluorescent dye intervention

Investigators notified community leaders of the event dates, who then notified participants by phone and word of mouth and asked them to participate in spraying if the event dates coincided with their crops' needs and agricultural calendar. Some farmers who were already planning to spray on event days were recruited by community leaders and consented on the day of the event.

At the first spraying event in July 2017, a total of 15 participants completed the study protocol; 12 had previously registered on the day of the skit, and three were new to the study. One additional participant was provided with dye but failed to return for post-spraying visualization.

These recruitment procedures were repeated in October 2017. At the second spraying event, a total of 14 participants completed the study protocol. Of these, seven had participated in the first event ("experienced group") and seven had not participated previously ("naïve group"). Among the naïve group, three had previously registered on the day of the skit, and four were new to the study. Two additional participants were provided with dye but failed to return for post-spraying visualization. A total of 25 distinct participants completed the study protocol during the spraying events (See Fig. 1).
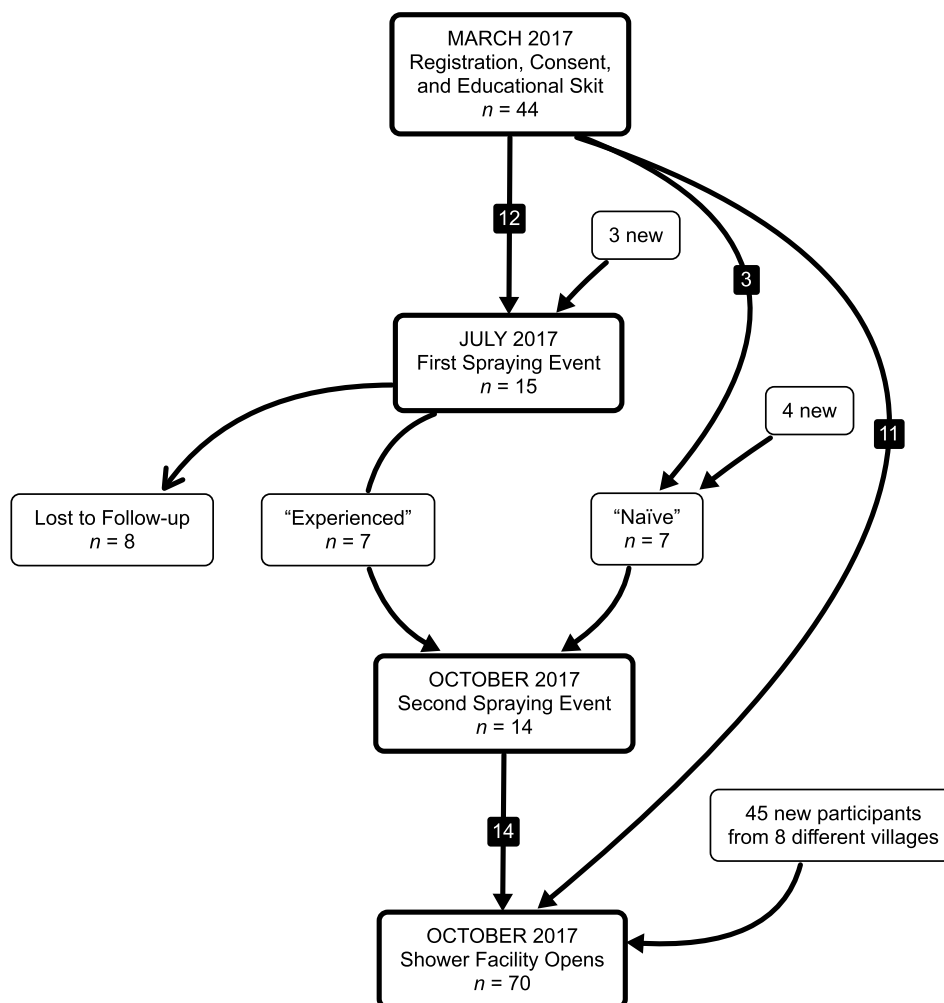

Fig. 1. Flow chart of study events.

2.4.1. Dye acquisition

Tinopal CBS-X (CAS# 27344-41-8) was acquired from Aurum Pharmatech LLC, Franklin Park, New Jersey, USA. Tinopal, a stilbene fluorescent dye found in many laundry detergents and long used as a groundwater tracer due to its low ecotoxicity and biodegradability, was previously reported to be useful for pesticide contamination studies because it is invisible to the naked eye, strongly fluorescent at low concentrations, binds strongly to skin, and is relatively non-toxic (Licha et al., 2013). Prior studies reported using a concentration of 260 mg/L (Aragón et al., 2006). Preliminary laboratory tests found a lower concentration of 200 mg/L to be adequate for visualization without loss of deposition information. Dye solution was tested using a UV spectrophotometer and found to exhibit maximal absorbance in the 365–375 nm range.

2.4.2. Dye preparation and spraying

On the morning of spraying days, before arriving at plots to be sprayed, study participants were given a 1-liter beverage-type plastic bottle containing 0.6 L of a concentrated 18 g/L aqueous solution of Tinopal CBS-X (below the solubility limit of 25 g/L). Bottles were marked with lines at gradations of 0.2 L, and participants were instructed to agitate the solution before adding 0.2 L of the concentrated dye to each of three 18 L tank loads that they were applying that day in order to achieve the target dye concentration of 200 mg/L. PPE including a long-sleeved shirt, goggles, gloves, and disposable 3M respirators (appropriate for oily mists) were made available to all participants. Participants were instructed to mix their agrochemical products and to spray their crops as they normally would, then return to the clinic for dye visualization immediately after completing their field tasks.

2.4.3. UV lamps and dye visualization

Clinic exam rooms were darkened using opaque black material over windows. Portable Convoy S2+ lamps using a 365 nm Nichia UV emitter diode with a ZWB2 visible light filter and powered by a single rechargeable 18650 Li-ion cell were acquired from Shenzhen Convoy Electronics Co., Ltd., Shenzhen, China. Study participants and investigators wore UV-blocking protective eyewear compliant with the ANSI Z87.1 standard. Participants' skin was briefly examined using the UV lamps and contamination was mapped and photographed using an iPhone™ 6 rear-facing 8-megapixel camera with high dynamic range enabled. Participants were also invited to use a mirror to view the fluorescent dye present on their person.

2.4.4. Quantification of dermal exposure

Dermal exposure revealed by the tracer dye was quantified using a visual scoring system, adapted from Aragón et al., (2006), that accounts for both the extent (surface area) of contamination, as well as the intensity of that contamination.

The extent of contamination of each segment of the body was scored by two separate reviewers on a scale from 1 to 5 (corresponding to estimated surface area coverage of 0–20%, 20–40%, 40–60%, 60–80%, and 80–100%, respectively). The mean of the two ratings, or mean extent score (MES), was then divided by the total possible extent score (5) to give the proportion of that segment that appeared contaminated. This proportion was then multiplied by a given segment's percent contribution to total body surface area (%BS) in order to calculate a weighted extent score (WES). The sum of the WES for all segments of a given subject's body is the contaminated body area (CBA), which is an estimate of the percent of the body's total surface area that was found to be contaminated.

The intensity of contamination of each segment of the body was classified by two separate reviewers as low, moderate, or high and assigned scores of 1, 3, or 5, respectively (Aragón et al., 2006). The mean of the two ratings, or mean intensity score (MIS), was then multiplied by the WES to yield the body segment score (BSS), which accounts for both the extent and intensity of contamination of a given segment. The sum of

the BSS for all segments of a given participant's body yields the total visual score (TVS) for that subject, which is the primary outcome measure.

$$MES/5 \text{ x } \%BS = WES$$

$$\sum WES \text{ (all segments)} = CBA$$

$$MIS \text{ x } WES = BSS$$

$$\sum BSS \text{ (all segments)} = TVS$$

2.5. Statistical analyses

Reliability analyses were performed to assess the consistency of ratings between the reviewers. Inter-group (experienced vs. naïve at second event) and intragroup (experienced at first event vs. experienced at second event) analyses were performed. Participants who attended only the first event were excluded from analysis.

2.5.1. Inter-rater reliability analysis

All extent scores (CBA) and intensity scores (TVS) calculated by Rater 1 were compared to those calculated by Rater 2 using a weighted kappa coefficient ($n = 551$) (Cohen, 1968; McHugh, 2012; Viera and Garrett, 2005).

2.5.2. Inter-group comparison (naïve ($n = 7$) vs. experienced ($n = 6$))

Mean TVS and CBA values of naïve and experienced participants at the second event were compared using a t-test with pooled variance. Mean BSS for each segment in both groups was also compared using t-test with pooled variance. One of seven participants from the experienced group was excluded due to self-report of "just helping" and not personally spraying during the second event.

2.5.3. Intra-group comparison (experienced at first vs. second event ($n = 5$))

Mean TVS and CBA values of experienced participants at the first event were compared to those at the second event using a paired t-test. Participants' mean BSS for each segment at the first event and the second event also were compared using a paired t-test. One of seven participants was excluded due to self-report of "just helping" and not personally spraying during the first event, and another (same participant described in 2.5.2) was excluded for the same reason during the second event.

2.6. Knowledge, attitudes, and behavior survey

A survey was developed to assess the degree to which participants agreed with and adopted the health behaviors that were depicted in the skit. Previous work within the community demonstrated that participants were more comfortable with questions that utilized a "true/false" format. There were two knowledge questions and 10 behavior questions. If a participant's reply indicated that he was not utilizing the healthy behavior, then he was asked if he would consider adopting the behavior. Surveys were carried out after the skit, and before and after the dye demonstrations. Because of logistical issues, baseline surveys prior to the skit were not collected. Surveys were conducted in Spanish by research staff members.

3. Results

3.1. Inventory of agrochemicals in use in the study community

A total of 17 different agrochemicals were reported in use in the community. Four of these compounds are classified by either the USEPA

or the IARC as "probable" human carcinogens. Two other compounds are considered "possible" human carcinogens. There were no WHO Class I highly acutely hazardous compounds reported in use in the community; the majority were considered moderately hazardous (Table 1).

3.2. Study subjects

A total of 51 farmers participated in the study. Their demographics are described in Table 2. All participants identified as male. Age ranged from 20 to 89 years with roughly normal distribution, with slight skew toward younger farmers, and 84% of all participants had started farming under the age of 20 years. In terms of education, 67% of farmers had an elementary-school level education, 12% had completed high school, 10% did not go to school (functionally illiterate in this community), and the education status of 12% was unknown. Total number of household members ranged from 2 to 14 (Table 2).

3.3. Fluorescent dye exposure data

3.3.1. Inter-rater reliability

Weighted kappa coefficient for inter-rater reliability of extent scores (CBA) was 0.86 (95% CI 0.83–0.88, $p < 0.0001$) and that for intensity scores (TVS) was 0.91 (95% CI 0.89–0.93, $p < 0.0001$), which indicate "strong" to "almost perfect" agreement (McHugh, 2012; Viera and Garrett, 2005).

3.3.2. Inter-group comparison, naïve vs. experienced

The results of the inter-group comparison of the extent and intensity of contamination is shown in Table 3. The mean TVS was lower among those in the experienced group as compared to those in the naïve group (*t*-test with pooled variance, $p = 0.02$; Table 3). The mean total CBA was also lower in the experienced group compared to the naïve group, though the difference did not reach statistical significance (*t*-test with pooled variance, $p = 0.06$; Table 3).

Table 2
Demographics of the study participants.

Characteristics	n (%)
Age (years)	
<20	0 (0)
20-39	15 (29.4)
40-59	24 (47.0)
>60	12 (23.5)
Age When Started Farming (years)	
<5	0 (0)
5-14	22 (43.1)
15-20	21 (41.1)
>20	2 (3.9)
unknown	6 (11.8)
Level of Education	
elementary school	34 (67.0)
high school	6 (11.8)
did not go to school	5 (9.8)
unknown	6 (11.8)
Total Household Members	
≤4	20 (39.2)
>5	25 (49.0)
unknown	6 (11.8)

An example of dye contamination is shown in Fig. 2. Stratifying contamination by body segment showed that contamination of the left anterior lower extremity (shin), dorsum of the right foot, and dorsum of left foot was lower among dye-experienced participants than dye-naïve controls (*t*-test with pooled variance, $p = 0.03$; Fig. 3, Table B1). All but one of the other segments (left anterior upper arm) showed either a trend towards decreased contamination among experienced sprayers, or no change (Table B1). In both groups, the posterior trunk was the segment with the highest mean BSS, therefore contributing most to overall exposure (Fig. 3, Table B1). No contamination was noted on any participants on the posterior proximal extremities. (Table B1).

Table 1
Most common agrochemicals used by subsistence farmers in El Rosario, Honduras.

Common Name	Use	USEPA[a]	IARC[b]	WHO[c]
2,4-D	Herbicide	Group D: Not classifiable as to human carcinogenicity	Group 2B: possibly carcinogenic to humans	II: Moderately hazardous
Azoxystrobin	Fungicide	Not likely to be carcinogenic to humans	NA	Unlikely to present acute hazard
Benzyladenine	Plant growth regulator	Not determined	NA	NA
Copper (II) sulfate, pentahydrate	Fungicide	Group D: Not classifiable as to human carcinogenicity	NA	II: Moderately hazardous
Cypermethrin	Insecticide	Group C: Possible human carcinogen	NA**	II: Moderately hazardous
Fluazifop-p-butyl	Herbicide	Not likely to be carcinogenic to humans	NA	III: Slightly hazardous
Fomesafen	Herbicide	Not likely to be carcinogenic to humans	NA	II: Moderately hazardous
Glufosinate ammonium	Herbicide	Not likely to be carcinogenic to humans	NA	II: Moderately hazardous
Glyphosate	Herbicide	Not likely to be carcinogenic to humans	Group 2A: probably carcinogenic to humans	III: Slightly hazardous
Haloxyfop-p-methyl ester	Herbicide	Group B2: Probable human carcinogen	NA	II: Moderately hazardous
Kinetin	Plant growth regulator	Not determined	NA	NA
Lambda-cyhalothrin	Insecticide	Group D: Not classifiable as to human carcinogenicity	NA	II: Moderately hazardous
Mancozeb	Fungicide	Group B2: Probable human carcinogen	NA*	Unlikely to present acute hazard
Paraquat	Herbicide	Group E: Evidence of non-carcinogenicity for humans	NA	II: Moderately hazardous
Propaquizafop	Herbicide	Not determined	NA	Unlikely to present acute hazard
Thiamethoxam	Insecticide	Not likely to be carcinogenic to humans	NA	II: Moderately hazardous
Thiodicarb	Insecticide	Group B2: Probable human carcinogen	NA	II: Moderately hazardous

NA = not assessed.
*moderate and **high priority for upcoming evaluation.
[a] United States Environmental Protection Agency weight-of-evidence carcinogenicity classifications.
[b] International Agency for Research on Cancer weight-of-evidence carcinogenicity classifications.
[c] World Health Organization acute hazard classifications.

Table 3
Comparison of Mean Dermal Exposure Between Experienced vs. Naïve Participants.

Outcome	Experienced ($n = 6*$)	Naïve ($n = 7$)	Difference (95% CI)	p-value[†]
TVS[a]	41.27	78.37	−37.10 (−66.26, −7.95)	0.02
CBA[b]	11.28	17.49	−6.20 (−12.82, 0.41)	0.06

*one of seven experienced participants was excluded from analysis due to self-report of helping, but not personally spraying, during the second event.
[†]using t-test with pooled variance.
[a] total visual score (TVS) reflects whole-body dermal exposure as a function of both the surface area and intensity of fluorescently traced contamination.
[b] contaminated body area (CBA) reflects the extent of fluorescently traced contamination, expressed as percent of total body surface area.

3.3.3. Intra-group comparison (experienced at first vs. second event)

Among the experienced group, the mean TVS decreased from first event to second event, though the change did not reach statistical significance (mean TVS of 55.86 versus 41.27, difference between means of −14.59 [95% CI -44.74, 15.56], paired t-test, $p = 0.25$). Stratifying by body segment, BSS for the right side of the face showed a significant decrease from first to second event (mean BSS of 5.08 versus 1.54, difference between means of −3.54 [95% CI -5.66, −1.41], paired t-test, $p = 0.005$). All other segments showed no statistically significant difference between the first and second event.

3.4. Surveys and behaviors

Forty-four surveys were conducted, and the results are reported in Table 4. In terms of knowledge of the risks posed by agrochemicals, all participants (100%) were aware, even before the intervention, that agrochemicals can have negative health consequences. However, the proportion of participants who felt that there are ways to mitigate the risk increased over time from 71% to 100%.

Survey results also revealed that individual participants were already practicing a number of risk reduction behaviors before the intervention and were somewhat open to learning new ones. After the dye interventions, participants appeared even more open to new risk reduction behaviors. Among the behavior items, participants reported using, on average, between 4 and 5 risk reduction behaviors (range 1–8). Taking a shower after spraying was the most frequently reported behavior

(93–100%), followed by keeping chemicals away from family members (71–80%), fixing leaky equipment (67–70%), having designated work clothes (57–71%), and not eating in the field (40–71%). Participants who had not adopted these risk reduction behaviors were consistently open to consider them (>55%). After each dye intervention the willingness to adopt these risk reduction behaviors, among those who had not been performing them previously, was >90%.

The behaviors in which the most subjects self-reported a change from before to after the dye interventions were a decrease in home storage of chemicals, an increase in the number of participants who said they do not spray when others are nearby, and an increase in those who said they do not spray on windy days. There was also a trend toward reporting that their equipment does in fact leak, which may reflect an awareness gained through the dye demonstration.

4. Discussion

The inventory of agents in the community revealed a spectrum of agricultural chemicals in use, including fungicides, herbicides, and insecticides. Three of these compounds are considered probable human carcinogens (Table 1), which raises concern that occupational exposure during their application may increase the risk of cancer for farmers who apply them. This is especially true for mancozeb and thiodicarb, which may have mutagenic properties. Mutagens, or chemicals that directly damage DNA, may not have a threshold of exposure below which they are considered safe (the no observed adverse effects level, NOAEL) and therefore all exposure, no matter how small, increases the theoretic risk of developing cancer (Sargent et al., 2010). In addition to increasing the long-term risk of various cancers, some of the agents in use also pose an acute risk to human health.

Participant gender distribution, age distribution, and educational attainment were comparable with those of previous studies among subsistence farmers using backpack sprayers in the neighboring Central American nation of Nicaragua (Aragón et al., 2001). Many participants began farming as teenagers, and in many cases continue to apply agrochemicals beyond age 60, creating the potential for a large additive exposure over time. The majority of participants reported elementary school education or less, which highlights the appeal of this visually engaging and participatory intervention that does not require formal education or advanced health literacy (Table 2).

In terms of fluorescently measured whole-body exposure, the mean TVS of 78.4 for naïve participants was consistent with the 74.7 mean TVS reported by Aragón et al., and somewhat lower than the TVS of 130

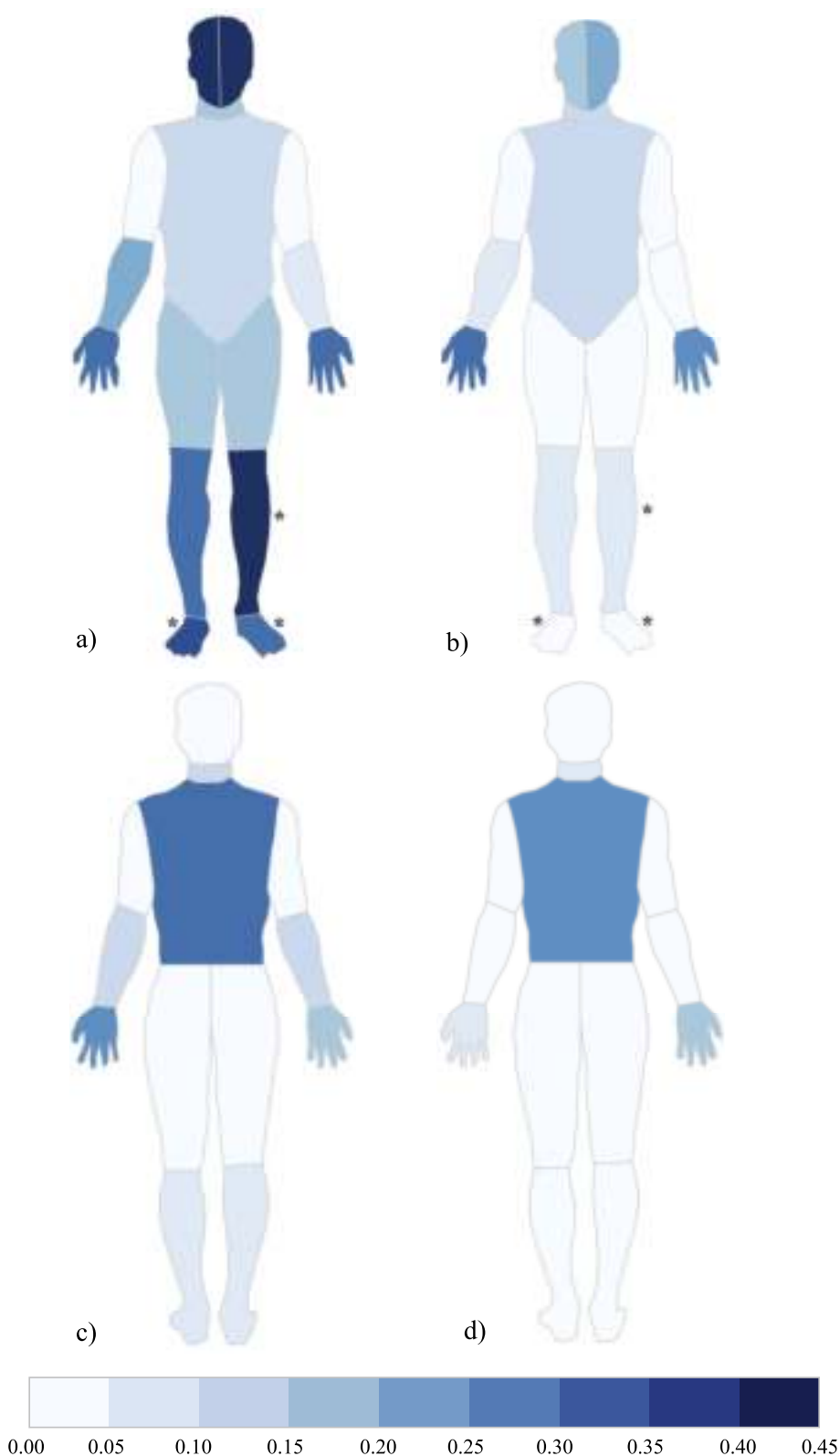**Fig. 2.** Fluorescent tracer deposition on contaminated hand and foot.

Fig. 3. Heat map of contamination by body segment. Anatomic heat maps showing the mean contamination of body segments for anterior aspect of the body for naïve (a) and experienced (b) subjects, and posterior aspect of the body for naïve (c) and experienced (d) subjects. Contamination is expressed as proportion of each segment's maximum possible body segment score (BSS). Naïve $n = 7$, experienced $n = 6$. *$p < 0.05$, pooled t-test.

reported by Blanco et al. (Aragón et al., 2006; Blanco et al., 2005). These findings, the first reported from Honduras, suggest that the dye-naïve group in this community had a baseline level of agrochemical exposure that was similar to or less than the previously studied Nicaraguan groups. These findings may reflect shared agricultural practices shaped by cultural, socioeconomic, geopolitical, ecologic, and climatic factors

common to the region; however, homogeneity in pesticide use across Central America should not be assumed as pesticide consumption varies greatly between countries, with Honduras importing twice as many kilograms of active ingredient per rural inhabitant per year compared with Nicaragua from 2000 to 2004 (Bravo et al., 2011).

Notably, we report a mean TVS for dye-experienced sprayers of

Table 4

Summary of knowledge and behaviors of study participants.

Surveys were conducted after skits, and before and after dye demonstrations #1 and #2. Numbers of participants responding correctly to each individual item is represented by *n*, and the percentage of the participants who responded correctly, when compared to the total survey participants, is found in parentheses. For behavior items, participants were asked *prior* to the intervention whether they had already adopted the specific behavior (*already reducing risk*). After the intervention, participants were asked whether they would consider adopting the specific behavior if they hadn't yet (*would consider doing.*)

Knowledge Items	Pre-Skit Survey Answered Correctly *n* (%)	Dye Demo #1 Answered Correctly *n* (%)	Dye Demo #2 Answered Correctly *n* (%)
Chemicals for agriculture can make you sick (true)	14 (100)	15 (100)	14 (100)
There is no way to keep from getting sick from chemicals (false)	10 (71)	12 (80)	14 (100)

Behavior Items	Pre-Skit Survey (*n* = 14)		Dye Demo #1 (*n* = 15)		Dye Demo #2 (*n* = 14)	
	Already reducing risk *n* (%)	Would consider doing *n* (%)	Already reducing risk *n* (%)	Would consider doing *n* (%)	Already reducing risk *n* (%)	Would consider doing *n* (%)
No use of chemicals for agriculture at all	3 (21)	6 (55)	2 (13)	13 (100)	1 (7)	12 (92)
Avoid mixing chemicals	1 (7)	0 (0)	1 (7)	14 (100)	3 (21)	10 (91)
Equipment does not leak	11 (79)	3 (100)	10 (67)	5 (100)	11 (79)	3 (100)
No smoking or eating in the field	10 (71)	4 (100)	6 (40)	9 (100)	8 (57)	5 (83)
Wear work clothes only for spraying chemicals.	10 (71)	4 (100)	9 (60)	6 (100)	8 (57)	6 (100)
Avoid spraying near others	3 (21)	7 (64)	10 (67)	3 (60)	8 (57)	5 (83)
Take a shower after spraying	14 (100)	–	14 (93)	1 (100)	14 (100)	–
I never spray on windy days	3 (21)	9 (82)	6 (40)	9 (100)	7 (50)	7 (100)
Keep chemicals out of home	5 (36)	7 (78)	13 (87)	2 (100)	12 (86)	2 (100)
Keep the chemicals away from my family members, children or pets in my home	10 (71)	3 (75)	12 (80)	3 (100)	11 (79)	3 (100)

41.27, which was significantly lower than that of the dye-naïve sprayers from the same community who applied the same volume of agrochemicals on the same day (Table 3). As noted above, this is also the lowest mean TVS yet reported for groups that have been assessed using this model (Aragón et al., 2006; Blanco et al., 2005). Therefore, our findings suggest that experience with the dye intervention has the potential to reduce the whole-body dermal exposure to carcinogenic agrochemicals experienced by subsistence farmers in Central America.

That there was a non-significant trend towards decreased CBA can be interpreted to mean that the reduction in TVS is not fully explained by decreased extent of contamination alone, and that a reduction in the intensity of contamination played a role as well. This result also may be due, at least partially, to the small sample size.

In terms of contamination of specific parts of the body, our findings are consistent with prior studies in identifying the posterior torso as the segment that contributes most to overall contamination, likely given its large surface area and vulnerability to leaking backpacks (Fig. 3, Table B1). Our data also provide further support for the notion that the anterior lower extremities and feet actually contribute a great deal to total body contamination, which prior authors noted had been previously under-recognized (Aragón et al., 2006; Blanco et al., 2005). Previous multivariate analyses found that holes in work boots and lack of use of long pants were linked with significant increases in total body exposure (Blanco et al., 2005).

Interestingly, while almost all body segments showed a trend towards decreased contamination among the dye-experienced group, it was the shins and feet where this reduction reached statistical significance (Fig. 3, Table B1), lending credence to the notion that improvements in this area are at least partly responsible for the reduction in whole-body contamination (TVS) that was observed. This finding suggests that the anterior lower extremities may be the most readily modifiable areas of exposure after undergoing the dye visualization intervention. However, whether this reduction resulted from improvements to footwear, clothing, spraying behavior, spraying equipment, or some combination of these factors remains unclear, and this finding may represent the collective effect of unique individual behavior changes rather than one specific change common to all participants.

Decreased facial exposure in those who underwent dye training, evidenced by a strong trend in inter-group comparison, and by being the only region of the body to show a statistically significant decrease in the intra-group comparison, suggests that increased awareness of pesticide contamination prompts efforts to protect the face (Table B1). This tendency may be echoed in survey data showing an increase in the proportion of respondents who say they do not spray near others or on windy days (Table 4). Provision of a mirror during the dye intervention may also have encouraged greater care in avoiding facial contamination, either through increased adherence to respirator mask usage or other means.

In contrast, little difference between groups was seen for palmar-hand and posterior torso contamination, suggesting that the scenarios leading to exposure of these areas may be less modifiable by increased awareness alone (Table B1). The need for bare-handed dexterity in opening tanks, mixing chemicals, adjusting nozzles, as well as leaking backpack sprayers identified both by our participants and in prior studies (Blanco et al., 2005) may be more difficult to rectify, and future work should address means of minimizing exposure to these parts of the body.

Strengths of this study included the use of dye during actual pesticide applications, as opposed to observing an instructor in a simulated skit or mock scenario, in that each farmer received personalized feedback that reflected their own spraying behavior, use or non-use of PPE, and specific areas on which to focus future prevention. While the investigators in this study were bilingual, this intervention would also have worked well across linguistic barriers.

As a small feasibility study, there were multiple limitations that should be addressed in future investigations. While the results suggest a reduction in exposure at least three months post-intervention, additional events at longer intervals would be needed to assess the longevity of the effect. The unpredictability of attendance at spraying events due to challenges inherent to the rural study area, such as decentralized communication, made recruitment and retention more difficult, limited the sample size, and did not allow for randomization, opening the possibility of self-selection in the experienced group towards more motivated participants. Difficulty controlling for attendance at skits and educational events was also a potential confounder for similar motivational reasons. However, these same difficulties also did not allow the investigators to assign participants to a group, thereby reducing the potential for selection bias on the part of the investigators.

Though intra-individual comparison between first and second dye events was the initial intent of the investigators, this comparison was

later thought to be less valid given no ability to control for potentially confounding factors such as differences in growing seasons, crop height, or weather conditions between the two time points. This comparison was also limited by the low number of two-time participants. For these reasons, inter-group comparisons at the second event were investigated.

Another limitation of this study was lack of standardization of the PPE worn by study subjects, as logistical constraints did not allow for direct observation of spraying. However, all participants had equal access to PPE if desired, and therefore participants' use or non-use of PPE can be regarded as another behavioral choice that may have been influenced by the intervention and ultimately reflected in the levels of dermal contamination observed.

5. Conclusion

To our knowledge, this is the first study that combines two well-described roles for fluorescent tracer dye, both as a means of quantifying dermal exposure to pesticides and as a tool for pesticide safety demonstrations, and provides preliminary evidence that participating in dye visualization demonstrations can lead to measurable reductions in future exposure. This novel method, termed fluorescence-augmented contamination for individualized learning (FACIL), is a low-cost, practical, and portable intervention that cuts across linguistic and cultural barriers and relies upon participants' direct experience rather than a didactic or precautionary approach. When employed as part of a multimodal, participatory campaign that includes support for sustainable changes in infrastructure and the promotion of a culture of safety at the community level, it constitutes a scalable model for Honduras and other LMIC that addresses a modifiable cancer risk factor, and could help mitigate excess disease burden among vulnerable populations.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113734.

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The European Human Biomonitoring Initiative (HBM4EU): Human biomonitoring guidance values for selected phthalates and a substitute plasticizer

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ABSTRACT

Ubiquitous use of plasticizers has led to a widespread internal exposure of the European population. Until today, metabolites are detected in almost every urine sample analysed. This raised the urgent need for a toxicological interpretation of the internal exposure levels. The European Human Biomonitoring Initiative (HBM4EU) contributes substantially to the knowledge on the actual exposure of European citizens to chemicals prioritised within HBM4EU, on their potential impact on health and on the interpretation of these data to improve policy making. On that account, human biomonitoring guidance values (HBM-GVs) are derived for the general population and the occupationally exposed population agreed at HBM4EU consortium level. These values can be used to assess phthalate exposure levels measured in HBM studies in a health risk assessment context. HBM-GVs were derived for five phthalates (DEHP, DnBP, DiBP, BBzP and DPHP) and for the non-phthalate substitute Hexamoll® DINCH. For the adult general population, the HBM-GVs for the specific metabolite(s) of the respective parent compounds in urine are the following: 0.5 mg/L for the sum of 5-oxo-MEHP and 5-OH-MEHP; 0.19 mg/L for MnBP, 0.23 mg/L for MiBP; 3 mg/L for MBzP; 0.5 mg/L for the sum of oxo-MPHP and OH-MPHP and 4.5 mg/L for the sum of OH-MINCH and cx-MINCH. The present paper further specifies HBM-GVs for children and for workers.

1. Introduction

Human Biomonitoring (HBM) has increasingly been established globally as an instrument to inform policy and citizens about the exposure of the general public to anthropogenic chemicals. The European Human Biomonitoring Initiative (HBM4EU) is a joint effort of 30 European countries, and the European Environment Agency, co-funded by the European Commission under Horizon 2020 with the goal to improve chemical safety. Many countries in Europe and worldwide already run HBM programs to monitor exposure levels of environmental chemicals, some of them on a regular basis (WHO, 2015). In Europe, these programs had previously worked independently of one another. As a result, comparability of the national HBM data is limited. HBM4EU has created a European network that improves knowledge for the European Union's environmental and chemical policy by harmonizing the planning and implementation of HBM studies, as well as sample and data analysis across national borders (Ganzleben et al., 2017). HBM4EU also aims to establish a sustainable Europe-wide HBM that provides comparable results tailored to directly feed into the development of European policies in the fields of health, environment and chemical safety to protect human health more effectively (David et al., 2020; HBM4EU Website).

The HBM4EU consortium identified 18 substances and substance groups, including phthalates and Hexamoll® DINCH, as of high priority to answer open policy relevant questions by targeted research. To interpret the results of HBM studies, up-to-date health-related assessment values are a useful tool. Such values (as HBM-I values from the German HBM Commission or biomonitoring equivalents (BE) by Summit Toxicology and Health Canada) have been applied in national HBM programs in the past (Angerer et al., 2011; Apel et al., 2017; Aylward et al., 2013; Ewers et al., 1999; Faure et al., 2020; German HBM Commission, 2007a, b, c, 2014a; St-Amand et al., 2014). In HBM4EU, a broad consented methodology for so-called HBM guidance values (HBM-GVs)

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Abbreviations

AF	Assessment Factor
AGD	Anogenital Distance
ANSES	FrenchAgency for Food, Environmental and Occupational Health and Safety
BE	Biomonitoring Equivalent
BBzP	Butyl benzyl phthalate (CAS No.: 85-68-7)
CAS No	Chemical Abstract Service Number
DEHP	Diethyl hexyl phthalate (CAS No.: 117-81-7)
DFG	German Research Foundation
DiBP	Diisobutyl phthalate (CAS No.: 84-69-5)
DiDP	Diisodecyl phthalate (CAS-No.: 26761-40-0/68515-49-1)
DiNP	Diisononyl phthalate (CAS-No.: 28553-12-0/68515-48-0)
DnBP	Di-n-butyl phthalate (CAS-No.: 84-74-2)
DNEL	Derived No Effect Level
DPHP	Bis(2-propylheptyl) phthalate (CAS-No.: 53306-54-0)
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
F1 generation	First Filial generation
F2 generation	Second Filial generation
F_{ue}	Fractional urinary excretion coefficient

GD	Gestational Day
HBM	Human Biomonitoring
HBM-I value	Human biomonitoring I value from the German HBM Commission
HBM4EU	The European Human Biomonitoring Initiative
HBM-GV	Human Biomonitoring Guidance Value
HBM-GV$_{GenPop}$	Human Biomonitoring Guidance Value for the general population
HBM-GV$_{Worker}$	Human Biomonitoring Guidance Value for workers
Hexamoll® DINCH	1,2-Cyclohexane dicarboxylic acid diisononyl ester (CAS No.: 166412-78-8)
LoC	Level of Confidence
LO(A)EL	Lowest Observed (Adverse) Effect Level
MnBP	Mono-n-butyl phthalate (CAS-No.: 131-70-4)
NO(A)EL	No Observed (Adverse) Effect Level
PBTK	Physiologically-based toxicokinetic
POD	Point of Departure
RSD	Relative Standard Deviation
SCOEL	Scientific Committee on Occupational Exposure Limits
TDI	Tolerable Daily Intake
TRV	Toxicity Reference Value

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was established. These values, derived from epidemiological or toxicological data, indicate the concentration of a compound or its metabolite(s) in a biological matrix (e.g. blood, urine) at/under which a health risk is not anticipated, according to current knowledge (Apel et al., 2020a). Used at the population level, they can not only help to refine the public health risk assessment by identifying exposures of potential concern, but also indicate potential regulatory priorities and the need for (additional) measures to reduce exposure. Within HBM4EU, a methodology has been elaborated on the derivation of these guidance values not only for the general population (HBM-GV$_{GenPop}$) but also for occupationally exposed adults (HBM-GV$_{Worker}$). HBM-GV$_{GenPop}$ are equivalent to the HBM-I values from the German HBM Commission and similar to the BE values introduced by Summit Toxicology (Apel et al., 2017; German HBM Commission, 2007a, b, 2014a; Hays and Aylward, 2009; Hays et al., 2008, 2007). The HBM-GV$_{Worker}$ are similar to the biological limit values derived by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) as well as by the former Scientific Committee on Occupational Exposure Limits (SCOEL) or also to the biological tolerance values (BAT) set by the Working Group on the Setting of Threshold Limit Values in Biological Material of the German Research Foundation (DFG) (ANSES, 2014; Apel et al., 2020a; Bolt and Thier, 2006; DFG, 2014).

Each HBM-GV derived within HBM4EU underwent a consultation process with national experts to ensure a high degree of both scientific integrity and acceptance (for more information please see Apel et al., 2020a). As HBM-GVs are set for exposure biomarker(s) levels in a biological matrix, they can be directly compared to exposure levels measured in HBM studies, if the same biomarker(s) are measured with comparable quality-assured analytical methods. They are thereby an easy-to-use tool for performing an integral health risk assessment (covering all known and unknown sources and routes of exposure), and are complementary to external health-based guidance values (e.g. Tolerable Daily Intake (TDI)) that usually focus on specific sources or exposure routes (e.g. food).

The regulation of reprotoxic phthalates resulted in a change in use and in the exposure pattern within the general population over time. Whereas the exposure levels of old, well-known phthalates (DEHP, DnBP, BBzP) have decreased, an increase in exposure levels of high molecular weight phthalates such as DiNP, DiDP and DPHP has been observed over the last two decades. The exposure levels of the non-

phthalate substitute Hexamoll® DINCH have also increased in some European countries (Frederiksen et al., 2020; Gyllenhammar et al., 2017; Kasper-Sonnenberg et al., 2019; Koch et al., 2017; Schmidtkunz et al., 2019; Shu et al., 2018). Thus, the widespread use of phthalates and their substitute still leads to their ubiquitous presence in the environment (Przybylińska and Wyszkowski, 2016; Szewczyńska et al., 2020) and in humans. The frequency of detection of the phthalate metabolites mentioned ranges from over 80 up to 100% of all urine samples analysed (Apel et al., 2017, 2020b; Correia-Sá et al., 2018; Dewalque et al., 2014; Frederiksen et al., 2011; Hartmann et al., 2015; Husøy et al., 2019; Kasper-Sonnenberg et al., 2019; Katsikantami et al., 2016; Koch et al., 2017; Myridakis et al., 2016; Schoeters et al., 2017; Schütze et al, 2014, 2015; Schwedler et al., 2019, 2020). Therefore, it is still urgently needed not only to monitor plasticizers in the European population but also to be able to interpret these results to adopt measures at European scale.

With this paper, the published general methodology for deriving HBM-GVs (see Apel et al., 2020a) is implemented for phthalates and a substitute plasticizer. HBM-GVs agreed on within HBM4EU for DEHP, DiBP, DnBP, BBzP, DPHP and the substitute Hexamoll® DINCH are presented.

2. Methods

The methodological approaches applied to derive HBM-GVs for the general and for the occupationally exposed population are outlined in detail in the strategy paper to derive HBM-GVs (Apel et al., 2020a). The strategy paper underwent a consultation process within the HBM4EU consortium to include the expertise of scientists from HBM4EU partner countries. Briefly, the strategy comprises that a literature research on recent toxicological and epidemiological data is conducted to find a robust exposure-health relationship to derive an HBM-GV. If present and reliable, human data is preferred over animal data. If it is not possible to establish a relationship between the internal concentration of the substance of concern or its metabolite(s) and a selected critical health effect in human data, a toxicity reference value (TRV) established by acknowledged authorities or committees can be used to derive an HBM-GV by means of toxicokinetic extrapolation. If there is no TRV available, an HBM-GV can be derived based on a point of departure (POD) identified in an animal toxicity study. Here too, information on

the toxicokinetics of the substance of concern is needed to calculate the internal concentration for the exposure biomarker. Depending on the information that is available, an HBM-GV can be calculated by using a simple toxicokinetic model such as the mass balance approach or by using a more sophisticated physiologically-based toxicokinetic (PBTK) model assuming a steady-state exposure condition in any case. The general formula used for the derivation of an HBM-GV based on an established TRV or on a TRV-like value calculated from an animal POD for a single metabolite using a mass-balance equation is shown in Formula 1.

Formula 1. Mass balance equation for the derivation of an HBM-GV for a single exposure biomarker based on an established TRV or based on a TRV-like value (calculated from an animal POD by applying assessment factors to the selected animal POD)

$$HBM - GV = \frac{TRV \text{ or } TRV - \text{like value} \cdot \left[\frac{(MW \text{ Metabolite}) \cdot (F_{ue} \text{ Metabolite})}{MW \text{ Parent compound}} \right]}{\text{average daily urinary flow rate adjusted to bw}}$$

TRV = toxicity reference value; MW = molecular weight; F_{ue} = fractional urinary excretion coefficient; bw = body weight. Average daily urinary flow rates adjusted to bodyweight of 0.03 and 0.02 L/kg bw/d for children and adults, respectively as proposed by the German HBM Commission were used. To address the underlying uncertainties in the data used for the HBM-GV derivation, an overall level of confidence (LoC) is attributed to each HBM-GV. The LoC is estimated to be "low", "medium" or "high". The overall LoC is obtained by combining single LoC associated with the following criteria: 1) nature and quality of the epidemiological and toxicological data; 2) choice of critical effect and mode of action; 3) choice of the key study; 4) selection of the critical dose; and 5) extrapolations across and within species (see Apel et al., 2020a). The LoC aims at pointing out the uncertainties underlying the HBM-GV, as well as providing guidance to prioritise research activities needed for removing those uncertainties. Where new evidence is available, the values should be revised, especially those with lower LoC. It is important to stress out that the LoC does not relate as such to the level of protection towards adverse effects conferred by the value (a value with a low LoC for example may have been derived considering very conservative default assumptions) (Apel et al., 2020a).

3. Results

In the following the main approach in deriving HBM-GVs for the general population and for workers is detailed using DnBP as exemplary case. Details on input data for the derivation of the HBM-GVs for each compound can be found in the supplementary material 1. All HBM-GVs derived for the general population are summarised in Table 1 and HBM-GVs to be used in an occupational setting are displayed in Table 2. A detailed report on the derivation of HBM-GVs for DEHP and Hexamoll® DINCH has been published previously in an HBM4EU Deliverable and can be found on the HBM4EU website (see Apel and Ougier, 2017).

3.1. Selection of biomarkers of exposure for Di-n-butyl phthalate, DnBP

To select suitable biomarkers of exposure for which an HBM-GV can be derived, the toxicokinetic information on DnBP was reviewed with the emphasis on human data. A toxicokinetic study on one human volunteer by Koch et al. (2012) showed that within 24 h most of the dose (5.38 mg of D4-labelled DnBP) was excreted as total MnBP (84%) in urine, whereas oxidized metabolites were excreted to a minor extent (approximately 8%) (Koch et al., 2012). Seckin et al. (2009) applied a total dose of 3.6 mg in form of a capsule to 17 volunteers (male and female; including 4 children) and determined that within 24 h, 78% of the dose (median) was excreted as total MnBP via urine (Seckin et al., 2009). Anderson et al. (2001) demonstrated in a human voluntary toxicokinetic study on eight individuals per dose group (single dose of 0,

225 or 510 µg) that within 24 h, a mean of 69% of the ingested DnBP (molar basis) was excreted in urine as total MnBP, which is well in line with the other studies mentioned. On average 73% of the high dose (n = 6; two sample were not usable) and 64% of the low dose (n = 7; one sample was lost) administered was excreted in urine. The relative standard deviations (RSD), which give an estimation on the inter-individual variations were relatively low with 28% and 29% for the high and low dose, respectively (Anderson et al., 2001). MnBP is selected as biomarker of exposure suitable for the HBM-GV derivation, as it is identified as most suitable biomarker for determining DnBP exposure within HBM4EU (see Thomson et al., 2017) and total MnBP is excreted in relevant quantities in urine after DnBP intake. It needs to be noted, that MnBP was also found in urine after exposure to BBzP, but only when high doses were administered and only to a minor extent (Anderson et al., 2001). The fractional urinary excretion coefficient (F_{ue}) is a crucial parameter for the HBM-GV derivation to predict the daily excretion rate of the biomarker. The F_{ue} is the share of the orally absorbed compound that is excreted in form of the respective biomarker. Anderson et al. (2001) determined the mean F_{ue} for MnBP over all participants and doses to be 0.69. Both, Seckin et al. (2009) and Anderson et al. (2001) investigated the metabolism of DnBP by including several volunteers. The study by Anderson (2001) was the only study in which two doses were tested and the inter-individual variance of the excretions was given. Additionally, the doses tested in the Anderson study were the lowest and thus are more realistic to the levels of exposure of the general population not receiving capsule medication. Therefore, the F_{ue} determined in the Anderson study is considered in the HBM-GV calculation.

3.2. Selection of the TRV or POD and calculation of the HBM-GV for DnBP

3.2.1. General population

A literature search of the toxicological and epidemiological database was performed to identify the most sensitive endpoint (i.e. critical effect) for DnBP. The epidemiological studies on DnBP did not allow for establishing a relationship between the internal biomarker concentration and the critical health effects. In line with the methodology for deriving HBM-GVs, the second option was explored: whether a TRV is available, adequate to derive an HBM-GV. In the Annex XV restriction report on four phthalates (ECHA, 2016), a derived no effect level (DNEL) of 0.0067 mg/kg bw/d was derived based on the LOAEL of 2 mg/kg bw/d for developmental toxicity observed in the oral toxicity study in rats by Lee et al. (2004). Critical effects were reduction of testicular spermatocyte development and mammary gland changes in the male adult offspring (ECHA, 2016; Lee et al., 2004). An overall assessment factor (AF) of 300 was applied to this LOAEL, accounting for inter- and intraspecies differences (AF of 10 each) and for the extrapolation from a LOAEL to a NOAEL (AF of 3) (ECHA, 2016). As a review of the literature revealed no new findings that would justify an update of this POD, the use of the derived DNEL is supported. Thus, the derivation of an HBM-GV$_{GenPop}$ for the single metabolite MnBP is based on this DNEL of 0.0067 mg/kg bw/d as the TRV. The HBM-GV was calculated according to Formula 1 by using the molecular weights of the parent compound DnBP and the monoester metabolite MnBP, its corresponding F_{ue} and the adjusted urinary flow rates for adults and children each. The resulting HBM-GV$_{GenPop}$ for DnBP is 0.12 mg/L and 0.19 mg/L for children and adults, respectively (see Table 1).

3.2.2. Working population

The exposure protocol for the underlying key study used in the HBM-GV derivation for the general population (Lee et al., 2004) does not correspond to an occupational exposure scenario that should occur for pregnant working women. The exposure period in the Lee et al. (2004) study lasted from gestational day (GD) 15 to the end of lactation on postnatal day (PND) 21 and thereby included perinatal exposure

conditions (Lee et al., 2004). Therefore, the HBM-GV$_{Worker}$ for the selected biomarker of exposure (MnBP) is derived based on an animal POD, representing the third option in the HBM-GV derivation as outlined by Apel et al. (2020a). In the toxicity study conducted by Lehmann et al. (2004), rats were exposed from GD12 to GD19 (Lehmann et al., 2004). This *in utero* period of exposure, when extrapolated to humans, corresponds to the critical window of exposure for the male reproductive system, that is during the 1st trimester of pregnancy. The reduction of foetal testicular testosterone in combination with the reduction in the expression of key genes encoding proteins involved in cholesterol transport and steroidogenesis observed in this study is selected as critical effect for which a POD of 10 mg/kg bw/d was identified (Lehmann et al., 2004). As absorption of DnBP is assumed to be similar for both the inhalation and oral routes, the retrieved urinary total MnBP concentration (free and glucuronidated) is anticipated to be the same for both routes. For this reason, a route-to-route extrapolation is not deemed necessary. A total AF of 100 accounting for inter- and intraspecies differences is applied to the NOEL, resulting in a TRV-like value of 0.1 mg/kg bw/d. The DnBP HBM-GV$_{Worker}$ relating to urinary MnBP is calculated by using Formula 1 and is 3 mg/L (see Table 2).

3.3. HBM-GVs derived for 5 phthalates and the substitute Hexamoll® DINCH for the general population and workers

HBM-GVs have been derived according to the overall methodology agreed upon at HBM4EU consortium level previously published by Apel et al. (2020a). HBM-GV$_{GenPop}$ for children and adults including adolescents and HBM-GV$_{Worker}$ are summarised in Tables 1 and 2, respectively. The key toxicokinetic, epidemiological and/or toxicological data underlying the calculation of these values can be found in supplementary material 1.

Table 1

Human biomonitoring guidance values for the general population (HBM-GV$_{GenPop}$) derived for selected phthalates and the substitute Hexamoll® DINCH.

Parent compound	Biomarker(s)	HBM-GV$_{GenPop}$ in mg/L[a]	
		Children[b]	Adults incl. adolescents[c]
DEHP	5-oxo-MEHP + 5-OH-MEHP	0.34	0.5
	5-cx-MEPP + 5-OH-MEHP	0.38	0.57
DnBP	MnBP	0.12	0.19
DiBP	MiBP	0.16	0.23
BBzP	MBzP	2.0	3.0
DPHP	oxo-MPHP + OH-MPHP	0.33	0.5
	oxo-MPHP	0.19	0.29
	OH-MPHP	0.14	0.22
Hexamoll® DINCH	OH-MINCH + cx-MINCH	3.0	4.5

5-oxo-MEHP: mono(2-ethyl-5-oxohexyl)phthalate (CAS No.: 40321-98-0); **5-OH-MEHP**: mono(2-ethyl-5-hydroxyhexyl) phthalate (CAS No.: 40321-99-1); **5-cx-MEPP**: mono (5-carboxy-2-ethylpentyl) phthalate (CAS No.: 40809-41-4); **MnBP**: monobutyl phthalate (CAS No.: 131-70-4); **MiBP**: monoisobutyl phthalate (CAS No.: 30833-53-5); **MBzP**: monobenzyl phthalate (CAS No.: 2528-16-7); **oxo-MPHP**: mono(propyl-6-oxo-heptyl) phthalate*; **OH-MPHP**: hydroxy-mono-propylheptyl phthalate*; **OH-MINCH**: cyclohexane-1,2-dicarboxylic acid-mono(hydroxyl-iso-nonyl) ester*; **cx-MINCH**: cyclohexane-1,2-dicarboxylic acid-mono-(carboxy-iso-octyl) ester*. Please note, that deriving an HBM-GV$_{GenPop}$ for the subgroup of children under 6 years of age is not appropriate, considering the lack of relevant toxicokinetic data.
*no CAS number available.
[a] Rounded value.
[b] Including children 6–13 years of age.
[c] Including women of child-bearing age.

Table 2

Human biomonitoring guidance values for the working population (HBM-GV$_{Worker}$) derived for selected phthalates.

Parent compound	Biomarker(s)	HBM-GV$_{Worker}$ in mg/L[a]
		Adults[b]
DEHP	5-cx-MEPP	0.62
DnBP	MnBP	3
DiBP	MiBP	3.5
BBzP	MBzP	3
DPHP	oxo-MPHP + OH-MPHP	0.7
	oxo-MPHP	0.4
	OH-MPHP	0.3

5-cx-MEPP: mono (5-carboxy-2-ethylpentyl) phthalate (CAS No.: 40809-41-4); **MnBP**: monobutyl phthalate CAS No.: 131-70-4); **MiBP**: monoisobutyl phthalate (CAS No.: 30833-53-5); **MBzP**: monobenzyl phthalate (CAS No.: 2528-16-7); **oxo-MPHP**: mono(propyl-6-oxo-heptyl) phthalate*; **OH-MPHP**: hydroxy-mono-propylheptyl phthalate*.
*no CAS number available.
[a] Rounded value.
[b] Including women of child-bearing age.

3.4. Global levels of confidence (LoC) attributed to each HBM-GV

As indicated in Table 3 of the supplementary material 2, the overall LoC for the HBM-GVs set for DEHP, DnBP, BBzP and Hexamoll® DINCH were evaluated to be "medium", and the overall LoC for the HBM-GVs for DiBP and DPHP were set to "low". The specific uncertainties considered to assign these overall LoC to the derived HBM-GVs are discussed in detail in supplementary material 2.

The derivation of HBM-GV$_{GenPop}$ for DEHP and Hexamoll® DINCH, as well as an HBM-GV$_{Worker}$ for DEHP have been published in a Deliverable (Apel and Ougier, 2017). However, the LoC for some of the single criteria have been adapted in the meantime for DEHP and Hexamoll® DINCH to be consistent in the assessment of the different criteria. As a result, the overall LoC for the HBM-GV$_{GenPop}$ for Hexamoll® DINCH and also the HBM-GV$_{Worker}$ set for DEHP was changed from low to medium. For more details please see supplementary material 2.

4. Discussion

The HBM-GVs for five phthalates and the non-phthalate substitute Hexamoll® DINCH presented in this paper allow for a direct toxicological interpretation of measured exposure biomarker levels of these compounds in urine. They constitute an easy-to-use screening tool for scientists and risk assessors. They enable the assessment of the chemical burden of the general and the occupational population, prerequisite for the identification of regulatory and scientific needs and priorities. Health-based guidance values for the general population that refer to the internal concentration of a biomarker have been introduced in the past for some phthalates and Hexamoll® DINCH with the HBM-I values derived by the German HBM Commission (values exist for DEHP, DPHP and Hexamoll® DINCH) (German HBM Commission 2007c; 2014b; 2015; summarised in Apel et al., 2017) and the BE values by Hays and Aylward from Summit Toxicology (values exist for DEHP, BBzP, DiNP and DnBP) (Aylward et al., 2009a, 2009b; Hays et al., 2011). Although the derivation concepts for the HBM-GVs, HBM-I and BE values are comparable (Angerer et al., 2011), some of the already existing values differ from those presented here, as some values have been derived years ago and new information has become available.

For DEHP, previous values, either BE or HBM-I were already set a decade ago, in 2009 and 2007, respectively. Both, the German HBM Commission (HBM-I value of 0.3, 0.5 and 0.75 mg/L for women of child-bearing age, children and men aged 14 years and older as well as the rest of the general population, respectively) and Summit Toxicology (BE value of 0.66 mg/L) proposed values based on the TDI established by EFSA in 2005 (Aylward et al., 2009b; German HBM Commission,

2007c). As no new evidence has been identified that would justify a different starting point, the TDI from 2005 is retained as POD for deriving the HBM-GV$_{GenPop}$. However, these derivations are based on human toxicokinetic information from studies by Koch et al. (2004; 2005) in which only one male volunteer participated. For the derivation of an HBM-GV$_{GenPop}$ for DEHP, current and more robust human toxicokinetic data were included in the calculation. The study of Anderson et al. (2011) included 20 human volunteers of both sexes and thereby providing a more robust data basis for the determination of an F$_{ue}$. Presented HBM-GV$_{GenPop}$ are lower than the BE value and the HBM-I values, except for the HBM-I value of 0.3 mg/L exclusively set for women of child-bearing age (German HBM Commission, 2007c). Please note, that the BE value for DEHP is set for the sum of 3 metabolites, MEHP, 5-OH- and 5-oxo-MEHP, whereas the HBM-GV$_{GenPop}$ referred to here are set for the sum of two metabolite combinations each (5-OH-MEHP & 5-oxo-MEHP; 5-OH-MEHP & 5-cx-MEPP; see Table 1).

Similarly, new toxicokinetic information became available for DPHP, for which the German HBM Commission derived HBM-I values in 2015 (German HBM Commission, 2015). The F$_{ue}$ used to extrapolate the concentration of the metabolites in urine corresponding to the TRV-like value of the parent compound was based on the study by Leng et al. (2014) (F$_{ue}$ of 10.7% for OH-MPHP and 13.5% for oxo-MPHP after 48 h). Klein et al. (2018) obtained a F$_{ue}$ about a factor of 4 lower (F$_{ue}$ of 2.3% for OH-MPHP and 3.6% for oxo-MPHP after 46 h) than in the study by Leng at al. (2014). As the laboratory that performed the analysis and the analytical method were the same and similar doses were administered, both results were evaluated as equally reliable. Average F$_{ue}$ of 5.97% for OH-MPHP and 7.95% for oxo-MPHP after ~24 h were calculated from the F$_{ue}$ values indicated in the two studies (at 24 h for Leng et al., 2014 and 22 h for Klein et al., 2018) and used for the HBM-GV$_{GenPop}$ derivation. Furthermore, the POD selected for deriving an HBM-GV$_{GenPop}$ was different from the one chosen by the German HBM Commission for deriving an HBM-I value (German HBM Commission, 2015). In the present paper, the derivation is based on a TRV, i.e. the RfD of 0.1 mg/kg bw/d calculated by Bhat et al. (2014), who have considered effects on the thyroid (follicular hypertrophy/hyperplasia) observed in F1 adults in a two-generation feeding study (BASF, 2009). The German HBM Commission based their HBM-I values of 1 and 1.5 mg/L (sum of OH- and oxo-MPHP), for children and adults respectively, on a NOAEL of 40 mg/kg bw/d determined in a subchronic feeding study (BASF, 1995), in which effects on the thyroid and pituitary gland were considered critical. As a result, the HBM-GV$_{GenPop}$ presented here is lower than the HBM-I value.

For Hexamoll® DINCH, there are HBM-I values for children and adults, set in 2014 (German HBM Commission, 2014b), but no BE values. As no new toxicological evidence was identified, the HBM-GV$_{GenPop}$ are at the same level as the HBM-I values.

In 2009, Aylward et al. presented BE values for DnBP and BBzP based, among others, on the TDIs set by EFSA in 2005 (Aylward et al., 2009a). The HBM-GV$_{GenPop}$ for DnBP and BBzP presented in this paper were derived based on different PODs (and endpoint, respectively). Thus, the HBM-GVs differ from previously set BE values (0.2 and 12 mg/L for DnBP and BBzP, respectively). With regard to DnBP, the DNEL of 0.007 mg/kg bw/d set by ECHA (2012, 2016) is based on the LOAEL of 2 mg/kg bw/d observed in the study by Lee et al. (2004). ECHA applied an overall AF of 300, accounting for interspecies and intraspecies differences (each AF = 10) and for the LOAEL-NOAEL extrapolation (AF = 3) according to the ECHA R8 guidance. EFSA however, used an AF for the LOAEL-NOAEL extrapolation of 2 for setting the TDI (EFSA, 2005, 2019). For the HBM-GV$_{GenPop}$ derivation, the approach of ECHA was followed (ECHA, 2012, 2017), resulting in a similar value of 0.19 mg/L for adults and a lower value for children with 0.12 mg/L compared to the BE value for DnBP.

In case of BBzP, the TDI set by EFSA, 2005 based on reduced AGD in F1 and F2 rat offspring was not used as POD for the HBM-GV$_{GenPop}$ derivation, nor another TRV. Instead, the LO(A)ELs of 100 mg/kg bw/d

for foetal testicular testosterone suppression observed in the study by Furr et al. (2014) and for reduced serum testosterone levels as well as reduced epididymal sperm count and motility in F1 adult rats after *in utero* exposure observed in the study by Ahmad et al. (2014) were identified as substantial (Ahmad et al., 2014; Furr et al., 2014). It needs to be noted, that within HBM4EU the significance of smaller changes in testosterone levels in the foetus and the resulting possibility of adverse outcomes was controversially discussed. Therefore, another endpoint besides foetal testosterone suppression was considered when the POD was selected (i.e. epididymal sperm changes). As a result, the HBM-GV for BBzP is more than one order of magnitude higher than the values for DEHP and DnBP. Considering similar potencies for anti-androgenic effects (Howdeshell et al., 2008), this value must be used with caution. Further toxicity studies for BBzP are needed to evaluate, whether critical effects not assessed so far (i.e. testicular and mammary histology; dysgenesis of external genitalia) are occurring at low-level exposure to BBzP. Compared to the BE value, the HBM-GV$_{GenPop}$ of 3 mg/L for adults and 2 mg/L for children presented here are lower.

Lastly, neither HBM-I, nor BE values exist for DiBP, making the HBM-GV$_{GenPop}$ for DiBP presented in this paper the first health-related guidance value referring to the internal concentration of its metabolite MiBP.

The HBM-GVs derived under HBM4EU underwent a consultation with national experts nominated by the partner countries and the EU Policy Board on an HBM4EU consortium level and have been mutually agreed upon (see Apel et al., 2020a). This ensures the acceptance of these values by the European partner institutions and a comparable assessment of HBM data gathered in this project and presumably beyond, contributing to a harmonised approach towards a joint improvement of European chemical policy. The adoption and implementation at EU level remain to be discussed by the responsible EU authorities.

The presented HBM-GVs are rather intended to be used for the interpretation of results from HBM studies reflecting exposure of the general population (or workers sub-populations). The interpretation of HBM results towards the risk for the occurrence of an adverse health effect is more complex at the individual level. On the one hand, urinary concentrations of an individual measured in HBM studies are assumed to be subject to large within-day variations due to physiological factors of that individual (hydration status). In addition, within-day variations in urine samples are likely to occur due to the short biological half-lives of the biomarkers of exposure (Aylward et al., 2009a, 2017; Hays and Aylward, 2009). Therefore, for the analysis of individual samples, 24 h urine collections instead of spot urine samples is recommended. However, on a population basis, spot and 24 h samples produce comparable results (Christensen et al., 2012). On the other hand, there are underlying uncertainties in the HBM-GV derivation itself. These include general assumptions made regarding typical values for urinary flow rates and the origin of urinary excretion fraction data. These data often originate from a small number of volunteers, mostly men, only and data on inter-individual variability (e.g. due to sex or age, genetic polymorphism) often lacks (Angerer et al., 2011; Aylward et al., 2009a). The HBM-GVs are of limited interpretability in regard to multiple individual and health-associated factors and this needs to be clearly communicated. Nevertheless, they are helpful in raising awareness towards the possible health risks of chemical exposure as long as information is well prepared for lay people and guidance is given.

The HBM-GVs have their limitations as they allow only a single substance risk assessment. Various phthalates are frequently detected in investigated populations all over Europe (Cullen et al., 2017; Dewalque et al., 2014; Frederiksen et al., 2020; Gyllenhammar et al., 2017; Hartmann et al., 2015; Husøy et al., 2019; Schwedler et al., 2020) and thus a widespread concurrent exposure to multiple phthalates is given. Phthalates that show similar anti-androgenic effects are assumed to share a common mode of action and can act in a dose-additive manner as shown in rats (Howdeshell et al., 2007, 2008; Rider et al., 2010). Therefore, a cumulative risk assessment is rather warranted to protect

the population from the adverse health effects arising from exposure to phthalates (Apel et al., 2020b; Kortenkamp and Koch, 2020). In order to close this gap, a proposal for a methodology using the HBM-GVs in a cumulative risk assessment for anti-androgenic phthalates within HBM4EU will be established.

5. Conclusion and perspective

The restrictions and regulations of reprotoxic phthalates in the EU have led to a change in their exposure patterns. The population's exposure to older, well-known and reprotoxic phthalates (DEHP, DnBP, BBzP) has decreased, while exposure inter alia to DPHP and the non-phthalate substitute Hexamoll® DINCH thought to be less harmful has increased (Apel et al., 2020b; Frederiksen et al., 2020; Gyllenhammar et al., 2017; Hartmann et al., 2015; Kasper-Sonnenberg et al., 2019; Schwedler et al., 2019). Exposure levels of the population to phthalates and Hexamoll® DINCH must be assessed to timely implement necessary reduction measures. Furthermore, the monitoring of restricted phthalates and their alternatives is warranted to evaluate the effectiveness of the regulations in place and adopt new regulations if necessary. The HBM-GVs for the six substances presented in this paper form the basis for a harmonised health risk assessment of the European population. These values will be used to perform single-substance risk assessments by comparison with aligned HBM datasets from across Europe generated under the HBM4EU project. Furthermore, it is currently explored how HBM-GVs can be used to perform risk assessments for chemical mixtures (e.g. mixture of phthalates) to evaluate real-life exposure scenarios.

In addition to the six HBM-GVs described in this paper, HBM-GVs have been derived for cadmium and bisphenol A (Lamkarkach et al., 2021; Ougier et al., publ. submitted). It is anticipated that further HBM-GVs will be derived for bisphenol S, aprotic solvents (n-methyl-2-pyrrolidone, n-ethyl-2-pyrrolidone, dimethylformamide, dimethylacetamide), mercury and selected pyrethroids.

HBM-GVs are a valuable tool for the evaluation of exposures in a health-risk assessment. They constitute a common basis for assessment and they are easy to use as they can directly be compared to HBM data. The HBM4EU project was the first initiative proposing HBM-GVs agreed upon in a broader European consensus. In addition, the LoC assigned to each derived HBM-GV highlight data gaps and thus can help to foster research activities for future HBM-related international initiatives.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113722.

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The European human biomonitoring platform - Design and implementation of a laboratory quality assurance/quality control (QA/QC) programme for selected priority chemicals

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ABSTRACT

A fundamental objective of the human biomonitoring for Europe initiative (HBM4EU) is to progress toward comparable and robust exposure data for a wide variety of prioritized chemicals in human samples. A programme for Quality Assurance/Quality Control (QA/QC) was designed in HBM4EU with the purpose of creating a network of European laboratories providing comparable analytical data of high quality. Two approaches were chosen for two sets of prioritized chemicals with different timelines: (i) Scheme 1, where interested candidate laboratories participated in multiple rounds of proficiency tests (ii) Scheme 2, where selected expert laboratories participated in three rounds of interlaboratory comparison investigations. In both cases, the results were used to identify laboratories capable of generating consistent and comparable results for sample analysis in the frame of HBM4EU. In total, 84 laboratories from 26 countries were invited to participate in Scheme 1 that covered up to 73 biomarkers from Hexamoll® DINCH, phthalates, bisphenols, per- and polyfluoroalkyl substances, halogenated flame retardants (HFRs), organophosporous flame retardants (OPFRs), polycyclic aromatic hydrocarbons (PAH), cadmium, chromium and aromatic amines. 74 of the participants were successful for at least one biomarker in Scheme 1. Scheme 2 involved 22 biomarkers and successful results were obtained by 2 expert laboratories for arsenic, 5 for acrylamide, 4 for mycotoxins, 2 for pesticides and 2 for UV-filters in skin care products. The QA/QC programme allowed the identification of major difficulties and needs in HBM analysis as well of gaining insight in the analytical capacities of European laboratories. Furthermore, it is the first step towards the establishment of a sustainable European network of HBM laboratories.

1. Introduction

Human biomonitoring (HBM) is the gold standard for assessing the

actual, overall exposure to chemicals in individuals or populations, irrespective of the detailed knowledge of contributing exposure sources or pathways. Differences in body burdens are mainly reflective of diet,

consumer goods and lifestyles as the main exposure determinants for many environmental or product-use related chemicals (Scherer, 2005; Ginsberg and Balk, 2016; Pacyga et al., 2019). These differences are generally small, whereas occupational exposure to chemicals could result in relatively high body burdens. The chemical analysis of HBM samples is facing a number of challenges, related to the environmental exposure at low levels, to the complexity of mixtures of different chemicals (ubiquitous in some cases) and the complex biological matrices. Being a relatively young discipline, with a rapidly growing number of exposure biomarkers in various human tissues and an increasing number of laboratories venturing into the field of HBM, there is a growing demand for establishing a common platform for HBM laboratories, facilitating knowledge exchange, cross-validations, and an international standardization/harmonization of appropriate biomarkers and analytical methods. In this context, it is crucial to have the confidence that the observed differences in multicentre HBM studies are due to variations in exposure and not due to variability or artefacts in the analytical or pre-analytical phase.

In the majority of the EU countries, HBM has been applied as a tool in research projects, often focusing on specific populations, with the exception of some countries such as Germany, Belgium or France, which have established full-scale HBM programmes (Kolossa-Gehring et al., 2012; Schoeters et al., 2012; Dereumeaux et al., 2017). Although significant HBM data have been generated in European countries in the last few decades, the available information appears somewhat fragmented and not always fully comparable. The differences range from the study design (e.g. target population, selection of biological matrices, etc.) to the pre – analytical phase (e.g. sampling procedures, type of samples, etc.) and to the applied analytical methods. With regard to the latter, unlike in some other fields, no standard reference methods do exist for HBM surveillance purposes yet, as there is currently no structure/network of European and/or National Reference Laboratories as it exists in other fields, such chemical food safety (von Holst et al., 2016; Parvaneh et al., 2017; Broothaerts et al., 2020). In addition, sustainable procedures and schemes for proficiency testing applied to human matrices have not yet been extensively developed and there are only few suppliers of proficiency tests for HBM biomarkers (e.g. G-EQUAS, QMEQAS, OSE-QAS), offering a limited (though increasing) range of biomarker/matrix combinations and relevant environmental and product-use related exposure levels (Göen et al., 2012).

This lack of harmonization was already addressed during the preparation of the EU Environment and Health Action Plan 2004–2010 (COM 416, 2004) and as a consequence, efforts were made to harmonize HBM in Europe. The first steps were implemented by ESBIO (Expert Team to Support Biomonitoring in Europe), followed by COPHES (Consortium to Perform Human Biomonitoring on a European Scale) (Becker et al., 2014; Schindler et al., 2014; Esteban López et al., 2015) and DEMOCOPHES (DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale) (Den Hond et al., 2015) and most recently, by the Human Biomonitoring for Europe Initiative (HBM4EU, www.hbm4eu.eu).

HBM4EU is an EU Joint Programme that has developed its research programme for priority substances as defined by EU services and partner countries' policy makers to answer open policy relevant questions. HBM4EU aims to harmonize and use HBM to understand human exposure to chemicals, in occupational settings, through the use of consumer products or behavioural choices and the related health risks to improve the chemical risk management and to support policy-making (Ganzleben et al., 2017). Based on policy-related research needs regarding chemical exposure and potential health effects, two sets of priority chemicals were selected in HBM4EU. First set, including phthalates and their substitute 1,2-cyclohexane dicarboxylic acid diisononyl ester (Hexamoll® DINCH), bisphenols, per- and polyfluoroalkyl substances (PFAS), halogenated flame retardants (HFRs), organophosphorus flame retardants (OPFRs), polycyclic aromatic hydrocarbons (PAHs), cadmium, chromium and aromatic amines and the second, acrylamide, aprotic

solvents, arsenic, diisocyanates, lead, mercury, mycotoxins, pesticides and UV-filters in skin care products.

Because the chemical analyses in HBM4EU and its predecessors have been organized in a decentralized manner, involving multiple laboratories in several countries the need to ensure data comparability has been a central aspect of the project early on. First steps in this direction were undertaken in COPHES/DEMOCOPHES where a programme consisting of Interlaboratory Comparison Investigations (ICIs) and External Quality Assessment Schemes (EQUAS) was implemented, supporting the generation of comparable HBM data in 17 EU countries (Schindler et al., 2014; Esteban López et al., 2015). In HBM4EU the challenge has been even greater since the number of laboratories, countries and chemicals are significantly higher. Also, as the conclusions on exposure differences in HBM4EU will have important public health consequences at policy-making level, the quality and comparability of the analytical results has to be guaranteed by strictest Quality Assurance and Quality Control (QA/QC) measures. Based on the two list of priority substances established along the project and the time frame, two different approaches were applied to ensure the full comparability of the analytical results. The first one comprised 4 rounds of proficiency tests with a high number of participating laboratories while the second approach was an intensive interlaboratory comparison investigation with a reduced number of expert participants.

This paper presents the main results and compares the two approaches developed in HBM4EU to obtain high quality and comparable analytical results in multicentre HBM studies for a variety of chemicals and laboratories with different degrees of expertise.

2. Material & methods

2.1. Quality Assurance Unit and QA/QC programme

A Quality Assurance Unit (QAU) was established to discuss and decide all issues related to the QA/QC of the chemical analyses in HBM4EU, including the design of the QA/QC programme. The QAU was formed by experts in the field of HBM and analytical chemistry and included the leaders of the COPHES/DEMOCOPHES QAU, to ensure the continuation of previous successful approaches.

The prime objective of the HBM4EU QA/QC programme was to identify (and in the end certify) analytical laboratories that could analyse the HBM4EU samples accurately, precisely and the most important in a comparable way. For that, the QAU designed two different schemes for each set of prioritized compounds mainly to address the time constraints (Fig. 1). Scheme 1 covered the substances on the 1st priority list and involved four rounds of proficiency tests. Participants were free to decide for which biomarkers they participated. The exercises were organised and evaluated as ICIs or EQUAS, depending on the needs and situation for each substance group. In both cases, the exercises involved the assessment of the comparability of analytical results for the same control material analysed in parallel by multiple laboratories, with their own analytical method. As measure of proficiency, Z-scores were calculated using an assigned value, and a preset target standard deviation (e.g. fit-for-purpose standard deviation). In case of ICIs, the assigned value was derived from the participants' results, in case of EQUAS, the assigned value was the mean concentration as established from data generated by designated expert laboratories (ELs).

Scheme 2 addressed a reduced list of chemicals, compared with the original 2nd list of prioritization, to match the studies planned in HBM4EU: acrylamide, arsenic, mycotoxins, pesticides and UV-filters. Scheme 2 included three rounds of ICIs and laboratories should participate for all the biomarkers within a substance group.

For both schemes, two control materials were sent to the participants in each round. The target concentrations of the biomarkers in the control materials was in the range commonly observed in the general population (between P25–P90 percentile in available national reference values of
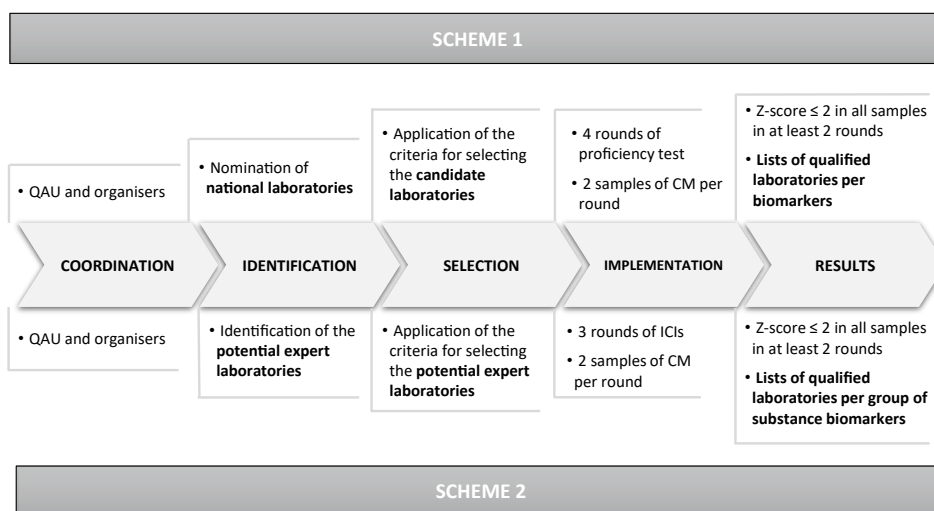
Fig. 1. Steps followed in the two schemes of the HBM4EU QA/QC programme. CM: control materials. ICI: interlaboratory comparison investigation.

EU countries (Den Hond et al., 2015), occupational exposure in the case of Cr) (table S7).

The rounds were spread out over time in such a way that laboratories received feedback on their performance well before the next round, allowing them to perform corrective actions, if needed, before participation in the next round.

To achieve satisfactory results in the schemes and take part in the analysis of the samples in HBM4EU, participants had to obtain successful results in at least two rounds.

2.2. Identification of the supporting and participating laboratories

The objective of this part of the study was to identify laboratories that could support the QA/QC programme by organising the proficiency tests for a specific group of substances, as well as those laboratories that like to analyse samples in HBM4EU.

The potential candidate laboratories were identified by the HBM4EU National Hub Contact Points (i.e. the contact point for each participating country), who provided information on laboratories performing chemical analyses of the prioritized compounds in human matrices and announcing the activity at country level. Additionally, an announcement was launched on the websites of HBM4EU (www.hbm4eu.eu) and the European Environment Agency (EEA) and in different scientific societies and fora, requesting interested laboratories to sign up as potential candidate laboratories in HBM4EU.

Questionnaires were sent to all nominated laboratories to collect information on their experience in the analysis of the target chemicals in human matrices as well as in organising proficiency tests. The responses were evaluated according to the criteria previously defined by the QAU, having as first criterion the experience in the chemical analysis of the target compound group in human samples (Tables S2 and S3). This process resulted in a list of candidate laboratories who were invited to participate in Scheme 1 or to support the QA/QC programme. Fig. 1 summarises the process.

In Scheme 2, it was agreed to select a reduced number of expert laboratories according to technical and practical criteria defined by the QAU (Table S4). The ELs were invited to join the ICIs and alerted about the tight time frame of these ICIs. All the HBM4EU analyses of the 2nd set of priority substances would be performed only in the ELs obtaining satisfactory results (Fig. 1).

The QA/QC programme was coordinated by the QAU and the supporting selected laboratories were responsible for preparing and sending the control materials to the participants, establishing the communication with the participants, evaluating the results and preparing the reports.

2.3. Selection of the biomarker/matrix combinations in the QA/QC programme

Specific exposure biomarkers and most suitable matrices to be included in the QA/QC programme were selected for the first group of prioritized chemicals as described in Vorkamp et al. (2021). Briefly, compound-independent criteria were developed for the selection of most suitable biomarkers and matrices for HBM, for example considering the specificity, biological sensitivity and stability of a certain biomarker/matrix combination. These criteria were then applied to review the scientific literature of the last ten years approx., with a view to identify the most suitable biomarkers and matrices for each of the prioritized chemical. This evaluation resulted in a first list of pairs of biomarker/matrix (typically serum or urine) for each compound group (Vorkamp et al., 2021). In the next step, the list of exposure biomarkers was further reduced based on technical feasibility, expected body burdens and policy-related research needs, as evaluated by the QAU. This shortlist was used in the QA/QC programme.

The same procedure was applied to define the biomarkers for the 2nd list of priority substances addressed in Scheme 2.

2.4. Organization of the proficiency tests

In order to provide a harmonised approach for the organization and evaluation of the different ICI/EQUAS exercises, protocols were drafted and described in standard operating procedures (SOPs). These SOPs were based on existing protocols originated from ISO17043 accredited organisations, and included detailed instructions for all aspects of the QA/QC programme, such as the description of the roles and responsibilities of the organisers, timeline of the exercises, definitions of different terms or templates for communication with the participants and reporting of the results. Additional SOPs were drafted for the preparation and characterisation of control materials and for the evaluation of participants' results. Details for the preparation of the various control materials are available in the online library of the HBM4EU website (www.hbm4eu.eu). The characterization of the control materials included homogeneity and stability testing.

Homogeneity testing was based on ISO13528:2015 and Fearn and Thompson (2001). This involved duplicate analysis of 10 randomly selected test samples of a control material. The control material was considered sufficiently homogeneous if the between-sample standard deviation did not exceed a critical value (0.3 x target standard

deviation).

The stability of the control materials during the period from shipment to the deadline for submission of the participants' results was assessed in line with ISO 13528:2015 and the international harmonised protocol for the proficiency testing of analytical laboratories (Thompson et al., 2006). For this, the organiser stored test samples under the conditions recommended to the participants (typically freezer < -18 °C), and optionally an additional set at −80 °C. Stability was assessed by comparison of the mean of six stored samples (−18 °C, t = after receiving all participants' results), with the mean of a reference set of six samples. The reference was either the mean as obtained at/before shipment of the samples, or the mean obtained for samples stored at −80 °C (assumed stable) analysed concurrently with the stored samples.

2.5. Evaluation of laboratory performance

The laboratory performance was assessed by calculation of z-scores for each biomarker in the test samples according to the following formula:

$$Z = \frac{x - A}{\sigma_T} \tag{1}$$

with Z = z-score
x = participant's result
A = assigned value
σ_T = standard deviation for proficiency, with $\sigma_T = 0.25*A$

A z-score of $|Z| \leq 2$ was interpreted as satisfactory, $2<|Z|<3$ as questionable, and $|Z| \geq 3$ as unsatisfactory performance. The assigned value was either the consensus value derived from the participants (used in ICI) or a value derived from analysis by selected expert laboratories (used in EQUAS). The parameters from equation (1) are briefly explained l below.

- Standard deviation for proficiency, or target standard deviation (σ_T), determines the performance boundaries of the ICI/EQUAS. The performance boundaries should be fit-for-purpose and take into account the interlaboratory variability (reproducibility relative standard deviation, RSD_R) currently considered achievable in HBM analysis. The available data on the latter is scarce and variable. Schindler et al. (2014) reported RSD_Rs ranging from 6% to 32% for cadmium in urine, and 31%–45% (even higher in some cases) for phthalate biomarkers in urine. For selected highly experienced reference laboratories, Göen et al. (2012) reported RSD_Rs in the range 7%–19% for cadmium in urine. Outside the HBM domain, a generic relationship between expected RSD_R and concentration was originally proposed by Horwitz et al. (1980) and later modified by Thompson (2000), and has often been used as a fitness-for-purpose criterion in proficiency testing. The modified Horwitz equation suggests a constant RSD_R of 22% for concentrations below 120 μg/kg, and a decrease of RSD_R for higher concentrations. The validity of the modified Horwitz function has been a matter of debate (Linsinger and Josephs 2006). A constant RSD_R of 25% over a range of 1 μg/kg to 10 mg/kg has been suggested as more appropriate (Alder et al., 2001). Based on the literature it appears that, as long as concentrations are (well) above the method limit of detection (LOD), there is no consistent relationship between concentration and RSD_R. It may depend on the analyte and technique, but at this stage, in lack of exhaustive data for achievable RSD_Rs in HBM analysis and based on the data and discussions from the literature, it was decided to apply a fixed RSD of 25% as fit-for-purpose criterion to be used as target relative standard deviation in the ICI/EQUAS programme.

- Assigned value = consensus value (ICI). For determination of the consensus value, robust statistics was performed in accordance with Thompson et al. (2006), the guidelines from (Analytical Methods Committee, 1989a&b), and ISO 13528. The robust mean was taken as consensus value when the following requirements were met: the number of results submitted for a biomarkers had to be at least seven, the uncertainty (u) of the consensus value should be negligible (not exceed $0.3*\sigma_T$), with u being 1.25 times the standard deviation of the participants' results, divided by the square root of the number of participants. When the uncertainty of the consensus was not negligible, but not exceeding $0.7*\sigma_T$, the consensus value was still used for calculation of z-scores, but the uncertainty of the consensus value was taken into account for calculation of the z-scores using the following formula:

$$Z' = \frac{x - A}{\sqrt{\sigma_T{}^2 + u^2}} \tag{2}$$

with Z' = z-score $(0.3*\sigma_T < u \leq 0.7*\sigma_T)$
u = uncertainty of consensus value

In case the uncertainty of the consensus exceeded $0.7*\sigma_T$, the variability of results was considered too high to derive a meaningful consensus, and, consequently, also z-scores using such consensus value were considered unfit for evaluating individual participants' performance.

- Assigned value = Expert value (EQUAS). Establishment of the expert value to be used as assigned value involved the analysis of six replicates of the control material by at least three selected laboratories with a high level of expertise in the determination of the biomarker. For each expert laboratory, the mean value was calculated. Based on these means, the mean of the expert laboratories was calculated, the RSD, and the relative uncertainty (RSD divided by the square root of the number of expert laboratories). The expert value was considered suitable for use as assigned value as long as the uncertainty did not exceed $0.7*\sigma_T$ (i.e. 17.5%). For EQUAS, equation (1) was used for calculation of z-scores.

2.6. Programme coordination and follow – up

Monthly web conferences were organised among the QAU, the programme coordinator and the organisers of the different exercises to discuss the problems encountered and to exchange experiences. A help desk was available for the participating laboratories during the whole duration of the scheme and web conferences were offered to the participants when necessary in order to solve the main analytical problems faced during the exercise. Furthermore, participants also received recommendations after each round in the result reports. Nevertheless, some groups (i.e. Hexamoll® DINCH and phthalates) required a more intense and continuous support and a specific training school was organised.

3. Results

3.1. Details of the QA/QC programme

Both approaches were designed under common premises, but there were some important differences, for example in the number of biomarkers and matrices included (Fig. 2). Scheme 1 covered 73 biomarkers in 3 different human matrices while Scheme 2 involved 22 biomarkers in urine. The duration of Scheme 1 was 18–20 months depending on the substance group. The time for implementing Scheme 2 was shorter, from 3 up to 7 months depending on the group of substances.

3.2. Identification of the supporting and participating laboratories

For Scheme 1, a total of 183 questionnaires were sent to identify candidate laboratories for supporting the QA/QC programme and for

SCHEME 1	SCHEME 2
- Phthalates, Hexamoll® DINCH, bisphenols, PFAS, BFRs, PFRs, PAHs, Cd, Cr, aromatic amines	- As, acrylamide, mycotoxins, pesticides, UV-filters
- 73 biomarkers in total	- 22 biomarkers in total
- 4 rounds of proficiency tests	- 3 rounds of ICI
- Urine, blood, serum	- Urine
- Participation per biomarker	- Participation per group of substance

Fig. 2. Main characteristics of the two approaches followed in the HBM4EU QA/QC programme.

participating in the proficiency tests. 115 replies were received (63% response rate), some of them from the same laboratory to participate for different substance groups. Fig. 3 presents the number of candidate laboratories for Scheme 1 per group of substance. Approximately one year later, and after the 1st round of the proficiency test, the list of candidate laboratories was updated (Fig. 3). Questionnaires were sent to 229 potentially interested laboratories, including those already registered as candidate laboratories. The response rate was lower (37%) since some of the laboratories already included on the list of candidate laboratories did not update their data. In order to increase the participation, the selection process was simplified and the only criteria applied for participating in Scheme 1 was the exclusive criterion in table S2 ("have experience in analysing human samples for the given chemical").

With regard to the origin of the candidate laboratories, almost half of them (48%) were from universities or university hospitals, followed by governmental laboratories with 39% of the total number. Private laboratories accounted for 9%.

Of the laboratories completing the questionnaire for supporting the HBM4EU QA/QC programme, 19% reported experience in organising proficiency tests and 18 laboratories were selected in the first call, based on the criteria in Table S3. In the update, 11 participants out of 48 (23%) provided a positive answer and only three new laboratories were added to the list of potential supporting laboratories. Finally, five laboratories were involved as organisers (Table 1). In case of Scheme 2, the organisers were selected from those supporting the previous scheme based on their proven expertise (Table 1). The proficiency test for aromatic amines required a different organization as so, it will not be addressed in this publication.

Table 2 shows the biomarkers covered in the programme (except those for aromatic amines) as a result of the selection process, grouped by substances classes. While Scheme 1 offered a broad range of biomarkers and the participants decided for which they reported results, Scheme 2 offered a more limited number of biomarkers and the ELs had to participate for all biomarkers included in the programme. However, while the biomarkers were pre-defined, the laboratories were free to choose their own analytical method.
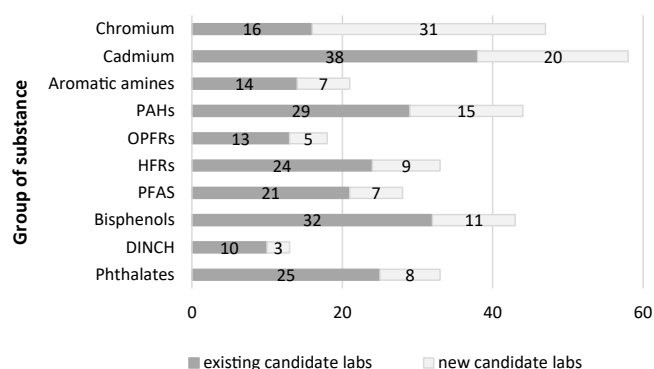
Table 1
Laboratories supporting the HBM4EU QA/QC programme. (Acronyms are defined in Table S1).

Substance group	Organiser	Laboratory preparing & testing control material
1st list of prioritization – Scheme 1		
Phthalates	RIKILT	RIKILT, IPA
Hexamoll® DINCH	RIKILT	RIKILT, IPA
Bisphenols	INRAE	INRAE
PFAS	IPASUM	IPASUM
HFRs	UCT	UCT
OPFRs	UCT	IPASUM
PAHs	IPASUM	IPASUM, UCT[a], ABF[b]
Cadmium	IPASUM	IPASUM
Chromium	IPASUM, JSI[a]	IPASUM, JSI[a]
2nt list of prioritization – Scheme 2		
Acrylamide	IPASUM	IPASUM
Arsenic	IPASUM	IPASUM
Mycotoxins	RIKILT	RIKILT
Pesticides	RIKILT	RIKILT, IPA
UV-filters	IPASUM	IPA, Region H

RIKILT, current name: Wageningen Food Safety Research, part of Wageningen University & Research, The Netherlands.
IPA, Institute for Prevention and Occupational Medicine of the German Social Accident Insurance - Institute of the Ruhr-University Bochum, Germany.
INRAE, Laboratoire d'Etude des Résidus et Contaminants dans les Aliments, LABERCA, Oniris-INRAE, France.
IPASUM, Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany.
UCT, University of Chemistry and Technology, Czech Republic.
ABF, ABF GmbH Analytisch-Biologisches Labor, Planegg, Germany.
JSI, Jozef Stefan Institute, Slovenia.
Region H, Dep. of Growth and Reproduction, Rigshospitalet, University of Copenhagen.
Selection of the parameters in the programme.
[a] Only the 1st round.
[b] Only 3-BaP in the 1st and 2nd round.

3.3. Participation

A total of 84 laboratories from 26 countries were invited to participate in Scheme 1 but not all of them confirmed their participation. The percentage of invited laboratories that registered in the course of the complete scheme per group of substance varied from 85% for Hexamoll® DINCH to 35% for OPFRs (considering the highest number of registrations in each group). In the case of Hexamoll® DINCH the registration was constant in the four rounds while for the rest of substances, in general, the number of registered laboratories increased after the first two rounds, up to twice or more in case of cadmium and chromium, and decreased after the 3rd round. In total 9 laboratories from Canada, Japan and United States collaborated as reference laboratories in the rounds that were organised as EQUAS (from the 2nd to the 4th round, except for Cr in which all rounds were ICIs) (Table S5).

Looking at the participation of the laboratories in Scheme 1 for different groups of chemicals, more than the half (61%) of the

Fig. 3. Number of candidate laboratories in the two calls.

Table 2
Biomarkers covered in the HBM4EU QA/QC programme.

Substance group	Matrix	Biomarkers
1st list of prioritization – Scheme 1		
Phthalates	urine	MEP, MBzP, MiBP, MnBP, MCHP, MnPeP, MEHP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP, MnOP, OH-MiNP, cx-MiNP, OH-MiDP, cx-MiDP
Hexamoll® DINCH	urine	OH-MINCH, cx-MINCH
Bisphenols	urine	BPA, BPF, BPS
PFAS	serum	PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFBS, PFHxS, PFHpS, PFOS (sum of all isomers)
HFRs	serum	BDE-47, BDE-153, BDE-209, α-HBCD, γ-HBCD, TBBPA, Syn-DP, Anti-DP, DBDPE, 2,4,6-TBP
OPFRs	urine	DPHP, BDCIPP, BCEP, BCIPP
PAHs	urine	1-naphthol, 2-naphthol, 1,2-DHN[a], 2-FLUO, 3-FLUO, 9-FLUO, 1-PHEN, 2-PHEN, 3-PHEN, 4-PHEN, 9-PHEN, 1-PYR, 3-BaP[a]
Cadmium	urine blood	Cd
Chromium	urine blood serum	Cr
2nt list of prioritization – Scheme 2		
Acrylamide	urine	AAMA, GAMA
Arsenic	urine	As total, As (III), As (V), MMA, DMA, AsB
Mycotoxins	urine	DON (total)
Pesticides	urine	TCPy, glyphosate, AMPA, cis-DBCA, cis-DCCA, trans-DCCA, 3-PBA, 4-F-3-PBA, ClF3CA
UV-filters	urine	BP1, BP2[b], BP3, BP7[b]

[a] Only in the 1st and 2nd round.
[b] Only in the 1st round.

laboratories participated in the proficiency test for one or two substance groups (36% and 25% respectively) while a very limited number of laboratories (3%) participated for six or more substance groups.

The registration of the laboratories in the biomarkers offered in Scheme 1 varied considerably and showed a high intra-group variation for some substances. The low number of participants sometimes led to insufficient data for results evaluation and a detailed revision was required, including different statistical evaluations in order to obtain reliable results and conclusions. The specific difficulties encountered in each group of substances and the solutions applied will be addressed elsewhere. Table S6 shows the number of laboratories that registered in the four rounds as well as those reporting results and those consistently achieving satisfactory performance in Scheme 1.

Considering the global results, 74 participants reported successful results for at least one biomarker. The maximum number of biomarkers for which a laboratory reported successful results was 47. The average and P90 were 11 and 30 biomarkers, respectively.

Regarding participation in Scheme 2, five expert laboratories participated for acrylamides, three for arsenic, six for mycotoxins, four for pesticides and three for UV-filters, however, this was reduced to two in the second and third round (Table 3). At least two thirds of the participating laboratories returned satisfactory results (Table 3). All participants in the acrylamide exercise obtained satisfactory results.

4. Discussion

Although QA/QC is an essential component in any analytical laboratory, robust results that are comparable between laboratories can still be a challenge, in particular in the context of human biomonitoring of the general population, including low concentrations and the co-occurrence of a multitude of chemicals. For the first time, two different QA/QC approaches were implemented to ensure the quality and comparability of the analytical results in a multicentre EU-wide HBM project.

The design of the HBM4EU QA/QC programme had to be adapted to certain predefined characteristics of the project, mainly the time constraints and the support of capacity building in the participating countries. Scheme 1 offered the possibility of including a high number of laboratories (including less HBM experienced laboratories) and improving their analytical performance while Scheme 2, had to be done in a shorter time period and focused on assessing comparability of results for a small pre-selected group of expert laboratories. Thus, the two approaches were designed according to different priorities.

This work has allowed the identification of a high number of EU laboratories with experience in human biomonitoring and created the first HBM laboratory network in Europe. Nevertheless, despite the two calls and different communication channels employed to reach the laboratories, the authors are aware that a number of analytical laboratories from the different participating countries were not involved in the programme. This could be due to the information not reaching the laboratories or to a lack of interest in participating in the programme (e. g. not aligned with the laboratory interests or because it was a non-funded activity). However, the number of participants allowed to achieve the objectives of HBM4EU, i.e. obtain high quality and comparable HBM results and to provide the capacities for a Europe wide HBM study.

The process of identifying the candidate laboratories revealed interesting information. Significant differences were observed in the number of candidate laboratories for the different groups of substances, primarily reflecting expertise in the analysis of the substances involved. For example, the analysis of cadmium in human samples has been established for years, and validated analytical methods are available. As a consequence, the highest number of candidate laboratories to participate in the QA/QC programme was found for cadmium analysis. However, for biomarkers related to chemicals of more emerging concern the number of laboratories with experience is lower and therefore the number of candidate laboratories was reduced e.g. for Hexamoll® DINCH or OPFRs. A kind of specialization or interest in certain substances was observed for the majority of the laboratories since in general the participation was restricted to 1–3 groups of substances while a reduced number of them covered a wider spectrum. Nevertheless, this could be influenced by other factors such as individual interests and therefore not reflect the real situation in terms of expertise and capacities of the laboratories. Independently of that, there was a clear difference between the participation and results of the inorganic and organic chemicals selected in the project.

A great challenge during the first stages of the programme was the identification of the laboratories to support the QA/QC programme (proficiency tests organisers) since only a limited number of laboratories

Table 3
Participants and results in Scheme 2.

	ROUND 1	ROUND 2	ROUND 3	no.reporting satisfactory results [a] (%)
	no. registered/no.reporting	no. registered/no.reporting	no. registered/no.reporting	
Arsenic and compounds	3/3	3/3	3/3	2 (67%)
Acrylamide	5/5	5/5	5/5	5 (100%)
Mycotoxins	6/6	6/5	5/5	4 (67%)
Pesticides	4/4	4/4	4/4	2 (50%)
UV-filters	3/3	2/2	2/2	2 (100%)

[a] Achieving satisfactory z-scores for the biomarker in both control materials from a round, in at least two rounds from the QA/QC programme.

meet the HBM4EU criteria to support the QA/QC programme. In addition the short timeframe did not help neither since proficiency test had to be organized in parallel for 73 biomarkers in 3 matrices for more than 80 laboratories across 30 countries. In some cases, the laboratories did not have experience in organising proficiency tests for the target compounds. In others, the laboratories had wide experience even in the target chemicals but in other research areas, including in non-human matrices. In addition, although some laboratories had experience in organising these exercises, they could not prepare and test the homogeneity and stability of the control materials employed in the programme and, for some substances (phthalates, Hexamoll® DINCH, OPFRs and PAHs in Scheme 1 and for pesticides and UV – filters in Scheme 2), it was necessary to involve both an organiser and an expert laboratory able to prepare and test an adequate and reliable control material to use in the QA/QC programme. This was indeed another great challenge since, due to the lack of reference materials (i.e. target matrix and biomarkers in the concentrations expected in the general population) the preparation (and test the homogeneity and stability) of the control material for all the exercises increased the time period of the programme. This process was done under strict QA/QC measures and precisely described in the corresponding SOP, to ensure that organisation and evaluation by the different parties involved were done in a harmonised way.

In general, the adherence to the programme was good, although in the first rounds of phthalates, HFRs, OPFRs and PAHs the percentage of registered laboratories reporting results was low for certain biomarkers. In case of phthalates, this occurred for OH-MiNP, OH-MiDP and cx-MiDP, with reporting percentages below 90%. This could probably be explained by initial difficulties in the laboratories that were solved after the first round. The same tendency was observed for BPS and PFPeA. The HFRs also showed an increase in the reporting percentage after the first round except for TBBPA, DBDPE and 2,4,6-TBP. For these compounds, the potential analytical problems were not solved as the number of laboratories with satisfactory results remained low. The situation was similar for OPFRs with a low number of participants reporting results (and high variability among them) for the four biomarkers, making the evaluation of the results difficult. The highest variability in the participation per biomarker and the percentage of registered laboratories reporting results was found in the PAHs group, not only due to the technical difficulties but also due to the specialization of the participants in specific biomarkers.

To achieve satisfactory results in the programme and take part in the analysis of samples in HBM4EU, participants had to obtain successful results in at least two rounds of the proficiency tests and this could explain the general decrease in the number of participants in the 4th round, especially for Cd and Cr. However, this reduction was not so clear in the PAHs group.

Looking at the laboratories that obtained satisfactory results in the programme, the overall goal of analysing the samples in HBM4EU in a comparable way was achieved for all the target chemicals with a high improvement in the number of biomarkers with satisfactory results per laboratory (Figs. 4 and 5). For Hexamoll® DINCH, around 70% of participating laboratories obtained successful results for the two biomarkers (OH-MINCH and cx-MINCH). The phthalates group had higher variability with 31–95% of the participants with satisfactory results depending on the biomarker. There was a set of phthalate metabolites (MEP, MBzP, MnBP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP and cx-MiDP) with a satisfactory percentage above 75% while a second set had fewer participants and poorer results (MCHP, MnPeP, MnOP, OH-MiNP, cx-MiNP and OH-MiDP). Issues encountered for the phthalates group included the diversity in coverage of biomarkers (ranging from 3 to all prioritized 15) and limits of quantification (LOQs) (0.02–3.5 ng/ml), as well as background contamination for some of the biomarkers. For biomarkers of the long-chain phthalates (OH-MiNP, cx-MiNP, OH-MiDP, cx-MiDP) and Hexamoll® DINCH (OH-MINCH, cx-MINCH) initially a very high variability of results was observed. The reason for this was that
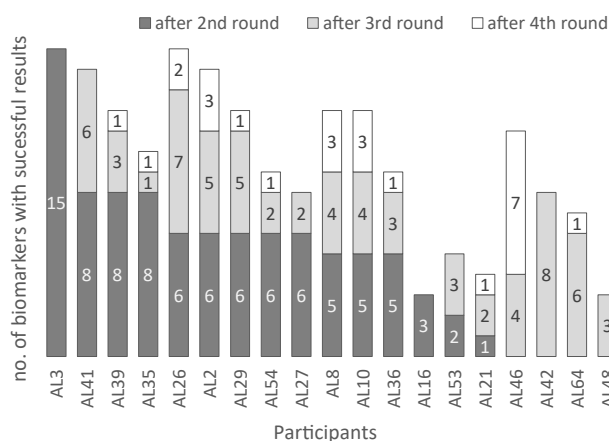

Fig. 4. Progress in the satisfactory results of phthalate biomarkers.
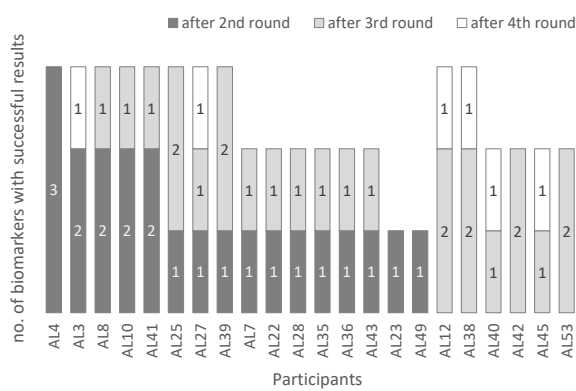

Fig. 5. Progress in the satisfactory results of bisphenols.

the parent compounds are mixtures of isomers resulting in multiple and/ or broad peaks in real samples, and because the transition used for quantification in LC-MS/MS analysis affected the results. Standardizing to prescribed mass-transitions for quantification and recommendations regarding the acquisition window to ensure all relevant isomer peaks were included in the measurement reduced the variability. The inter-laboratory variability (RSD_R) derived from the participants' results improved during the programme. Details will be presented in a future paper.

For bisphenols while no major differences were found in terms of participation, the laboratories with satisfactory results varied with the highest number for BPA (83%) and lowest for BPF (50%). Some issues were encountered for this group, especially during the 1st round. Firstly, BPA results appeared overestimated for some participants, probably impacted by an external contamination source. Secondly, BPS and especially BPF were more rarely included and reported by participants, leading to a non-achievable performance assessment especially for BPF during the 1st round, together with a high variability of the results reported by this limited number of laboratories. However, a significant improvement of the results for both BPA, BPS and especially BPF was observed between the 1st and 4th round, demonstrating a good capacity building and methodological consolidation after considering lessons learnt from each round (detailed results and discussion will be presented in a future paper). Globally, the whole exercise for bisphenols finally permitted to attest the existence of a core network of competent HBM laboratories for BPA, BPS and BPF.

For the PFAS group, in general, laboratories showed a high reporting rate for all the biomarkers and the percentage of successful results was above 70% (except for PFHxA) and up to 100% for PFHpA, PFOA, PFNA, PFDA, PFHxs and PFOS. While laboratory performance in the 1st round

(ICI) varied considerably for the individual PFAS biomarkers (PFPeA: 54%; PFOA and PFHxS: 94% each, of laboratories with successful results), the switch to EQUAS from the 2nd round on led to an overall improvement and better homogeneity of results. However, despite this general improvement, especially the analysis of PFHxA at low levels and PFDoDA at high levels proved to be challenging for some laboratories. Detailed results and discussion will be presented in a future paper.

For HFRs, BDE-47, BDE-153 and BDE-209 showed the highest registration in all rounds, thus the calculation of consensus value in the 1st round was possible unlike the other HFR biomarkers. From the 2nd round, organized as EQUAS, an expert assigned value was established for BDE-47, BDE-153, BDE-209, anti-DP and syn-DP. For others (α-HBCD, γ-HBCD, DBDPE, TBBPA and 2,4,6-TBP) the calculation of an assigned value was not possible because of the limited scope of reported results by experts or too high uncertainty of the assigned value. In this case, the calculation of the consensus value from the results submitted by experts and candidates was successful only for α-HBCD and γ-HBCD. Due to the low number of candidates and expert results the calculation of consensus or assigned value was not possible for DBDPE, TBBPA and 2,4,6-TBP (the laboratory in table S6 correspond to the laboratory preparing and testing the CM for this exercise). The highest number of satisfactory results was achieved for BDE-47 and BDE-153. For BDE-209 the success rate was not as high, because of a higher number of results assessed as questionable or unsatisfactory.

The group that presented most difficulties due to the low number of participants and high variability of the results was the OPFRs. The calculation of consensus or assigned values according to standardized ICI/EQUAS approach was not possible at all (BDClPP, BClPP and BCEP) or only to a limited extent (DPHP) in the first three rounds. It was necessary to apply a more flexible approach, in order to draw conclusions. It is worth noting, that following discussions of main analytical difficulties after the 1st and 3rd round, the 4th round was very successful. The calculation of assigned values and the evaluation of results using the ICI/EQUAS approach was realized for BDClPP, BClPP and DPHP. Finally, from a total of six laboratories, which participated in any or the four rounds, five laboratories were successful for DPHP and BDClPP and four for BClPP. Details of the flame retardants results will be presented in a future paper.

The PAHs group had the highest variability in the number of participants per biomarker and also in the laboratories reporting satisfactory results. The main difficulties with PAHs metabolites was that no evaluation was possible for some biomarkers (1,2-DHN, 3-FLUO, 9-FLUO, 9-PHEN, 3-BaP) as the number of participating laboratories was too small (<7). Even after switching to EQUAS from the 2nd round on, no z-scores could be obtained for 9-FLUO, 9-PHEN and 3-BaP, while the only laboratory to analyse 1,2-DHN could not provide quantitative results. From the 3rd round on, the initial scope of 13 biomarkers were reduced to 11 (1,2-DHN and 3-BaP were omitted), but still no z-scores could be provided for 9-FLUO and 9-PHEN in the remaining two rounds. A general improvement in results from the 2nd to the 4th round is not discernible, in fact for some biomarkers even the opposite development is noticeable (especially for 2-FLUO). Details will be presented in a future paper.

The groups involving inorganic biomarkers were those with the highest rate of participation probably due to a more well-established and robust methodology for the analysis of these metals, although the percentage of satisfactory results were in line with those observed in other groups (e.g. PFAS, HFRs). The proficiency tests on cadmium (in blood and urine) were characterized by a large number of participants and satisfactory results from the 1st round on. Thus, there was no noticeable improvement from one round to the next. The main problem encountered was that some laboratories had a too high LOQ for their analytical method and thus failed at low analyte concentrations (Nübler et al., 2021). Unlike the other substance groups, no EQUAS was performed for chromium from the 2nd round onwards, but an ICI was performed in all four rounds. The ICI exercises on chromium (in blood, urine and serum)

were characterized by satisfactory results throughout, with too high LOQs being the only problem encountered for some laboratories. Detailed results and discussion will be presented in a future paper.

Globally, the participation in Scheme 1 improved the capacities of the laboratories since the number of laboratories obtaining satisfactory results and the number of biomarkers with successful results per laboratory increased from the first to the last round.

For Scheme 2, the main difficulty was the selection of a reduced number of laboratories with enough expertise because in addition to the technical capability, practical aspects had to be considered in order to ensure the availability of the analytical results by the deadline defined within HBM4EU. The reduced time for implementing Scheme 2 was challenging for both the organisers and the participating ELs. The unexpected shutdown due to the first wave of covid-19 sanitary crisis put further strain on the scheme. The first round was implemented as planned from January to February 2020. The second round started in February–March, but the shutdown of laboratories and restrictions in the shipment of samples because of the covid-19 caused a significant delay in the ICI for acrylamide, UV-filters, pesticides and mycotoxins (samples for As had been sent before the shutdown). Furthermore, some laboratories withdrew their participation in the ICI as a result of the difficulties derived from this situation. Despite these delays, Scheme 2 was implemented between 3 months (arsenic) and 7 months (mycotoxins), so the objective of reducing the time for identifying laboratories with comparable results that could analyse the HBM4EU samples was achieved (Scheme 1 lasted at least one year and a half). In general, Scheme 2 presented less difficulty related to technical aspects since the laboratories involved had wide experience in the analysis of the target biomarkers and a baseline for their selection was defined (e.g. limit of quantification). Nevertheless, some interesting observations were made, for example the differences in total DON levels reported depending on the enzyme used for deconjugation. In several control materials, significantly lower concentrations of total DON were obtained when using β-glucuronidase/sulfatase from *Helix Pomatia* than when using β-glucuronidase from *E. Coli*. As expected, the pesticide group was the one with more difficulties, with consequences for the results evaluation. While for glyphosate and AMPA results were comparable in general, for chlorpyrifos and pyrethroid biomarkers the relative uncertainty of the mean was too high in several cases for a straightforward statistical evaluation of the reported results.

5. Conclusions

The QA/QC programme designed and implemented in the frame of the HBM4EU initiative can be termed a success and, as in previous studies, the need and utility of this kind of activities in HBM studies was evident. As long as there are no commercial proficiency tests offering a wide of biomarkers of interest in HBM at the concentrations in the range observed in the general population, the proposed approach appears as the best tool to investigate and improve results comparability. However, its implementation in the framework of such research project is complex and sustainability beyond the project is an issue regardless of the approach applied. Apart from the time constraints, questions such as the experience and capacities of the partners for supporting these activities should be considered, as well as the issues related to the funding. The organization of and participation in proficiency tests require a large amount of resources that cannot always be justified within a research project, possibly limiting the participation and thereby, the results achieved.

The main challenges of Scheme 1 approach were the time required for completing the scheme and, for some biomarkers, the rather low number of valid results, which hampered the evaluation of the ICI/EQUAS. Scheme 2 approach permitted to reduce the time required for having a set of laboratories with comparable results and, since it is based on the participation of laboratories fulfilling specific technical criteria (e.g. having a minimum LOQ for all the biomarkers in a group), the

potential problems related to the low experience were avoided. However, there was no opportunity for capacity building and supporting the national hubs in HBM4EU. Therefore, the design of the QA/QC programme has to consider the specific requirements or objectives for each situation.

Although the development and implementation of Scheme 1 required more efforts and time, it is the preferred one in the HBM4EU context since it allows the participation of more laboratories, and provides an opportunity to improve their analytical skills. This approach therefore boosts capacity building in EU laboratories, which in the end contributes to the sustainability of human biomonitoring in Europe. Major milestones and challenges for the future in this field are the definition of standard analytical methods and the establishment of a sustainable and periodical HBM QA/QC programme in Europe to support research activities and analytical laboratories. In line with this, the creation of an institution/network to prepare and provide certified control material in different human matrices would be very helpful for analytical laboratories working in human biomonitoring.

The HBM4EU QA/QC programme has revealed the utility and need in establishing a European network of analytical laboratories for human biomonitoring. This network would support the increasing human biomonitoring and risk assessment studies providing expertise for new method development and high quality analytical results. The network of laboratories created in HBM4EU can be considered as the project's legacy for future human biomonitoring actions in Europe.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113740.

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Water and health seminar and special issue highlight ideas that will change the field

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The 11th international "Water & Health" seminar took place from June 24–26, 2019 in Cannes, France. The seminar is sponsored annually by Suez, a 150-year-old multinational water and waste management company, to support research into water quality and health carried out by advanced doctoral students. It promotes ongoing communication among students, established scientists, and industry practitioners from all over the world. The seminar aims to: enhance dialogue between junior scientists and senior mentors, spotlight early-breaking research concepts and results, cross disciplinary boundaries to contribute to the advancement of water safety, and identify new critical research needs.

This special issue contains a number of articles on challenges such as emerging pollutants and antimicrobial resistance, as well as both high-tech and nature-based solutions. Some key topics from the seminar and this special issue include advancing data modeling to consider heterogeneity of populations, recognizing synergies among environmental health interventions, optimizing health by building resiliency through measured risk exposure, and understanding the role of contextual factors in tailoring intervention strategies to a given population or setting.

The winning seminar presentation came from Tanguy Pouzol, who modeled the release of 15 pharmaceuticals in effluents from a semi-urban area of 16,000 inhabitants and a 450-bed hospital. Pharmaceuticals have been detected in all types of water bodies worldwide and have been the subject of numerous risk assessments since the 1970s, as described in several recent comprehensive reviews (Ebele et al., 2017; Kümmerer, 2016; Li, 2014; Palli et al., 2019; Yang et al., 2017). Gosset et al. (2020) report a comparison of ecotoxicological risk assessment approaches for a "cocktail" of chemical pollutants, including pharmaceutical residues, discharged by urban wastewater treatment plants near Lyon, France. Their findings demonstrate the importance of jointly considering all the components of a pollutant mixture for accurate assessment of environmental risks. However, measuring concentrations of pharmaceutical residues in water remains a complex task. Analytical techniques under development that may improve environmental detection of pharmaceuticals include DNA nanobiosensors, which are reviewed by Soukarié et al. (2020) in this special issue. DNA nanobiosensors present a promising approach for an array of water quality monitoring applications, including the detection of microorganisms, as

described by one of the review co-authors, Diana Soukarié, in a seminar presentation on her ongoing research. The ongoing challenge of environmental measurement also highlights the need for complementary approaches to characterize pharmaceutical concentrations in environmental waters, such as simulation modeling. The model proposed by Dr. Pouzol (Pouzol et al., 2020) was based on the monthly sales recorded in 6 pharmacies and the daily consumption of the hospital, over 2.5 years, with hypotheses on the frequency of consumption, metabolism and release in wastewaters. For most molecules, the modeled concentrations matched well with the concentrations observed during several monitoring campaigns. The model allowed prediction of wastewater flows and changes in concentrations of pharmaceuticals in the effluents, on an hourly time scale, and thus prediction of important concentration peaks that cannot be observed by random or integrated sampling. Compared to classical approaches, such a model improved the average performance by one-third while giving additional information. Future improvements and perspectives should focus on improving knowledge on pharmaceutical loads, especially their variability at both daily and hourly scales, including in-sewer processes and improving the usability of the model by simplifying it while keeping its stochastic nature.

The recent Sustainable Development Goal (SDG) Synthesis Report on Water and Sanitation emphasized that the world is not on track to achieve global SDG 6 targets by 2030 and highlighted the need for improved understanding of the baseline status and trends of global indicators to support renewed progress (UN Water, 2018). The seminar and special issue addressed the challenges and interactions among development goals that must be considered to achieve sustainable water and sanitation for all. A microbial source tracking (MST) study conducted by Dr. David Holcomb in Mozambican households found widespread human fecal contamination, highlighting the obstacles to improving sanitary conditions through simple interventions directed at households (Holcomb et al., 2020). A study characterizing human fecal pollution of Thai surface waters using host-associated bacteriophages identified opportunities to reduce diarrheal disease risks at a broader scale through improved coastal water quality management (Chyerochana et al., 2020). These and other recent evaluation studies reflect a growing awareness of the synergies among water and health risks and other development interventions.

For instance, efforts to mitigate environmental transmission of fecal pathogens must address sustainable management of fecal wastes throughout the sanitation chain. The decentralized sanitation infrastructure common in rapidly growing urban areas in particular requires effective solutions for fecal sludge removal and treatment. An intervention impact study in Maputo, Mozambique demonstrated how subsidized pour-flush sanitation systems can increase hygienic emptying of fecal sludge, but also highlighted that the use of such services was unaffordable for many users (Capone et al., 2020).

Dr. Paul Hunter presented a discussion on how our ongoing knowledge of threats posed by waterborne noroviruses is shifting to account for population variability and the factors that influence susceptibility. Along the same lines, Sital Uprety demonstrated geographic variation in diarrheal disease risk in rural Nepal through a quantitative microbial risk assessment (QMRA) informed by a cross-sectional evaluation of household drinking water quality in the aftermath of the 2015 earthquake disaster (Uprety et al., 2020). While Uprety et al. used the fecal indicator bacterium (FIB) *Escherichia coli* to characterize risk under a reference pathogen approach, the seminar featured a discussion of the limitations of QMRA for predicting pathogen-specific risks. Specifically, QMRA has been found to overestimate disease burdens for pathogens such as norovirus, for which there remains substantial uncertainty around both the dose-response relationships and the implications of population immunity (Van Abel et al., 2017).

The growing issue of antimicrobial resistant (AMR) organisms compounds the risks posed by enteric infections. In addition to selection pressure driving an increase in AMR organisms in clinical settings, the presentation and article by Voigt et al. (2020) presented evidence for the introduction of antibiotic residues, antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARG) to environmental waters downstream of wastewater treatment facilities. Such risks are not confined to surface water, as demonstrated in an article by Salazar et al. (2020), which found aerosolized ARB in proximity of a river passing through La Paz, Bolivia, where urban population densities imply an elevated risk of exposure.

A seminar presentation by Dr. Karen Setty discussed intervention design, specifically the evidence base to address contextual variability in Water Safety Plan implementation and scale-up (Setty et al., 2019; Setty, 2019). Water safety plans represent a risk management approach developed by the World Health Organization to support safe drinking water provision (Bartram et al., 2009). These plans can be adapted to different resource settings from a community to industrial scale, and have the potential to enhance equitable outcomes (Ross et al., 2019). Dr. Setty borrowed concepts from the emerging field of implementation science to understand and better explain why results of complex interventions often vary among locations. Recognizing these site-specific contextual constraints or "barriers" and matching them to active, proven strategies can help to extend water, sanitation, and hygiene services to all under SDG 6 (United Nations, 2015). Articles in this issue recognize the outstanding needs of underserved populations such as the Roma in Europe (Anthonj et al., 2020), sustainable pit latrine emptying practices in low-income settings such as Mozambique (Capone et al., 2020), and affordable drinking water treatment using local materials such as curry leaf (Mukherjee et al., 2020). Contextual clues also offer a bridge to potential "quality improvement" solutions through rapid experimental cycling of pilots, data collection, and adjusted approaches (Setty et al., 2019; Setty, 2019).

Finally, a short communication by Dr. Mark LeChevallier bridges the ideas and challenges raised in the 2019 seminar with other global phenomena underlying access to safe water (namely, climate change). Dr. LeChevallier illustrates how climate shifts are already impacting water and health by heightening risks and requiring innovative adaptation of existing systems (LeChevallier, 2020). Climate adaptation will draw increased attention as the central theme of a future Water and Health seminar.

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