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An exploration of the trajectory of psychological distress associated with exposure to smoke during the 2014 Hazelwood coal mine fire

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ABSTRACT

Due to climate change, catastrophic events such as landscape fires are increasing in frequency and severity. However, relatively little is known about the longer-term mental health outcomes of such events. Follow-up was conducted of 709 adults exposed to smoke from the 2014 Hazelwood mine fire in Morwell, Victoria, Australia. Participants completed two surveys evaluating posttraumatic distress, measured using the Impact of Events Scale-Revised (IES-R), three and six years after the mine fire. Mixed-effects regression models were used to evaluate longitudinal changes in distress. IES-R total scores increased on average by 2.6 points (95%CI: 1.2 to 3.9 points) between the two survey rounds, with increases across all three posttraumatic distress symptom clusters, particularly intrusive symptoms. This increase in distress was evident across all levels of fine particulate matter ($PM_{2.5}$) exposure to the mine fire smoke. Age was an effect modifier between mine fire $PM_{2.5}$ exposure and posttraumatic distress, with younger adults impacted more by exposure to the mine fire. Greater exposure to $PM_{2.5}$ from the mine fire was still associated with increased psychological distress some six years later, with the overall level of distress increasing between the two survey rounds. The follow-up survey coincided with the Black Summer bushfire season in south-eastern Australia and exposure to this new smoke event may have triggered distress sensitivities stemming from exposure to the earlier mine fire. Public health responses to disaster events should take into consideration prior exposures and vulnerable groups, particularly younger adults.

1. Introduction

Given that climate change has accelerated the rate of catastrophic events occurring across the globe, understanding the longer-term trajectory of posttraumatic distress after exposure to environmental disasters has become increasingly important (Hughes et al., 2016; Leaning and Guha-Sapir, 2013). Although a range of studies have evaluated longer-term mental health and wellbeing in the aftermath of disasters, such as earthquakes and hurricanes, there was little evidence on the impact of repeated exposures, threats of landscape fires, and protracted air pollution events on mental health outcomes.

In previous research, Norris et al. (2002) concluded that levels of psychological distress and rates of posttraumatic stress disorder (PTSD) peaked within the first year post-event and then declined over time. However, other longitudinal studies such as McFarlane (1988) have found a persistent effect of exposure to fire events on the presentation of PTSD symptoms. Bryant (2019) observed that as many as 25% of cases of PTSD may not become apparent until several years after exposure to a disaster event. Other studies found that levels of distress and PTSD had not returned to pre-disaster levels 4–7 years afterwards (Bryant et al., 2018; North et al., 2011; Paxson et al., 2012).

The Latrobe Valley region in south-eastern Australia is prone to bushfire events (Teague et al., 2014). The Hazelwood mine fire occurred in February and March 2014. The event was caused by wildfires that

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spotted into the Morwell open-cut coal mine adjacent to the Hazelwood power station and ignited the exposed coal (Teague et al., 2014). While the precipitating wildfires were quickly brought under control, the ensuing coal mine fire emitted heavy plumes of smoke that blanketed surrounding communities in smoke and ash for a period of approximately six weeks before it could be brought under control, in what is considered to be the largest and longest-running mine fire event to have occurred in the region (Teague et al., 2014). Burning coal emits a range of elements, including carbon monoxide, sulphur, volatile organic compounds, and particulate matter that are understood to be hazardous to human health (Castleden et al., 2011). In Morwell, the nearest town to the mine, concentrations of fine particulate matter 2.5 μm or less in diameter ($PM_{2.5}$) reached as high as 3700 μg/m^3, greatly exceeding the estimated average background $PM_{2.5}$ concentrations (6 μg/m^3) and breaching national pollution standards 23 times during the 45-day mine fire (Luhar et al., 2020).

Early reports following the mine fire indicated that physical symptoms and psychological distress were evident within the local community, and residents held significant concerns about potential long-term health consequences (Wood et al., 2015). In response, the Victorian government commissioned the Hazelwood Health Study (HHS; www.hazelwoodhealthstudy.org.au) to investigate the long-term impacts of the mine fire smoke on the health and wellbeing of people living in affected communities. Results from the HHS Adult Survey conducted approximately 2.5 years after the mine fire indicated a dose-response relationship between level of $PM_{2.5}$ exposure during the mine fire and severity of subsequent psychological distress, with the relationship strongest for the youngest adults (Broder et al., 2020; Ikin et al., 2020). Additional risk factors identified in the survey included prior physical and mental health diagnoses, and prior exposures to trauma.

The current study followed-up HHS Adult Survey participants to investigate for the continued presence of, and changes in, the relationship between $PM_{2.5}$ exposure during the Hazelwood mine fire and subsequent event-related psychological distress. Importantly, the data collection period for the follow-up survey overlapped with the Australian "Black Summer" bushfires that impacted south-eastern Australia beginning in September 2019 and continuing into February 2020 (Levin et al., 2021). Catastrophic fire activity elsewhere in the region caused periods of widespread smoke haze across the Latrobe Valley, particularly in January 2020 (Davey and Sarre, 2020; Vardoulakis et al., 2020). Smoke distributed during the Black Summer bushfire season is understood to have had significant health impacts across the state of Victoria (Borchers Arriagada et al., 2020). Furthermore, the threat and impacts of the Black Summer bushfire season, along with its possible causes and management, were salient features of media content and public discourse in Australia at the time (Linnenluecke and Marrone, 2021). Given the extensive period of smoke exposure and media saturation, it is likely that all survey participants would have been exposed to multiple potential triggers for activating posttraumatic distress symptoms during the event. Accordingly, the findings presented in this study also provide an opportunity to consider how longer-term psychological impacts of exposure to a stressful event present within the context of exposure to a subsequent event with comparable characteristics.

2. Methods

2.1. Study design

This study presents the analysis of longitudinal self-reported survey data, collected at two time points from a subset of the Adult Survey cohort (Ikin et al., 2020). The baseline Adult Survey, conducted between May 2016 and February 2017, represented round one (R1) of data collection. Round two (R2) data were collected in a Mental Health and Wellbeing Survey conducted three years later, between December 2019 and March 2020.

2.2. Participants

2.2.1. Eligibility

All Adult Survey participants who had been residents of Morwell and at least 18 years of age at the time of the mine fire (n = 3096) were eligible for selection into the Mental Health and Wellbeing survey, with the exception of 17 known to have deceased and two who had previously withdrawn their consent to follow-up.

2.2.2. Sampling

From 3077 eligible, a weighted random sample of 1512 people, stratified by age at R1 (<35; 35–65; >65 years) and tertiles of mine fire-related $PM_{2.5}$ exposure, were invited to participate in the R2 survey. Although females were over-represented in the Adult Survey, gender was not associated with IES-R scoring at R1, so was not included as a stratification variable for R2.

2.2.3. Recruitment methods

A staggered approach was taken to inviting participation in R2, starting with email, then SMS, and finally postal approaches (depending on the contact information provided by participants at R1). Within each contact mode, a survey invitation and a reminder were delivered approximately one week apart. Approaches continued in turn until either a response was received or all attempts (i.e., two approaches in each of the three modes) had been made. Participants completed the survey online, by telephone interview, or by mail-out.

2.3. Measures

The R2 survey focused on assessing mental health and wellbeing outcomes. Accordingly, the demographic questions, mental health history questions, and psychometric assessments administered as part of the R1 survey were repeated in the R2 survey to facilitate analysis of change in mental health and wellbeing between the two time-points.

2.3.1. Assessment of $PM_{2.5}$ exposure during the mine fire period

Retrospective modelling of high resolution, hourly mine fire-related $PM_{2.5}$ concentrations was conducted using a chemical transport model that incorporated data on air monitoring, coal combustion and meteorological conditions (Luhar et al., 2020). The modelled $PM_{2.5}$ distribution data were merged with time-location diaries for the 45-day mine fire period that were provided by participants as part of the R1 survey to generate an estimated mean level of $PM_{2.5}$ exposure during the mine fire period for each participant (Ikin et al., 2020). Three categories of $PM_{2.5}$ exposure were subsequently established based on tertiles of the mean daily $PM_{2.5}$ exposure of all R1 Morwell participants: "Low Exposure" (<8.56 μg/m^3), "Medium Exposure" (8.56 μg/m^3 to 14.15 μg/m^3), and "High Exposure" (>14.15 μg/m^3).

2.3.2. Assessment of event-related psychological distress

The Impact of Events Scale – Revised (IES-R) (Weiss and Marmar, 1997) was used to assess participants' levels of psychological distress specifically in relation to the 2014 Hazelwood mine fire. The 22-item IES-R has three subscales relating to the PTSD symptom clusters of Intrusion (unwanted thoughts or memories that are recurrent and unable to be controlled), Avoidance (withdrawal from situations and stimuli that might aggravate fears, panic or distress), and Hyperarousal (excessive elevation in physiological and psychological alertness or responsivity). Respondents rated their experience of each of the 22 symptoms over the previous week with regards to the Hazelwood event on a four-point scale (0 = "Not at all"; 1 = "A little bit"; 2 = "Moderately"; 3 = "Quite a bit"; 4 = "Extremely"). The primary outcome was the IES-R total score, which is the sum of scores for all 22 items. The three subscales were secondary outcomes, with intrusion and avoidance the sum of eight items and hyperarousal the sum of six items. Hyperarousal scores were scaled up by a factor of 8/6 to enable direct

comparison between subscale scores.

2.3.3. Assessment of risk factors

Demographic factors included in the analysis were age (at the time of the mine fire), gender, education, and employment status. Health factors included were self-reported doctor diagnosed physical conditions (cardiac events, asthma or chronic obstructive pulmonary disease [COPD]) and self-reported doctor/psychologist diagnosed prior mental health conditions (anxiety, depression, PTSD, or other mental health conditions diagnosed prior to the 2014 mine fire), measured as a part of the R1 Survey. Previous exposures to traumatic life-events were categorised as: none, one, or multiple traumatic events. Self-reporting of prior mental health diagnoses was repeated in the R2 survey. In some instances, participants disclosed diagnoses that pre-dated the mine fire, but which had not been previously disclosed by them in the earlier survey round. Disclosures of prior mental health diagnoses were accepted as valid irrespective of whether they were reported in R1 and/or R2, and participants' responses at the two time-points were consolidated to determine their mental health diagnosis histories.

2.3.4Ethics approval

The Monash University Human Research Ethics Committee granted approval to conduct the surveys in May 2015 and October 2019 (approval numbers CF15/872 and 21151 respectively).

2.4. Statistical analysis

Inverse probability weighting (also known as response propensity weighting) was used to account for longitudinal attrition bias (Chen et al., 2012). Weights were estimated using a probit regression model to predict survey participation based on R1 risk factors. These response weights are incorporated into all analyses.

Descriptive statistics are given for baseline covariates and IES-R outcomes, categorised by $PM_{2.5}$ exposure group (low; medium; high). Crude differences between the $PM_{2.5}$ exposure groups were compared using Pearson chi-squared tests for categorical measures, and analysis of variance (ANOVA) for continuous outcomes, with continuous data log-transformed where heavily skewed.

Linear mixed-effects regression models were implemented to evaluate changes over time in IES-R outcomes and to assess whether IES-R and changes in IES-R were associated with $PM_{2.5}$ exposure during the mine fire. An interaction between $PM_{2.5}$ exposure and survey round was evaluated to assess whether the impact of $PM_{2.5}$ exposure changed over time. As younger adults reported higher levels of distress in R1 associated with higher $PM_{2.5}$ exposure (Broder et al., 2020), interactions between age, mean $PM_{2.5}$ exposure, and survey round were evaluated using two and three-way models. Age was centred at the cohort mean age during the mine fire, and mean $PM_{2.5}$ exposure was centred at 10 $\mu g/m^3$.

All regression models were adjusted for baseline covariates. Missing data were imputed using multiple imputation (Rubin, 1996) with chained equations using the ICE package (Royston and White, 2011). The mixed-effects models were executed on 20 imputed datasets and the results combined using Rubin's rules (Rubin, 1996).

A sensitivity analysis was conducted of IES-R total scores using unimputed and unweighted mixed-effects regression models to evaluate the impact of multiple imputation and response weighting on estimated effects. As scores on the IES-R were right-skewed, mixed-effects modelling was carried out on log-transformed IES-R total scores to assess whether similar conclusions would be drawn to the main results. Statistical analyses were performed using Stata version 16 (Stata Corporation, College Station, Texas, 2019).

3. Results

3.1. Recruitment and participant characteristics

In total, 709 participants were recruited into R2 from 1512 invitations. The majority of participants completed the survey online (n = 587, 83%), with a further 112 participants (16%) completing the survey over the telephone, and 10 participants (1.4%) completing via a paper survey. After excluding a further 17 deceased or permanently cognitively impaired participants, this equated to an overall response rate of 47%. A flowchart of recruitment into the R2 survey is presented in Fig. 1. A comparison of responders to non-responders pre- and post-weighting is given in Table S1. Responders were slightly younger, with better self-rated health, higher employment and education levels. The non-participation bias related to these covariates was largely corrected with response weighting used in all analyses (see Table S1).

Participant characteristics by $PM_{2.5}$ exposure group are presented in Table 1. The mean age of the cohort during the mine fire was 48.7 years. At the time of the R1 survey, 49% had post-secondary school qualifications, which is comparable with the region's adult population statistics (Australian Bureau of Statistics, 2016). One third of the cohort reported a mental health diagnosis prior to the mine fire (pre-2014) with 88% concordance in reporting on prior mental health between the two rounds, indicating that participants' self-reporting was highly consistent. Overall, there was little change in participants' demographic characteristics between survey rounds. At the time of the R2 survey, almost all respondents were still living in Gippsland (93%), with most (82%) still resident in Morwell. In relation to education, 13% of participants reported that they had attained a higher level of qualification since R1. For employment, 8% had dropped out of employment whilst 6% had entered employment since R1. Participant characteristics were generally comparable between the three exposure groups, except for lower education levels in the medium exposure group and a slightly higher proportion of high exposure participants having experienced multiple traumatic life-events.

3.2. Longitudinal model of event-related psychological distress

As illustrated in Fig. 2, IES-R total scores had increased at the R2 survey compared with corresponding scores from R1, with the upward change in scores evident across all exposure groups. The median IES-R total score increased by 2 points in the low exposure group, by 4 points in the medium exposure group, and by 6 points in the high exposure group (see Table S2 in the supplement). Similar patterns were observed for the IES-R subscales (see Fig. 3 and Table S2).

Table 2 presents the results of the linear mixed-effects models for the IES-R total score, which included an interaction between age during the mine fire and fire-related $PM_{2.5}$ exposure. Coefficients presented are the mean change in IES-R total scores. After adjusting for exposure level and other confounding factors, participants' IES-R total scores increased on average by 2.6 points (95% CI: 1.2 to 3.9 points) from R1 to R2.

For each 10 $\mu g/m^3$ increase in mean $PM_{2.5}$ exposure, there was an estimated 1.0-point increase in IES-R total score (95% CI: 0.0 to 1.9 points) at the cohort mean age during the mine fire, 48.7 years. This exposure—outcome relationship was not found to have attenuated or strengthened at the R2 survey (the interaction between $PM_{2.5}$ exposure and survey round was not associated with the outcome, ($p = 0.48$) and not included in the final model).

The exposure—outcome relationship did vary by age (a strong interaction between $PM_{2.5}$ exposure and age, $p = 0.05$). The effect of fire-related $PM_{2.5}$ exposure was strongest for adults who were younger at the time of the mine fire, moderate for those around the cohort average age during the mine fire, and least for those who were older. This is illustrated in Fig. 4, which shows predicted IES-R total scores for people who were aged 25, 45, and 65 years at the time of the mine fire. For every 10 $\mu g/m^3$ increase in average daily $PM_{2.5}$ exposure, IES-R total

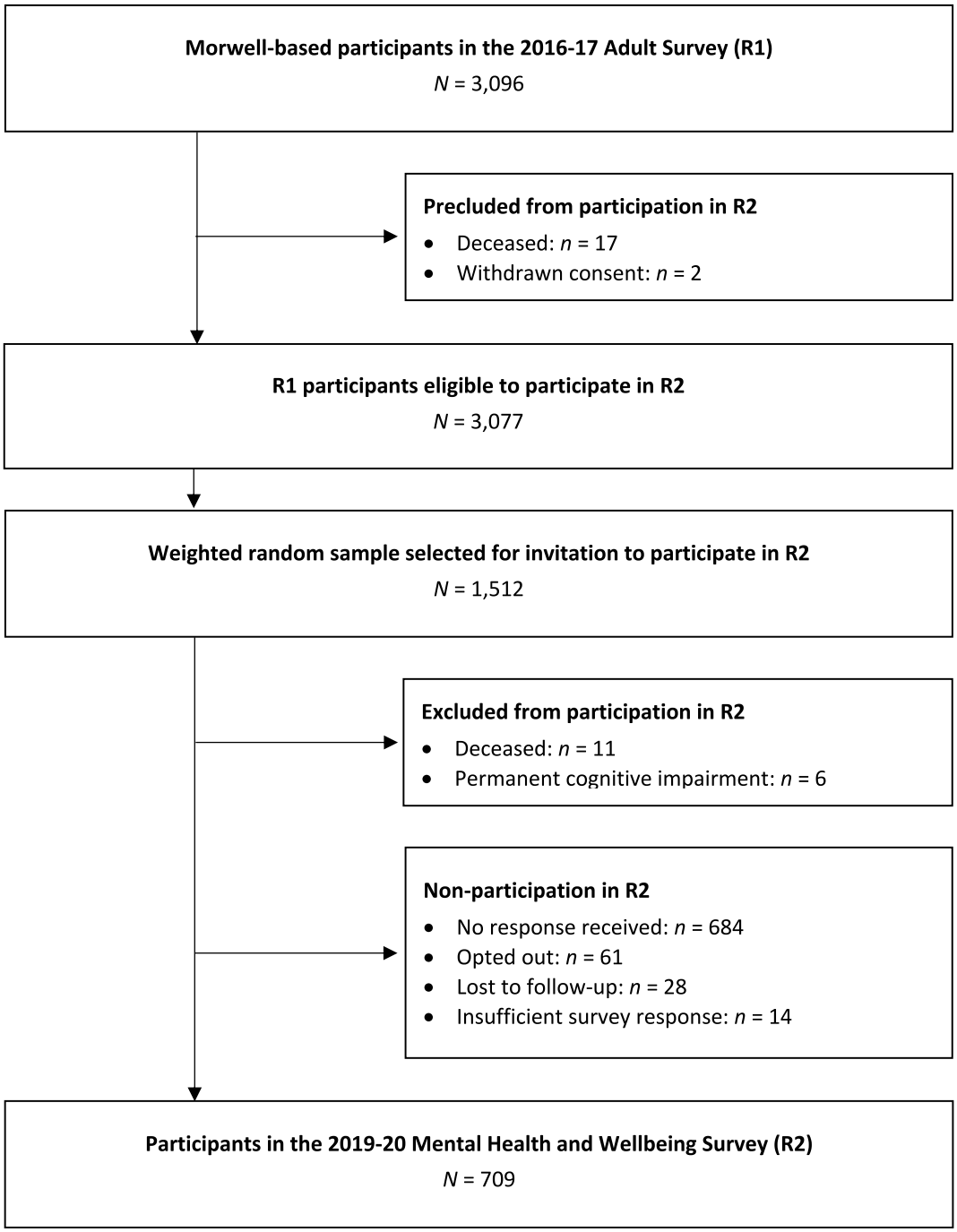

Fig. 1. Mental health and wellbeing survey recruitment flowchart.

scores increased by 2.2 points (95%CI: 0.6, 3.9) on average for participants aged 25 years, by 1.2 points (95%CI: 0.2, 2.2) for participants aged 45 years, compared with 0.1 points (95%CI: −1.1, 1.3) for participants aged 65 years.

Other covariates associated with a higher IES-R total score were self-reported diagnosis of asthma, self-reported COPD, having experienced multiple traumatic events, and being unemployed or unable to work. Having a certificate or tertiary qualification was observed to have a protective association with IES-R total score in comparison with having an education level of Year 10 or below. There was no evidence that IES-R total score was associated with past cardiovascular event(s) or mental illness diagnosed prior to the mine fire.

3.3. Longitudinal model of intrusion, avoidance, and hyperarousal symptoms

Table 3 shows the results of the linear mixed-effects models for the three IES-R subscales. Mean scores increased from R1 to R2 for each subscale. The largest change found was for the intrusion subscale scores, which increased on average by 1.3 points (95%CI: 0.8 to 1.9 points) after accounting for exposure and other confounding factors. In line with the IES-R total score model, younger age was also found to be associated with higher levels of intrusion symptoms. For each 10-year step younger from the average participant age during the mine fire, the impact of mean mine fire-related $PM_{2.5}$ exposure on intrusion subscale scores increased on average by 0.3 points (95%CI: 0.1 to 0.5 points). The estimated exposure-outcome relationship for ages 25, 45, and 65 years is

Table 1
Cohort characteristics, categorised by $PM_{2.5}$ exposure group, at the R1 survey[a].

Parameters[b, c]	Low exposure	Medium exposure	High exposure	Total	*p*-value
	N = 248	N = 236	N = 225		
Mean age during the mine fire (SD)	50.0 (17.7)	46.3 (18.4)	50.0 (17.2)	48.7 (17.9)	0.10
Male	104 (45%)	108 (48%)	107 (50%)	319 (47%)	0.53
Highest education level					
Secondary up to year 10	54 (27%)	62 (34%)	38 (20%)	154 (27%)	0.01
Secondary year 11–12	48 (21%)	59 (26%)	57 (27%)	164 (24%)	
Certificate, university or other tertiary degree	141 (53%)	108 (41%)	127 (53%)	376 (49%)	
Employment status					
Paid employment	136 (51%)	126 (49%)	129 (52%)	391 (51%)	0.29
Other: "student"; "volunteer"; "home-duties"; "retired"	86 (39%)	68 (34%)	67 (34%)	221 (36%)	
Unemployed or unable to work	23 (10%)	37 (17%)	29 (14%)	89 (13%)	
Asthma	76 (30%)	74 (31%)	60 (27%)	210 (30%)	0.66
COPD	9 (4%)	9 (6%)	12 (7%)	30 (5%)	0.53
Cardiovascular events[d]	41 (19%)	39 (19%)	43 (20%)	123 (20%)	0.98
Anxiety prior to 2014	60 (23%)	61 (27%)	57 (26%)	178 (26%)	0.69
Depression prior to 2014	57 (23%)	56 (24%)	64 (29%)	177 (26%)	0.32
PTSD prior to 2014	12 (5%)	11 (5%)	13 (5%)	36 (5%)	0.96
Any mental health diagnosis prior to 2014[a]	78 (31%)	76 (33%)	81 (37%)	235 (34%)	0.48
Number of traumatic life-events					
None	83 (33%)	83 (35%)	63 (27%)	229 (32%)	0.06
One	60 (24%)	50 (20%)	36 (16%)	146 (20%)	
Multiple	105 (43%)	100 (45%)	125 (56%)	330 (48%)	

[a] Prior mental health based on R1 survey responses with additional correction utilising R2 survey responses, where participants had entered further information about their mental health history.

[b] Missing data (%): education was missing for 15 participants (2.1%); employment was missing for 8 (1.1%); asthma was missing for 2 (0.3%); cardiac was missing for 8 (1.1%); anxiety was missing for 2 (0.3%); depression was missing for 2 (0.3%); PTSD was missing for 3 (0.4%); and traumatic life events was missing for 4 (0.6%).

[c] Statistics presented are number of participants (weighted %) and weighted mean (SD), with weighted chi-square tests performed for categorical variables and weighted ANOVA performed for continuous variables.

[d] Self-reported heart attack, heart failure, angina, irregular heart rhythm, stroke or other heart disease collected in the R1 survey.

shown for each of the subscales in Fig. 5. A similar pattern was observed for the avoidance and hyperarousal subscales, though to a lesser degree. Associations with the other covariates (asthma; COPD; prior traumatic events; employment status; education level) were also evident across each of the subscales.

3.4. Sensitivity analyses

Table S3 presents sensitivity analyses for (1) imputed and unweighted mixed-effects regression model, (2) unimputed and unweighted mixed-effects regression model, and (3) unimputed and unweighted mixed-effects regression with log-transformed outcomes. The findings were consistent across all models, suggesting the robustness of the main results.

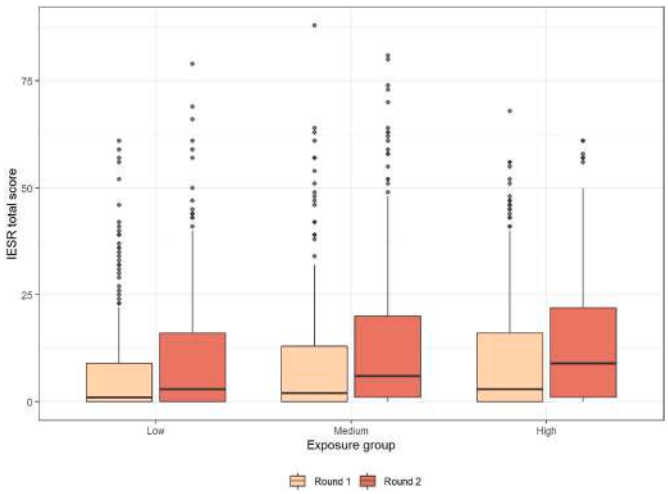

Fig. 2. Distribution of IES-R total scores, categorised by $PM_{2.5}$ exposure group, at each survey round.

4. Discussion

This paper reported a longitudinal adult cohort study examining the impacts of the Hazelwood mine fire. The main finding was that a dose-response relationship between participants' level of exposure to $PM_{2.5}$ during the 2014 Hazelwood mine fire and the psychological distress they attributed to that event is still evident six years later. In addition, the level of distress associated with the mine fire was found to have increased over the three years since the R1 survey. Once again, younger adults reported higher levels of ongoing distress in response to their exposure to the mine fire, now six years in the past. This interaction between exposure and age was stronger for the intrusion subscale. Also consistent with R1 results, greater event-related distress at R2 was associated with asthma, COPD, multiple prior traumatic events, and being unemployed or unable to work.

The overall increase in event-related distress between the two survey rounds contrasts with previous bushfire studies in Australia, which have reported decreasing levels of distress over time (Bryant et al., 2014, 2018; McFarlane et al., 1997). The finding also contrasts with earlier HHS research by Maybery et al. (2020). In qualitative interviews conducted in 2018, the majority of participants (22 of 26) reported that they were not presently experiencing symptoms of intrusion, avoidance, and/or arousal associated with having been exposed to the Hazelwood mine fire.

A potential explanation for why mine fire-related distress increased overall in the cohort at R2 is that it may be attributable to exposure to the Black Summer bushfire event which coincided with the data collection period. Previous research indicates that posttraumatic distress symptoms are most likely to present when situations reminiscent of the initial traumatising experience are encountered (Ehlers et al., 2004; Layne et al., 2006; Marks et al., 2018; McFarlane, 2010). Hence, because data collection period overlapped with a major smoke event and extensive media coverage about the fires, there was potential for mine fire-related distress to have been triggered at the time of the survey. In this light, it is interesting that the intrusive symptom domain was prominent amongst the results, both in terms of the increase of this symptom type between rounds and its continuing association with exposure to $PM_{2.5}$ from the Hazelwood mine fire. Exposure to a traumatic event may lead to the development of memories and

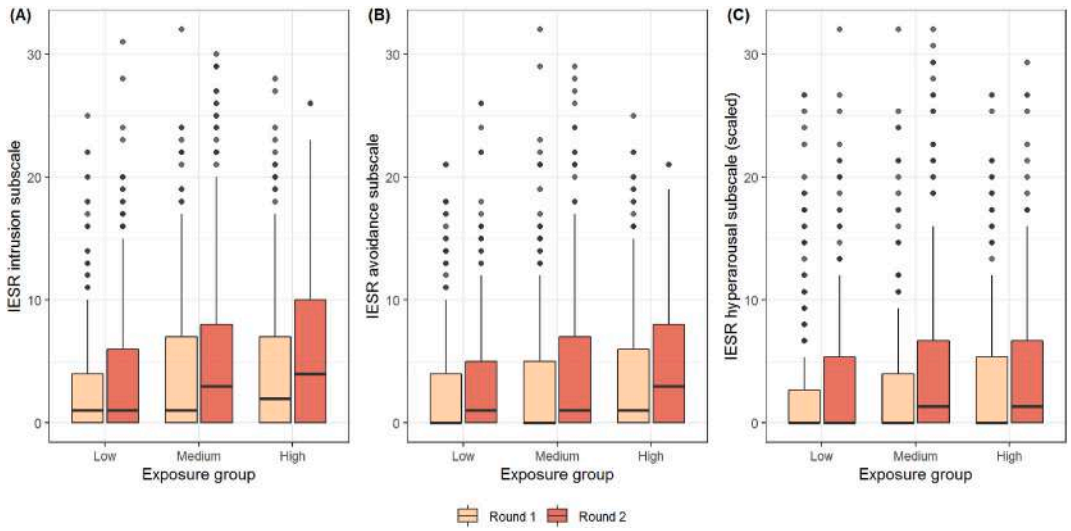

Fig. 3. Distribution of IES-R Subscale Scores, Categorised by $PM_{2.5}$ Exposure Group, at Each Survey Round. Note. Hyperarousal scores have been scaled (by multiplying 8/6) to enable direct comparison with scores for the other subscales.

Table 2
Linear mixed-effects model for IES-R total scores.

Risk factors at R1	IES-R total score	
	Coefficient[d] (95% CI)	*p*-value
Mean exposure to mine fire-related $PM_{2.5}$ (per 10 μg/m^3; at the mean age during the mine fire of 48.7 years)[a]	1.0 (0.0, 1.9)	0.048
Survey round (0 = R1; 1 = R2)	2.6 (1.3, 3.9)	<0.001
Age during the mine fire (per 10 years; at a $PM_{2.5}$ exposure level of 10 μg/m^3)[b]	0.3 (−0.6, 1.3)	0.49
Interaction between $PM_{2.5}$ exposure and age[c]	−0.5 (−1.1, 0.0)	0.050
Mental health conditions prior to 2014	1.3 (−1.2, 3.7)	0.32
Male	0.3 (−1.8, 2.4)	0.80
Cardiovascular event	1.5 (−1.9, 4.9)	0.39
Asthma	2.6 (0.3, 5.0)	0.030
COPD	7.4 (0.7, 14.2)	0.031
Number of traumatic life-events		
None	Ref	
One	1.3 (−1.4, 4.1)	0.33
Multiple	4.1 (1.6, 6.6)	0.001
Education level		
Secondary up to Year 10	Ref	
Secondary Year 11–12	−3.3 (−7.0, 0.5)	0.09
Certificate, university, or other tertiary degree	−5.4 (−8.4, −2.3)	<0.001
Employment status		
Paid employment	Ref	
Other: “student”; “volunteer”; “home-duties”; “retired”	1.7 (−1.3, 4.8)	0.27
Unemployed or unable to work	5.4 (1.3, 9.4)	0.010

[a] Mean $PM_{2.5}$ exposure was centred at 10 μg/m^3 and divided by 10 to give exposure units per 10 μg/m^3.
[b] Age during the mine fire was centred at mean age, 48.7 years, and divided by 10 to give age in 10-year units.
[c] The increase in the exposure effect per each 10-year increase in age.
[d] Coefficients were estimated from multivariate weighted mixed-effects regression with missing data imputed (see methods).

fear-conditioning that render an impacted person sensitive to re-experiencing distress following additional events (McFarlane, 2010). In line with the sensitisation effect described by McFarlane (2010), it is plausible that the Black Summer bushfires triggered intrusive memories associated with the earlier Hazelwood mine fire event. As noted earlier, it is likely that the entire cohort would have been exposed to some form of potentially distress-triggering stimuli relating to the Black Summer event, given almost all were still resident in the region, and all would likely have had access to media reporting which was ongoing throughout the data collection period.

The increasing frequency at which environmental disasters such as bushfires are occurring means that people living in areas prone to these types of events are vulnerable to recurrent exposure, which adds to the complexity of measuring and understanding levels of event-related distress and trauma. While we did not set out to assess the impact of a subsequent smoke event on distress associated with an earlier event, our findings suggest that the new event activated a sensitivity that has developed within this community, associated with exposure to the Hazelwood mine fire. We are not aware of any research that has explicitly examined the impact of exposure to multiple smoke events, so there is a clear need for research to address this knowledge gap. Regardless, our findings emphasise the need for targeted mental health screening in disaster-prone communities that takes into consideration the potential impact of accumulated exposures. In addition, this research identifies other risk factors for increased distress, including younger people being more vulnerable.

4.1. Strengths and limitations

This research has considerable strengths. Rather than relying on population-level pollution data, the current analysis utilised an individual-level $PM_{2.5}$ exposure estimate. This was a much more precise estimate of exposure that is largely free from the influence of the individual’s perception of the exposure (Glass and Sim, 2006). Additional strengths of this research include the large cohort recruited for R1, the strong recruitment rate for R2, and multivariate modelling that accounted for the impacts of other risk factors relevant to psychological distress, such as asthma, prior mental health diagnoses, and prior traumatic exposures.

However, there are also limitations to this research, including the potential for participant recall bias in the self-reported time-location information from the R1 survey, particularly given that this information was collected more than two years after the mine fire (Coughlin, 1990). Furthermore, there are well-recognised limitations to self-report data more generally (Althubaiti, 2016). It is possible that selection bias may persist even after the application of sample weighting in the research design and response weighting in the statistical analyses. However, sensitivity analyses showed consistency between results with or without response weighting and multiple imputation, suggesting our findings were fairly robust to potential bias.

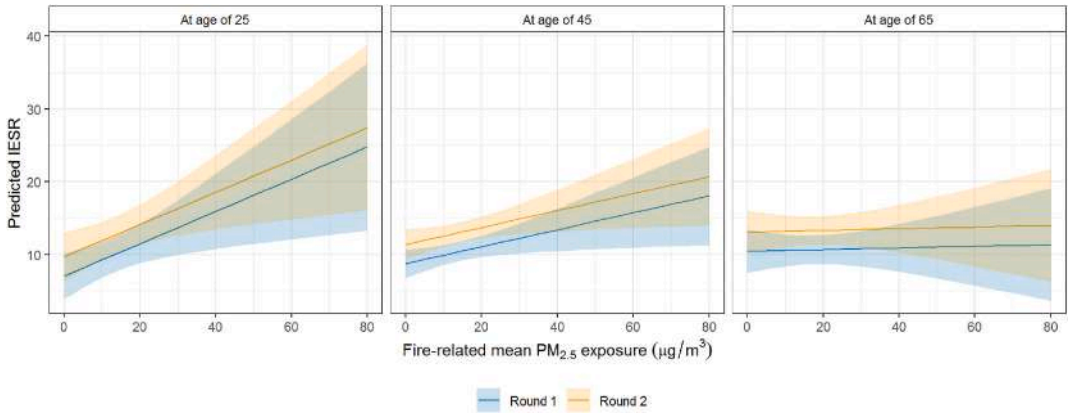

Fig. 4. Age-Dependence of the Relationship between $PM_{2.5}$ Exposure and IES-R Total Scores.

Table 3
Linear mixed-effects models for IES-R subscale scores.

Risk factors at R1	Intrusion		Avoidance		Hyperarousal[e]	
	Coefficient[d] (95% CI)	*p*-value	Coefficient[d] (95% CI)	*p*-value	Coefficient[d] (95% CI)	*p*-value
Mean exposure to mine fire-related $PM_{2.5}$ (per 10 μg/m^3; at the mean age during the mine fire of 48.7 years)[a]	0.5 (0.1, 0.9)	0.012	0.4 (0.0, 0.7)	0.037	0.1 (−0.2, 0.5)	0.45
Survey round (0 = R1; 1 = R2)	1.3 (0.8, 1.9)	<0.001	0.8 (0.3, 1.3)	0.002	0.7 (0.1, 1.2)	0.014
Age during the mine fire (per 10 years; at a $PM_{2.5}$ exposure level of 10 μg/m^3)[b]	0.2 (−0.2, 0.5)	0.40	0.2 (−0.2, 0.5)	0.33	0.0 (−0.4, 0.4)	0.98
Interaction between $PM_{2.5}$ exposure and age[c]	−0.3 (−0.5, −0.0)	0.018	−0.2 (−0.4, 0.0)	0.053	−0.1 (−0.3, 0.1)	0.28
Mental health conditions prior to 2014	0.6 (−0.3, 1.6)	0.19	0.3 (−0.6, 1.2)	0.56	0.5 (−0.5, 1.4)	0.32
Male	0.4 (−0.5, 1.2)	0.40	−0.2 (−1.0, 0.5)	0.53	0.2 (−0.6, 1.0)	0.65
Cardiac event	0.7 (−0.6, 1.9)	0.33	0.3 (−1.0, 1.5)	0.67	0.8 (−0.6, 2.1)	0.28
Asthma	1.0 (0.0, 1.9)	0.041	0.8 (−0.0, 1.7)	0.053	1.1 (0.2, 2.1)	0.022
COPD	2.1 (−0.4, 4.6)	0.104	2.4 (0.0, 4.8)	0.049	3.9 (1.1, 6.7)	0.006
Number of traumatic life-events						
None	Ref		Ref		Ref	
One	0.3 (−0.7, 1.4)	0.54	0.4 (−0.6, 1.4)	0.40	0.8 (−0.3, 1.9)	0.15
Multiple	1.6 (0.6, 2.5)	0.002	1.4 (0.5, 2.2)	0.003	1.6 (0.7, 2.6)	0.001
Education level						
Secondary up to Year 10	Ref		Ref		Ref	
Secondary Year 11–12	−1.2 (−2.7, 0.2)	0.10	−1.1 (−2.4, 0.2)	0.09	−1.3 (−2.8, 0.2)	0.10
Certificate, university, or other tertiary degree	−1.9 (−3.1, −0.7)	0.002	−1.7 (−2.7, −0.6)	0.002	−2.4 (−3.6, −1.2)	<0.001
Employment status						
Paid employment	Ref		Ref		Ref	
Other: “student”; “volunteer”; “home-duties”; “retired”	0.7 (−0.6, 1.9)	0.28	0.4 (−0.6,1.5)	0.45	0.8 (−0.3, 2.0)	0.17
Unemployed or unable to work	2.1 (0.5, 3.6)	0.01	1.6 (0.2,2.9)	0.025	2.3 (0.6, 4.0)	0.007

[a] Mean $PM_{2.5}$ exposure was centred at 10 μg/m3 and divided by 10 to give exposure units per 10 μg/m3.
[b] Age during the mine fire was centred at mean age, 48.7 years, and divided by 10 to give age in 10-year units.
[c] The increase in the exposure effect per each 10-year increase in age.
[d] Coefficients were estimated from multivariate weighted mixed-effects regression with missing data imputed (see methods).
[e] Hyperarousal scores have been scaled (multiplied by 8/6) to enable direct comparison with scores for the other subscales.

While the coincidental timing of the R2 survey with the Black Summer bushfires provided insights into the possible impacts of a new event on the experience of distress arising from a previous event, we were unable to say what the ongoing distress levels would have been in the absence of this event; a further round of surveys is planned, which will shed further light on the longer-term trajectory of event-related distress. In addition, as the survey was designed and launched without foreknowledge of the impending local environmental conditions that would result from the Black Summer bushfires, we were not positioned to capture information on individual participants' exposure to $PM_{2.5}$ during this event. Further research is planned to investigate the relationship between the Black Summer bushfire-related $PM_{2.5}$ exposure and attribution of distress to the earlier mine fire event.

5. Conclusions

The key messages are that higher levels of exposure to $PM_{2.5}$ during an extended mine fire were associated with greater psychological distress in relation to the event some six years later, and that the level of distress increased between the two survey rounds. Furthermore, it is plausible that this increase in distress was due in some part to the experience of a new smoke event at the time of the survey which triggered recall of the earlier mine fire in an already sensitised population. We recommend that public health agencies include warnings that people who have experienced similar events in the past may experience increased psychological distress in response to new events and that they should seek support if needed. This is particularly important given that the likelihood of residents in disaster-prone communities experiencing multiple disaster events is increasing due to climate change. In addition, the finding that multiple risk factors were associated with greater event-related psychological distress suggests that a more nuanced approach could be taken to identifying and supporting vulnerable groups in the aftermath of disaster. This need was reinforced by finding that younger adults appeared to be at greater risk of psychological distress following

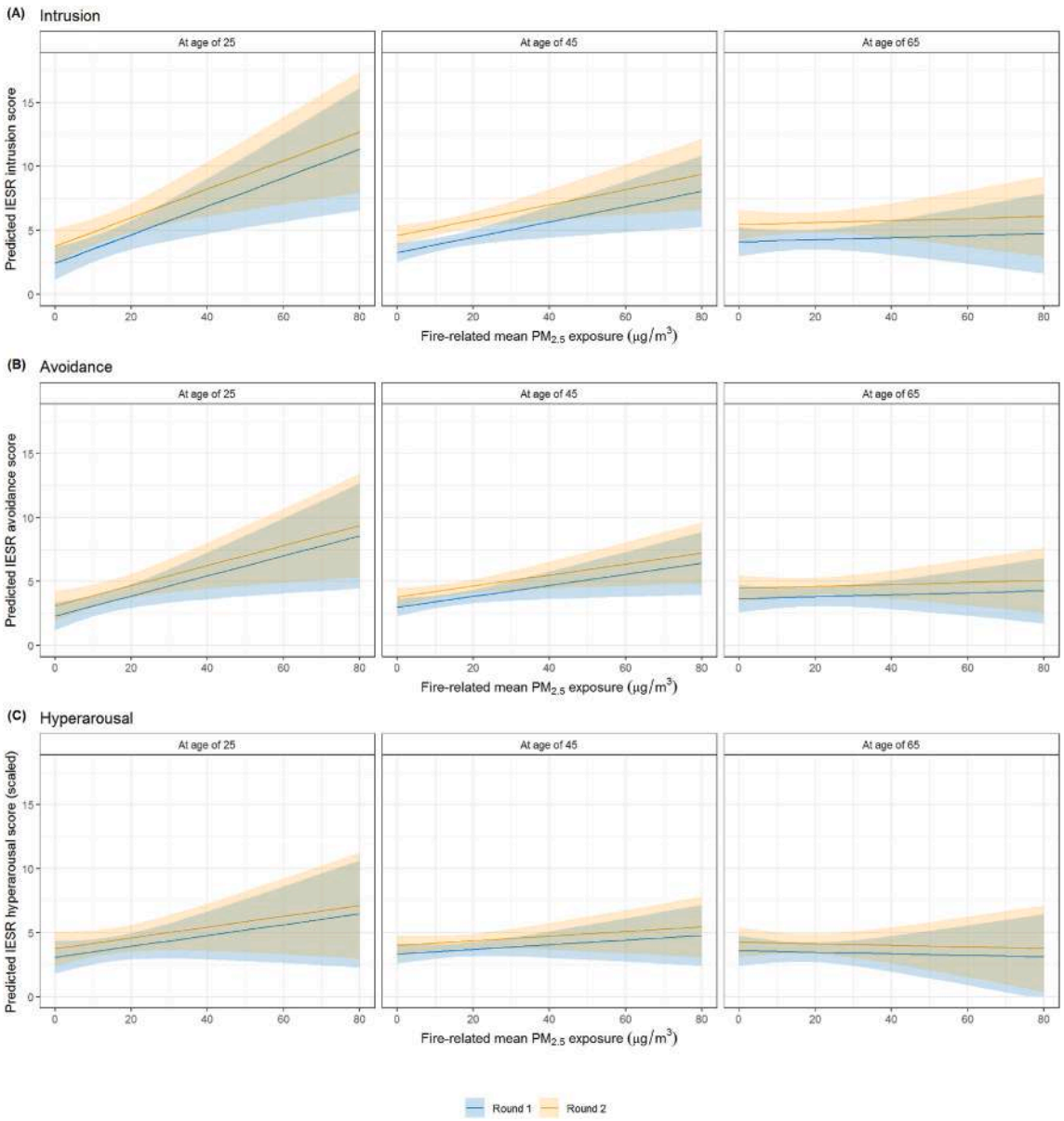

Fig. 5. Age-Dependence of the Relationships between $PM_{2.5}$ Exposure and IES-R Subscale Scores. *Note.* Hyperarousal scores have been scaled (by a factor of 8/6) to enable direct comparison with scores for the other subscales.

disaster.

Declaration of interests

Michael Abramson holds investigator initiated grants from Pfizer and Boehringer-Ingelheim for unrelated research. Michael has also undertaken an unrelated consultancy for Sanofi.

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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.2022.113946.

References

Althubaiti, A., 2016. Information bias in health research: definition, pitfalls, and adjustment methods. J. Multidiscip. Healthc. 9, 211–217. https://doi.org/10.2147/JMDH.S104807.

Australian Bureau of Statistics, 2016. 2016 Census Community Profiles (Latrobe - Gippsland), Commonwealth of Australia. https://quickstats.censusdata.abs.gov.au/census_services/getproduct/census/2016/communityprofile/205?opendocument.

Borchers Arriagada, N., Palmer, A.J., Bowman, D.M., Morgan, G.G., Jalaludin, B.B., Johnston, F.H., 2020. Unprecedented smoke-related health burden associated with the 2019–20 bushfires in eastern Australia. Med. J. Aust. 213 (6), 282–283. https://doi.org/10.5694/mja2.50545.

Broder, J.C., Gao, C.X., Campbell, T.C.H., Berger, E., Maybery, D., McFarlane, A., Tsoutsoulis, J., Ikin, J.F., Abramson, M.J., Sim, M.R., Walker, J., Luhar, A., Carroll, M., 2020. The factors associated with distress following exposure to smoke from an extended coal mine fire. Environ. Pollut. 115131 https://doi.org/10.1016/j.envpol.2020.115131.

Bryant, R.A., 2019. Post-traumatic stress disorder: a state-of-the-art review of evidence and challenges. World Psychiatr. 18 (3), 259–269. https://doi.org/10.1002/wps.20656.

Bryant, R.A., Gibbs, L., Gallagher, H.C., Pattison, P., Lusher, D., MacDougall, C., Harms, L., Block, K., Sinnott, V., Ireton, G., 2018. Longitudinal study of changing psychological outcomes following the Victorian Black Saturday bushfires. Aust. N. Z. J. Psychiatr. 52 (6), 542–551. https://doi.org/10.1177/0004867417714337.

Bryant, R.A., Waters, E., Gibbs, L., Gallagher, H.C., Pattison, P., Lusher, D., MacDougall, C., Harms, L., Block, K., Snowdon, E., 2014. Psychological outcomes following the Victorian Black Saturday bushfires. Aust. N. Z. J. Psychiatr. 48 (7), 634–643. https://doi.org/10.1177/0004867414534476.

Castleden, W.M., Shearman, D., Crisp, G., Finch, P., 2011. The mining and burning of coal: effects on health and the environment. Med. J. Aust. 195 (6), 333–335. https://doi.org/10.5694/mja11.10169.

Chen, Q., Gelman, A., Tracy, M., Norris, F.H., Galea, S., 2012. Weighting Adjustments for Panel Nonresponse. Columbia University. https://stat.columbia.edu/gelman/research/unpublished/weighting%20adjustments.

Coughlin, S.S., 1990. Recall bias in epidemiologic studies. J. Clin. Epidemiol. 43, 87–91. https://doi.org/10.1016/0895-4356(90)90060-3.

Davey, S.M., Sarre, A., 2020. Editorial: the 2019/20 Black summer bushfires. Aust. For. 83 (2), 47–51. https://doi.org/10.1080/00049158.2020.1769899.

Ehlers, A., Hackmann, A., Michael, T., 2004. Intrusive re-experiencing in post-traumatic stress disorder: phenomenology, theory, and therapy. Memory 12 (4), 403–415. https://doi.org/10.1080/09658210444000025.

Glass, D.C., Sim, M.R., 2006. The challenges of exposure assessment in health studies of Gulf War veterans. Phil. Trans. Roy. Soc. Lond. B 361 (1468), 627–637. https://doi.org/10.1098/rstb.2006.1822.

Hughes, L., Rickards, L., Steffen, W., Stock, P., Rice, M., 2016. On the Frontline: Climate Change and Rural Communities. Climate Council of Australia. https://www.climatecouncil.org.au/resources/ruralreport/.

Ikin, J., Carroll, M., Walker, J., Borg, B., Brown, D., Cope, M., Del Monaco, A., Dennekamp, M., Dimitriadis, C., Gao, C.X., Guo, Y., Johnston, F., Liew, D., Maybery, D., Thompson, B.R., Sim, M., Abramson, M.J., 2020. Cohort profile: the Hazelwood health study adult cohort. Int. J. Epidemiol. 49 (6), 1777–1778. https://doi.org/10.1093/ije/dyaa083.

Layne, C.M., Warren, J.S., Saltzman, W.R., Fulton, J.B., Steinberg, A.M., Pynoos, R.S., 2006. Contextual influences on posttraumatic adjustment: retraumatization and the roles of revictimization, posttraumatic adversities, and distressing reminders. In: Schein, L.A., Spitz, H.I., Burlingame, G.M., Muskin, P.R., Vargo, S. (Eds.), Psychological Effects of Catastrophic Disasters, first ed. Routledge, pp. 235–286. https://doi.org/10.4324/9781315821306.

Leaning, J., Guha-Sapir, D., 2013. Natural disasters, armed conflict, and public health. N. Engl. J. Med. 369 (19), 1836–1842. https://doi.org/10.1056/NEJMc1315507.

Levin, N., Yebra, M., Phinn, S., 2021. Unveiling the factors responsible for Australia's Black Summer fires of 2019/2020. Fire 4 (3), 58. https://doi.org/10.3390/fire4030058.

Linnenluecke, M.K., Marrone, M., 2021. Air pollution, human health and climate change: newspaper coverage of Australian bushfires. Environ. Res. Lett. 16 (12), 125003. https://doi.org/10.1088/1748-9326/ac3601.

Luhar, A.K., Emmerson, K.M., Reisen, F., Williamson, G.J., Cope, M.E., 2020. Modelling smoke distribution in the vicinity of a large and prolonged fire from an open-cut coal mine. Atmos. Environ. 229, 117471. https://doi.org/10.1016/j.atmosenv.2020.117471.

Marks, E.H., Franklin, A.R., Zoellner, L.A., 2018. Can't get it out of my mind: a systematic review of predictors of intrusive memories of distressing events. Psychol. Bull. 144 (6), 584–640. https://doi.org/10.1037/bul0000132.

Maybery, D., Jones, R., Dipnall, J.F., Berger, E., Campbell, T., McFarlane, A., Carroll, M., 2020. A mixed-methods study of psychological distress following an environmental catastrophe: the case of the Hazelwood open-cut coalmine fire in Australia. Hist. Philos. Logic 33 (2), 216–230. https://doi.org/10.1080/10615806.2019.1695523.

McFarlane, A.C., 1988. The longitudinal course of posttraumatic morbidity: the range of outcomes and their predictors. J. Nerv. Ment. Dis. 176, 30–39. https://doi.org/10.1097/00005053-198801000-00004.

McFarlane, A.C., 2010. The long-term costs of traumatic stress: intertwined physical and psychological consequences. World Psychiatr. 9, 3–10. https://doi.org/10.1002/j.2051-5545.2010.tb00254.x.

McFarlane, A.C., Clayer, J., Bookless, C., 1997. Psychiatric morbidity following a natural disaster: an Australian bushfire. Soc. Psychiatr. Psychiatr. Epidemiol. 32 (5), 261–268. https://doi.org/10.1007/BF00789038.

Norris, F.H., Friedman, M.J., Watson, P.J., 2002. 60,000 disaster victims speak: Part II. Summary and implications of the disaster mental health research. Psychiatry 65 (3), 240–260. https://doi.org/10.1521/psyc.65.3.240.20169.

North, C.S., Pfefferbaum, B., Kawasaki, A., Lee, S., Spitznagel, E.L., 2011. Psychosocial adjustment of directly exposed survivors 7 years after the Oklahoma City bombing. Compr. Psychiatr. 52, 1–8. https://doi.org/10.1016/j.comppsych.2010.04.003.

Paxson, C., Fussell, E., Rhodes, J., Waters, M., 2012. Five years later: recovery from post traumatic stress and psychological distress among low-income mothers affected by Hurricane Katrina. Soc. Sci. Med. 74 (2), 150–157. https://doi.org/10.1016/j.socscimed.2011.10.004.

Royston, P., White, I.R., 2011. Multiple imputation by chained equations (MICE): implementation in Stata. J. Stat. Software 45 (4), 1–20. https://doi.org/10.18637/jss.v045.i04.

Rubin, D.B., 1996. Multiple imputation after 18+ years. J. Am. Stat. Assoc. 91 (434), 473–489. https://doi.org/10.2307/2291635.

Teague, B., Catford, E.J., Petering, S., 2014. The Hazelwood mine fire inquiry report (parliamentary paper No. 342), Victorian government printer. https://www.parliament.vic.gov.au/file_uploads/8101_HAZ_Hazelwood_Mine_Inquiry_Report_BOOK_LR_f5Bp6wNh.pdf.

Vardoulakis, S., Marks, G., Abramson, M.J., 2020. Lessons learned from the Australian bushfires: climate change, air pollution, and public health. JAMA Intern. Med. 180 (5), 635–636. https://doi.org/10.1001/jamainternmed.2020.0703.

Weiss, D.S., Marmar, C.R., 1997. The impact of event scale-revised. In: Wilson, J.P., Keane, T.M. (Eds.), Assessing Psychological Trauma and PTSD. Guilford Press, pp. 399–411.

Wood, P.J., Duffy, M., Yell, S., Morrissey, B., Whyte, S., Walker, L., Carroll, M., Maybery, D., 2015. Coal Mine Fire Initial Impact on Community Health and Wellbeing Summary Report. Federation University Australia.

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Association of exposure to artificial light at night with atopic diseases: A cross-sectional study in college students

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ABSTRACT

The impact of artificial light at night (ALAN) exposure on health has become increasingly prominent. However, little is known about the effect of ALAN exposure on atopic diseases. In this study, a cross-sectional analysis of incoming students was conducted in 5 geographically disperse universities which locate in Changsha (south), Wuhan (central), Xiamen (east), Urumchi (west), and Hohhot (north), respectively. All incoming students who consented to participate were recruited, followed by a health examination and a questionnaire survey. Prevalent atopic diseases were diagnosed by clinicians. Mean ALAN (nanoWatts/cm^2/sr) during their adolescence was obtained from the remote sensing observed nighttime light data matching with their residence information, which was obtained from survey. Mixed generalized linear models (log-binomial) were used to estimate the associations, in terms of prevalence ratio (PR) with 95% confidence interval (CI). A total of 20106 participants were included in the analysis. Based on previous work, we chose factors including socioeconomic status, behavioural factors, major air pollutants, and air climatic parameters for adjustment. After full adjustment, the PR for atopic diseases was 1.35 (95% CI: 1.27–1.42; $P < 0.001$). The effect size of ALAN was the largest for asthma (PR = 1.80; 95% CI: 1.48–2.19; $P < 0.001$), followed by atopic rhinitis (PR = 1.42; 95% CI: 1.33–1.51; $P < 0.001$), and atopic dermatitis (PR = 1.20; 95% CI: 1.06–1.35; $P = 0.003$). Subgroup analyses by covariates showed consistent results. This study revealed that exposure to ALAN during adolescence may contribute to a higher risk of atopic diseases in young adulthood.

1. Introduction

Atopic diseases, including asthma, allergic rhinitis, and atopic dermatitis (AD), etc., represent a set of immunological disorders, with approximately 10–30% population in developed countries affected and global prevalence still increasing (Justiz Vaillant et al., 2021; Wesemann and Nagler, 2016). From a pathophysiological point of view, these sets of diseases usually result from the hypersensitive responses to allergens, exhibiting significant differences of predisposition among individuals. However, the etiology of atopic diseases and their predisposition remains largely elusive. One widely accepted view is that both genetic and environmental factors contribute to their occurrence, which is supported by a myriad of studies (Weidinger et al., 2018; Weidinger and Novak, 2016). Avoiding exposure to these factors, especially ones from

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extrinsic environments, becomes the fundamental measure to treat and further improve the life quality of patients.

Knowledge about environmental contributions to atopic diseases largely depends on the observation of its regional prevalence of different urbanization levels. In general, the incidence of atopic diseases tends to increase positively correlated to industrialization and urbanization, indicating that the occurrence of atopic diseases may be associated with the modern lifestyle (Okada et al., 2010). One well-known theory, 'hygiene hypothesis', attributes the occurrence of atopic diseases to the lowering pathogen exposure chances of modern lifestyle (Lambrecht and Hammad, 2017). This theory was supported by animal experiments but merely limited in the atopy rather than the occurrence of atopic disease (Weidinger et al., 2018). Besides, other environmental factors related to modern lifestyles or industrialization, such as postnatal tobacco exposure, routine childhood vaccinations, have also been identified but limited in effect size (Weidinger et al., 2018). Especially, owing to the application of ecological studies, air pollutants have received increased attention (Schikowski, 2022). Outdoor pollutants such as particulate matter and nitrogen oxides, have been shown to be associated with asthma, which may be attributed to their role in promoting systemic inflammation and oxidative stress (Schikowski, 2022). Although the correlation between air pollution and asthma is well established, limited evidences exist on air pollution and allergies and AD (Schikowski, 2022). Thus, it is still of significance to identify the potential environmental factors for both prevention and treatment.

In modern society, especially urban areas, artificial light at night (ALAN) has become an essential part of modern life. Annually, ALAN keeps increasing at a rate of 5%–20% in urban areas, leading to constant attention on ALAN's impact on health (Hölker et al., 2010). To date, multiple epidemiologic studies have indicated that ALAN exposure is associated with higher risks of malignancy, obesity, and psychological disorders (Franklin et al., 2020; Garcia-Saenz et al., 2018; Helbich et al., 2020; Zhang et al., 2021; Zhao et al., 2020; Zhong et al., 2020). Notably, ALAN was associated with an 87% increase in the risk of diffuse large B-cell lymphoma, and a 55% increase in thyroid cancer (Zhang et al., 2021; Zhong et al., 2020). Mechanically, overexposure to ALAN, also known as light pollution, is believed to resulted in circadian rhythm disturbance (Franklin et al., 2020; Obayashi et al., 2013). The latter, however, can subsequently lead to the alterations of hormone secretion, such as melatonin, which has been clarified as a modulator of allergic pathophysiology like allergic airway inflammation and type 2 immune responses (Marseglia et al., 2014; Wu et al., 2020; Yu et al., 2020). Nevertheless, little is known about the association between ALAN exposure and atopic disorders.

Adolescence serves as a bridge period during life with significant mental and physical transformations, which somehow influence and predict the health outcomes in the following adulthood (Marco et al., 2011; Shahar and Henrich, 2019). Observational studies on exposure during adolescence uncovered significant findings in diseases with complicated risk factors (Chen et al., 2019; Kemp et al., 2013). Besides, the rapid promotion of urbanization of China in the past decades can provide a representative example to study the relation between modern lifestyles and health. In the current study, we conducted a population-based cross-sectional analysis by correlating ALAN exposure during adolescence (2013–2018) with the risks of atopic diseases, including asthma, allergic rhinitis, and AD in young adulthood, in a group of incoming university students in China.

2. Methods

2.1. Study design

This was a population-based cross-sectional analysis in university students from most regions across China during September and October 2018. A cluster sampling framework was applied that incoming students from 5 geographically disperse universities which locate in Changsha, Wuhan, Xiamen, Urumchi, and Hohhot, respectively, were selected. With the electronic informed consent to participate, students received a health examination which included an evaluation for dermatologic conditions. An electronic questionnaire survey involving demographic and residential information, and behaviour were followed immediately. Details of the procedures could be found in a previous paper (Shen et al., 2020). This study was approved by the medical ethics committee of Xiangya Hospital, Central South University.

2.2. Outcomes

Clinical manifestation, disease history, and family history of the participants were inquired. Asthma was determined by self-reported history of doctor-diagnosed asthma or presentation of wheezing symptoms during the past year. Allergic rhinitis was determined by self-reported history or typical symptoms. All skin diseases were diagnosed by dermatologists during the health examination. AD was diagnosed according to the guideline from the American Academy of Dermatology (Eichenfield et al., 2014). Chronic urticaria was defined as having persistent or recurrent wheals for more than six weeks. Moderate-to-severe acne was defined as Grade 2 to 4 according to the grading system (Witkowski and Parish, 2004). Atopic disease was a combined health outcome, defined as having asthma, allergic rhinitis, or AD.

2.3. Exposure

Remote sensing technique has advantages of widely coverage and low cost for observing ANL. So far, there were two kinds of ANL data products widely used for human health research, Defense Meteorological Satellite Program's Operational Linescan System (DMSP-OLS) and Suomi National Polar-Orbiting Partnership Visible Infrared Imaging Radiometer Suite (NPP-VIIRS) (Paksarian et al., 2020; Sun et al., 2021). The DMSP-OLS were launched in 1992 and the data products were with the spatial resolution of 1 km × 1 km. Compared with DMSP-OLS, NPP-VIIRS data products have advantages of higher spatial resolution, no over-saturation problem and higher data quality (Shi et al., 2014). The transit time of NPP satellite is 1:30 a.m. at local time. The data were started in the April 2012, cover the wavelengths from 500 nm to 900 nm, the spatial resolution is 750 m × 750 m (Liao et al., 2013), and the unit is nanoWatts/cm^2/sr.

Thus, this study obtained monthly NPP-VIIRS nighttime light data during 2013–2018 from the Earth Observation Group (https://eogdata.mines.edu/products/vnl/), and the file type is "GeoTIFF". Then, mean ANL during 2013–2018 with original spatial resolution and the average nighttime light at county/district scale were calculated using the tool of "Raster Calculator", "Zonal Statistics" respectively in ArcGIS 10.2. Finally, matched the mean ANL to each individual adolescent period based on the codes of the participants' hometown, which was obtained from the survey. We also inquired the information about move during the past 6 years and if true, new address was collected and environmental data was calculated by averaging data by weighting for years of living.

2.4. Covariates

Previous studies identified socioeconomic status as a social determinant for atopic diseases (Xiao et al., 2019). Because ALAN is closely correlated with economic development, we included individual indicators or proxy measures of social stratum. We also included established risk factors for atopic diseases. In brief, socioeconomic status (annual household income) and behavioural factors (second-hand smoke exposure, physical activity, and intake of red meat) were self-reported through an online questionnaire survey (Jing et al., 2020; Li et al., 2021). Weight and height were measured by research nurses during the field survey, and body mass index was calculated as

weight/squared height [kg/m^2]. Besides, because nighttime light may correlate with insomnia and sleep quality, we included sleep disturbance as a covariate, measured by the Pittsburgh Sleep Quality Index (PSQI). Sleep disturbance was defined as PSQI ≥6 according to a previous validation study in the U.S. college students (Dietch et al., 2016).

Based on previous findings (Schikowski, 2022; Weidinger et al., 2018), environmental factors including humidity, temperature, air pollutants, and daytime ultraviolet (UV) radiation were obtained from public repositories. Relative humidity (%), temperature (°C), and concentrations of six major air pollutants including O_3 (μg/m^3), CO (mg/m^3), NO_2 (μg/m^3), SO_2 (μg/m^3), $PM_{2.5}$ (μg/m^3), and PM_{10} (μg/m^3) during 2013–2018 were collected from the Chinese Air Quality Reanalysis dataset, a high-resolution (10km × 10 km) gridded air quality dataset in China produced by the Institute of Atmospheric Physics, Chinese Academy of Sciences (Tang et al., 2020). The daily dose of daytime UV radiation (J/m^2) during 2013–2018 was selected from the Aura OMI UV product (OMI/Aura Surface UV Irradiance 1-orbit L2 Swath 13 × 24 km V003) which downloaded from NASA Goddard Earth Sciences Data and Information Services Center (https://disc.gsfc.nasa.gov/) with a spatial resolution of 13 km × 24 km. Individual data were linked to the environmental data by city distinct or county code of the participants' hometown.

Then, averagely daily exposure to air pollutants was estimated using the following procedures: (1) calculate the mean concentration of air pollutants during 2013–2018; (2) estimate basal metabolic rate by individual age and sex (Liu et al., 2014); (3) estimate the metabolic equivalents (METs) for school time (assuming 40 h/week for all students), physical activities (self-reported through a questionnaire, including type, frequency, and duration), and time of sleep (determined by PSQI); (4) adjust the basal metabolic rate by METs; (5) estimate the METs-weighted respiratory rate; (6) estimate averagely daily exposure (mg/kg·d or μg/kg·d) to pollutants according to the 6-year mean concentrations and respiratory rate (Ministry of Environmental Protection of China, 2017). Details can be found in our previous publication (Shen et al., 2021).

2.5. Statistical analyses

Continuous data with normal distribution were presented as mean ± standard deviation (SD) and compared using ANOVA for differences between groups. Continuous data with skewed distribution were presented as median (interquartile range, IQR) and compared using the Wilcoxon rank sum test. Categorical data were presented as number (%) and compared using the chi-square test. Comparisons of potential confounders including demographic, anthropometric, behavioural, meteorologic, and environmental factors across quartiles of ALAN during 2013–2018 were performed.

Cubic splines were used to determine whether ALAN is linearly associated with atopic disease. Because a nonlinear dose-response relationship was identified, several parametric model with different link functions were used, and Akaike information criteria (AIC) was used to select the best model (Supplementary Table S1). Finally, we used the $\log_{10}$-transformed ALAN as a continuous variable because it showed the best goodness of fit.

Owing to the potential intracluster correlations in cluster sampling (by university), the mixed generalized linear models with 'log' link function and 'binomial' distribution were applied to assess the association of ALAN with atopic diseases adjusting for potential confounders. Specifically, the random intercept at university level was modeled. The effect size of exposure was presented as crude and adjusted prevalence ratios (PRs) (Tamhane et al., 2016) and 95% confidence intervals (CIs).

To avoid biased estimation caused by the collinearity in the multivariable models, we performed principal component analysis (PCA) for environmental covariates (humidity, temperature, UV radiation, and six air pollutants) and extracted principal components with an eigenvalue >1. Then, the components were included in the adjusted models instead of the raw variables.

To investigate whether the trajectory of ALAN during 2013–2018 is associated with the risk of atopic diseases, we applied the group-based trajectory modeling, a finite mixture-based method which assumes that the population is composed of a mixture of distinct groups defined by their developmental trajectories (Nagin and Odgers, 2010). The groups were first identified statistically according to trajectories, and then correlated to the outcome using the two-level logistic model.

Effect modification by covariates were examined. Subgroup analysis for the association was performed within each stratum of categorical variables including sex, household income, second-hand smoke exposure, physical activity, intake of red meat, and university. Interactive effects were examined for continuous variables including body mass index and the principal component of air pollutants. Sensitivity analysis was performed by using the mean ALAN of a single year or combined period as the exposure variable, considering potential lag effect. The statistical analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, USA) using the 'glimmix' procedure and R 3.5.0 (R Core Team, 2018) using the 'ggplot2', 'FactoMineR', and 'interplot' packages. A P value < 0.05 was considered statistically significant.

3. Results

3.1. Characteristics of participants

A total of 27144 students were enrolled in the universities in 2018 according to the registry data, and 20138 (74.2%) consented to participate and completed the field study. Among them, 20106 participants (99.8%) from 2483 counties and city districts with complete individual and environmental data were included in the final analysis (Supplementary Fig. S1). The spatial distribution of mean ALAN during 2013–2018 is shown in Fig. 1. Regions with high levels of ALAN are concentrated in provincial capitals and cities along the east coast. The characteristics of the participants by ALAN are shown in Table 1. Most variables were significantly associated with ALAN ($P < 0.01$). Unexpectedly, ALAN was not associated with sleep disturbance ($P = 0.992$).

3.2. The pattern of a dose-response relationship

The prevalence rates of the diseases were increased in higher tertiles of ALAN except for acne vulgaris (Table 2). Cubic splines further indicate that ALAN was nonlinearly associated with atopic diseases including asthma, allergic rhinitis, and AD (Fig. 2). In contrast, ALAN was not significantly correlated with non-atopic diseases such as urticaria, allergies to drug/food/light, and acne. By comparing linear model, fractional polynomials, and logarithmic link functions, we identified the smallest AIC in the last model (Supplementary Table S1). As a result, we used $\log_{10}$-transformed ALAN in all subsequent analyses.

3.3. Dimension reduction for environmental covariates

Because Spearman correlation analysis indicates moderate-to-strong associations between the environmental covariates, PCA was conducted and two principal components were extracted and included in the multivariable models (Supplementary Fig. S2). Component 1 primarily represents air pollutants while component 2 represents meteorologic variables (humidity, temperature, and UV radiation).

3.4. Main findings

Unadjusted and adjusted estimates consistently showed that ALAN exposure was associated with atopic diseases (Fig. 3). After adjustment for all covariates, the PR for atopic diseases was 1.35 (95% CI: 1.27–1.42; $P < 0.001$), indicating that one-unit increase in the log-transformed ALAN was associated with a 35% additional risk of atopic diseases. The effect size of ALAN was the largest for asthma (PR = 1.80;

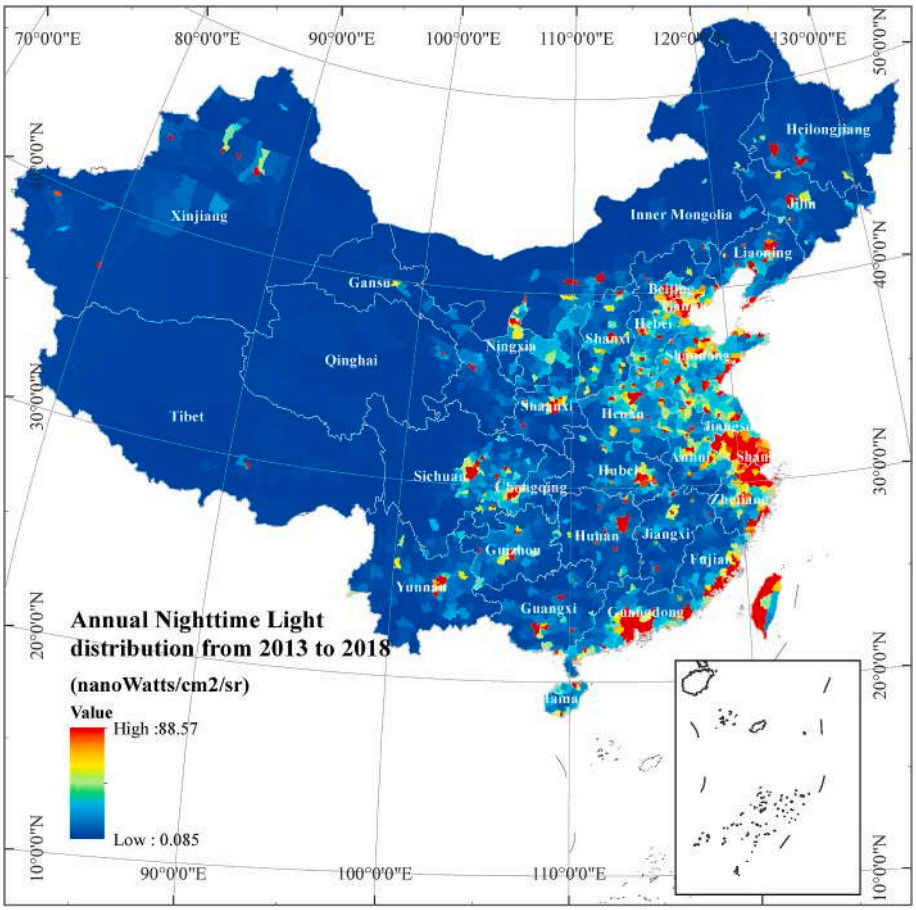

Fig. 1. Distribution of artificial light at night during 2013–2018.

95% CI: 1.48–2.19; $P < 0.001$), followed by allergic rhinitis (PR = 1.42; 95% CI: 1.33–1.51; $P < 0.001$), and AD (PR = 1.20; 95% CI: 1.06–1.35; $P = 0.003$). In contrast, eczema (including atopic and non-atopic eczema) was not significantly correlated with ALAN after full adjustment (PR = 1.07; 95% CI: 0.99–1.15; $P = 0.082$).

3.5. The trajectory of ALAN in association with atopic diseases

Group-based trajectory modeling identified three ALAN groups: "high-increasing" (3.1%), "medium-increasing" (14.6%), and "low-stable" (82.3%) (Supplementary Fig. S3). Compared to the "low-stable" group, the "medium-increasing" group (PR = 1.34; 95% CI: 1.23–1.46; $P < 0.001$) and the "high-increasing" group (PR = 1.39; 95% CI: 1.19–1.62; $P < 0.001$) showed a higher risk of atopic diseases after adjustments. Similar findings were observed for asthma, allergic rhinitis, and AD.

3.6. Subgroup and sensitivity analyses

The association of ALAN with atopic diseases was consistent across subgroups of the population, although some variations in the effect size were observed (Fig. 4). For example, the estimate was not significant in two of the universities, but the point estimate was close to that in other universities. We also examined the interaction between ALAN and body mass index and the component of environmental exposures and found no significant interactive effects (Supplementary Fig. S4).

In sensitivity analysis, we alternatively used the mean ALAN of a single year or combined period and found that the association of ALAN with atopic diseases was highly consistent. For example, the effect of ALAN in 2018 on atopic diseases in terms of PR was 1.39 (95% CI: 1.32–1.48; $P < 0.001$).

4. Discussion

In this population-based cross-sectional analysis, the associations between ALAN exposure during adolescence and the risks of atopic diseases in young adulthood were investigated, and a novel significant contribution of ALAN to atopic diseases was identified. The findings of our study fill the gap of the understanding about the effect of ALAN on atopic diseases.

So far, environmental factors related to modern lifestyles and urbanization have been widely acknowledged to involve in the development and manifestation of atopic diseases (Murrison et al., 2019). In general, living in modern and urbanized area usually is accompanied by higher exposure to air pollutants due to industrialization, higher exposure to house dust microbiota due to longer indoor time (Mahdavinia et al., 2021; Weinberg, 2000). Their roles in allergic prevalence have been clarified (Mahdavinia et al., 2021; Schikowski, 2022). However, shifting lifestyles often brings about changes of massive types of environmental factors, of which effect are complexed and heterogeneous on atopic diseases. Thus, careful adjustment with other factors is vital when establishing a correlation between certain environmental factors and atopic disease. In general, metrics in urban health can be classified into build environment, pollution, social factors, urban climates, etc (Prasad et al., 2016). In this paper, based on this analysis of young adults of the same age, we were able to largely avoid potential social confounders such as age, occupation, marriage, and social stress. Besides, to further avoid the interference from behavioral factors, we performed subgroup analysis according to smoking exposure, diets and physical activities, ect. As for environmental confounders, ambient air factors are among

Table 1
Comparison of characteristics of participants across nighttime light tertiles.

Characteristics	Total (N = 20106)	ALAN during 2013–2018 (nanoWatts/cm^2/sr)			*P*
		1st tertile (<0.65)	2nd tertile (0.65–4.02)	3rd tertile (>4.02)	
City of university					
Changsha	5017 (25.0)	1441 (28.7)	1818 (36.2)	1758 (35.0)	<0.001
Wuhan	5605 (27.9)	2199 (39.2)	1595 (28.5)	1811 (32.3)	
Xiamen	4186 (20.8)	775 (18.5)	1446 (34.5)	1965 (46.9)	
Urumchi	2924 (14.5)	1258 (43.0)	990 (33.9)	676 (23.1)	
Hohhot	2374 (11.8)	979 (41.2)	885 (37.3)	510 (21.5)	
Age (years), mean ± SD	18.3 ± 0.8	18.3 ± 0.9	18.3 ± 0.8	18.3 ± 0.7	0.003
Female sex, n (%)	9827 (48.9)	3156 (47.4)	3313 (49.2)	3358 (50.0)	0.011
Annual household income, n (%)					
<10,000 CNY (<1418 US$)	2167 (10.8)	1040 (15.6)	686 (10.2)	441 (6.6)	<0.001
10,000~ CNY (1418~ US$)	4374 (21.7)	1901 (28.6)	1521 (22.6)	952 (14.2)	
30,000~ CNY (4254~ US$)	3465 (17.2)	1330 (20.0)	1225 (18.2)	910 (13.5)	
50,000~ CNY (7091~ US$)	4412 (21.9)	1428 (21.5)	1618 (24.0)	1366 (20.3)	
≥100,000 CNY (≥14181 US$)	5688 (28.3)	953 (14.3)	1684 (29.6)	3051 (45.4)	
Body mass index (kg/m^2), mean ± SD	21.3 ± 3.5	21.2 ± 3.4	21.3 ± 3.6	21.4 ± 3.5	<0.001
Second-hand smoke exposure, n (%)					
Hardly	15873 (79.0)	5478 (82.4)	5318 (79.0)	5077 (75.5)	<0.001
1 day/week	2777 (13.8)	815 (12.2)	942 (14.0)	1020 (15.2)	
≥2 days/week	1456 (7.2)	359 (5.4)	474 (7.0)	623 (9.3)	
Intake of red meat, n (%)					
<1 time/week	6161 (30.6)	2487 (37.4)	2150 (31.9)	1524 (22.7)	<0.001
1–3 times/week	9305 (46.3)	3016 (45.3)	3066 (45.5)	3223 (47.9)	
>3 times/week	4640 (23.1)	1149 (17.3)	1518 (22.6)	1973 (29.4)	
Recreational physical activity, n (%)					
<60 min/week	8469 (42.1)	2975 (44.7)	2893 (43.0)	2601 (38.7)	<0.001
60–179 min/week	4243 (21.1)	1359 (20.4)	1408 (20.9)	1476 (22.0)	
≥180 min/week	7394 (36.8)	2318 (34.9)	2433 (36.1)	2643 (39.3)	
Sleep disturbance, n (%)	5875 (29.2)	1940 (29.2)	1969 (29.2)	1966 (29.3)	0.992
History of eczema, n (%)	723 (3.6)	201 (3.0)	230 (3.4)	292 (4.3)	<0.001
Humidity (%), mean ± SD	59.2 ± 13.3	58.5 ± 12.9	58.5 ± 13.0	60.7 ± 13.7	<0.001
Temperature (°C), mean ± SD	14.0 ± 5.5	12.7 ± 5.8	13.8 ± 5.2	15.4 ± 5.1	<0.001
O_3 (μg/kg·d), mean ± SD	11.1 ± 1.6	11.6 ± 1.5	11.3 ± 1.5	10.4 ± 1.7	<0.001
CO (mg/kg·d), mean ± SD	0.15 ± 0.06	0.12 ± 0.07	0.15 ± 0.07	0.18 ± 0.07	<0.001
NO_2 (μg/kg·d), mean ± SD	4.2 ± 2.5	2.6 ± 1.7	4.0 ± 2.2	6.0 ± 2.4	<0.001
SO_2 (μg/kg·d), mean ± SD	3.1 ± 2.0	2.4 ± 1.5	3.2 ± 2.0	3.7 ± 2.2	<0.001
$PM_{2.5}$ (μg/kg·d), mean ± SD	8.3 ± 3.5	7.4 ± 3.3	8.4 ± 3.6	9.0 ± 3.4	<0.001
PM_{10} (μg/kg·d), mean ± SD	12.9 ± 6.0	10.7 ± 5.3	13.2 ± 6.3	14.6 ± 5.9	<0.001
Daytime UV radiation (J/cm^2), mean ± SD	2861.8 ± 481.8	2876.1 ± 481.6	2821.8 ± 452.3	2887.9 ± 507.5	<0.001

ALAN, artificial light at night; CNY, Chinese yuan; SD, standard deviation; UV: ultraviolet.

Table 2
Crude prevalence rates of diseases across nighttime light tertiles.

Disease	Total (N = 20106)	ALAN during 2013–2018 (nanoWatts/cm^2/sr)			*P*
		1st tertile (<0.65)	2nd tertile (0.65–4.02)	3rd tertile (>4.02)	
Atopic diseases, n (%)	3061 (15.2)	759 (11.4)	966 (14.4)	1336 (19.9)	<0.001
Asthma, n (%)	303 (1.51)	50 (0.75)	79 (1.17)	174 (2.59)	<0.001
Allergic rhinitis, n (%)	2270 (11.3)	547 (8.2)	714 (10.6)	1009 (15.0)	<0.001
Atopic dermatitis, n (%)	772 (3.84)	209 (3.14)	245 (3.64)	318 (4.73)	<0.001
Eczema, n (%)	1819 (9.1)	526 (7.9)	579 (8.6)	714 (10.6)	<0.001
Chronic urticaria, n (%)	381 (1.89)	113 (1.70)	111 (1.65)	157 (2.34)	0.005
Allergies to food, drug, or light, n (%)	455 (2.26)	133 (2.00)	151 (2.24)	171 (2.54)	0.105
Acne, n (%)	2086 (10.4)	697 (10.5)	721 (10.7)	668 (9.9)	0.327

the best-clarified contributors to allergy (Burbank et al., 2017; Milligan et al., 2016). Thus, we included major air pollutants including O_3, CO, NO_2, SO_2, $PM_{2.5}$, and PM_{10} and air climatic parameters including humidity, temperature, air pollutants, and UV radiation for analysis. After adjustment for all covariates, the effect size of ALAN for atopic diseases remains considerable, indicating that ALAN is a highly independent variable among environmental factors related to urbanization. Meanwhile, including students from five university located in representative region in China, further avoiding the potential biases of regional or localized confounds related to enrolment and diagnosis. However, since environmental microbiome or allergen exposure are critical in atopic prevalence, future effort should be taken to investigate ALAN's effect considering these factors (Burbank et al., 2017; Milligan et al., 2016; Murrison et al., 2019).

In order to establish a more specific correlation of ALAN and atopic disease, we also included non-atopic but atopic diseases such as urticaria, allergies to drug/food/light, and inflammatory diseases such as acne. The insignificance of correlation between ALAN with these diseases further supports the specific effect of ALAN on atopic diseases. Although eczema or allergies to food/drug/light can also be considered as atopic diseases, but the significance of ALAN's effect merely limited in the three diseases that are more often occur as comorbidity. In fact, a term was widely used to describe the onset of these three diseases—atopic march (Paller et al., 2019; Yang et al., 2020). Surprisingly, the effect size also exhibits a rank similar to the onset of the three

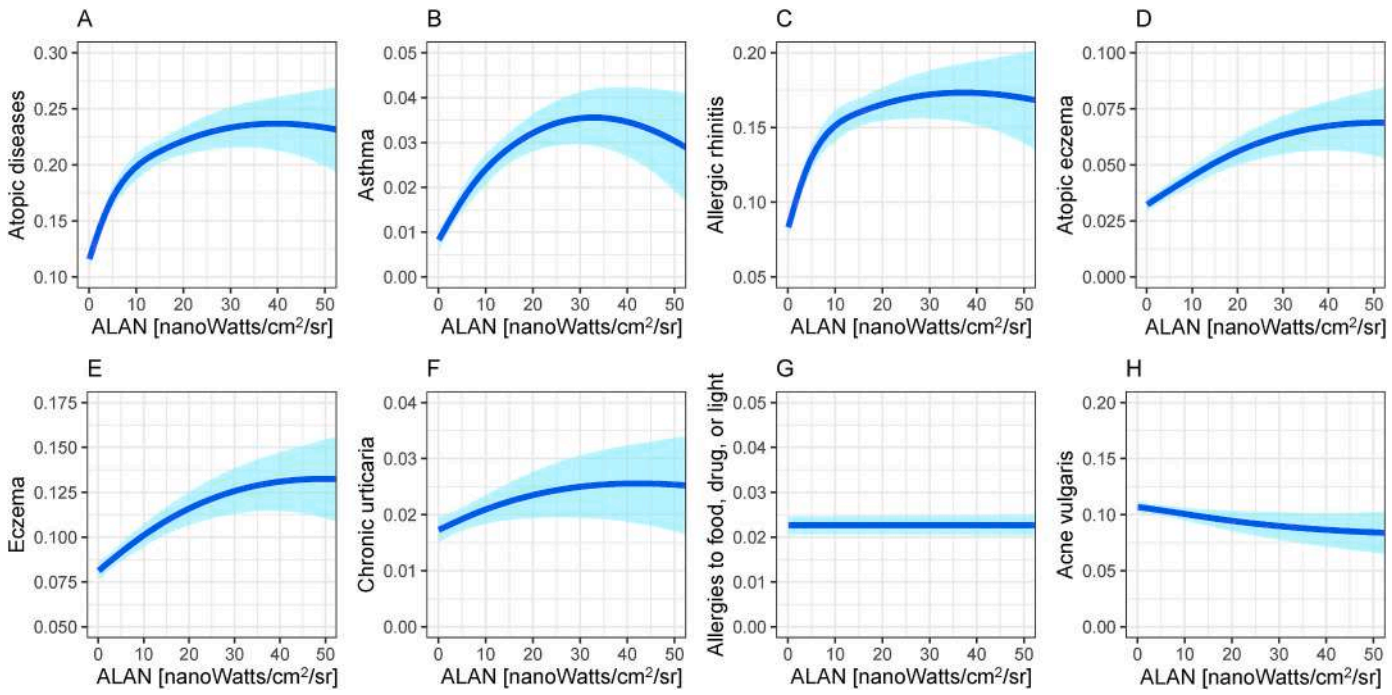

Fig. 2. Cubic splines for the associations of level of artificial light at night with the prevalence rates of atopic and non-atopic diseases. (A) Atopic diseases; (B) asthma; (C) allergic rhinitis; (D) atopic dermatitis; (E) eczema; (F) chronic urticaria; (G) allergies to drug/food/light; (H) acne vulgaris. X-axis refers to level of artificial light at night (ALAN) in nanoWatts/cm^2/sr; Y-axis refers to the prevalence rate of a disease.

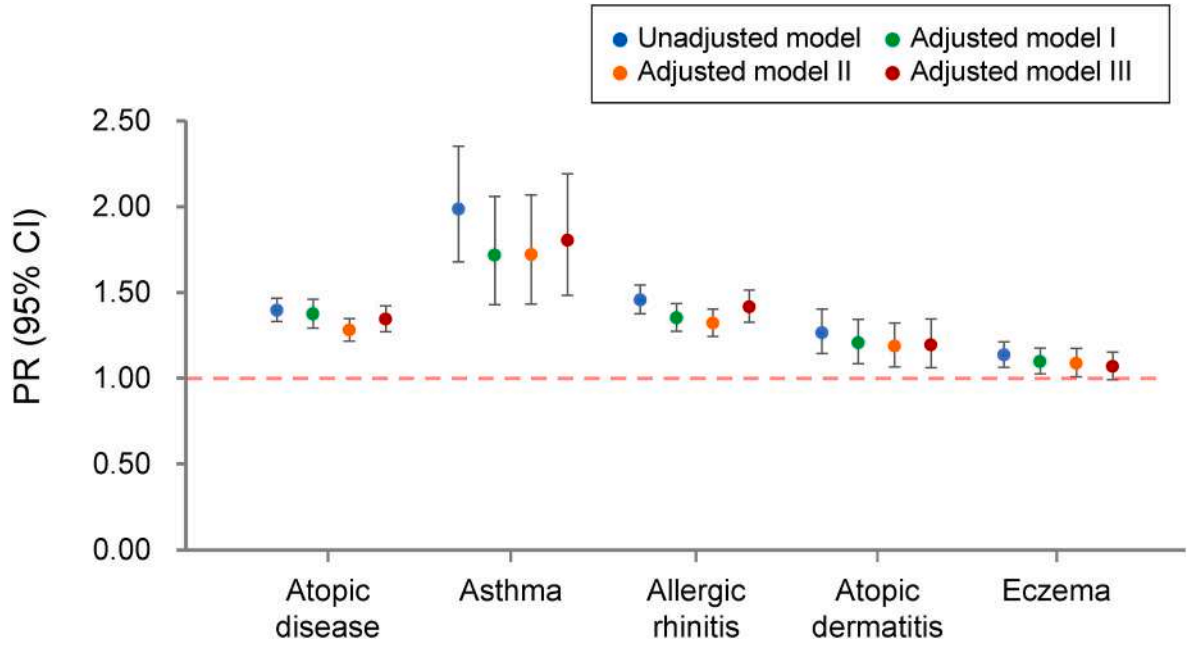

Fig. 3. Adjusted estimates for the associations of exposure to artificial light at night with the risk of atopic diseases. Adjusted model 1 includes demographic covariates (age, sex, and household income); adjusted model 2 further includes body mass index, second-hand smoke exposure, physical activity, and intake of red meat; adjusted model 3 further includes two principal components of the environmental covariates (humidity, temperature, daytime UV radiation, O_3, CO, NO_2, SO_2, $PM_{2.5}$, and PM_{10}). The prevalence ratio (PR)indicates an increased risk of the disease caused by a one-unit increment in the $\log_{10}$-transformed artificial light in nanoWatts/cm^2/sr.

diseases during the atopic march. The effect size of ALAN was the smallest for AD, which onsets at the earliest during life, followed by allergic rhinitis, and asthma, indicating an underlying time-dependent effect of ALAN (Paller et al., 2019). We, therefore, examined the trajectory of ALAN in association with atopic diseases and it turned out that the effect of ALAN on atopic diseases was strengthened by time accumulation. Unlike other environmental factors, such as infectious or microbial exposure, which mainly works at early life, the effect of ALAN exposure can be more constant and lasts until young adulthood at least (Han et al., 2017; Wesemann and Nagler, 2016). Thus, focusing on ALAN exposure can be more constructive in the management of atopic diseases.

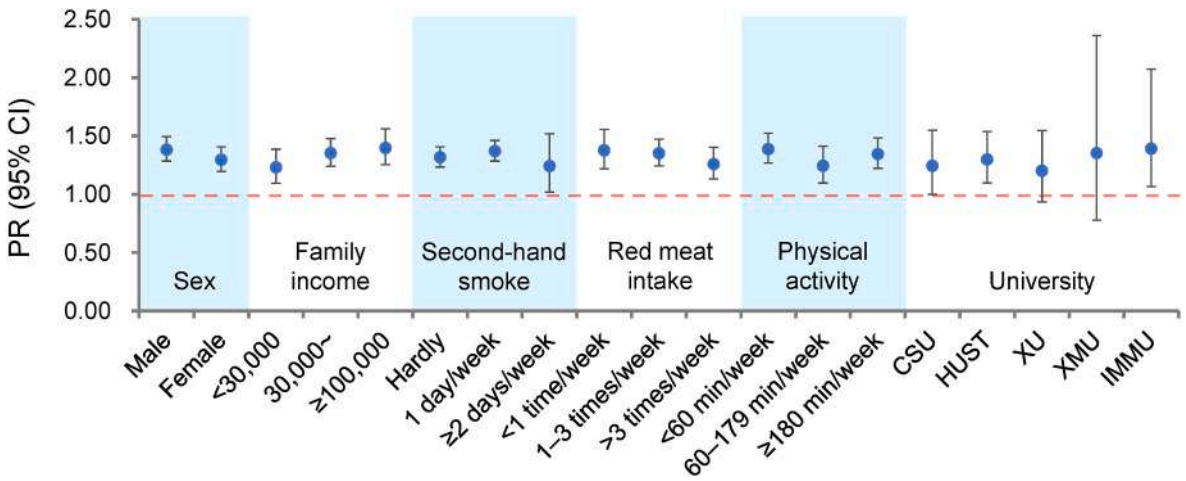

Fig. 4. Subgroup analysis for the association of exposure to artificial light at night with atopic diseases (asthma, allergic rhinitis, and atopic dermatitis) by categorical covariates after full adjustments. The prevalence ratio (PR) indicates an increased risk of the disease caused by a one-unit increment in the $\log_{10}$-transformed artificial light in nanoWatts/cm^2/sr.

The current understanding of the regulatory role of ALAN for human health mostly focuses on its relationships with circadian rhythm (Hussein et al., 2020; Touitou et al., 2017). A recent meta-analysis summarized the diverse impact of ALAN has on organisms, from individual aspects including organismal physiology, life-history traits, activity patterns, to the community or population level (Sanders et al., 2020). As for humans, ALAN-related circadian misalignment is believed to be mediated or featured by the abnormality of hormone secretion, sleep, food intake, etc (Touitou et al., 2017). Among these aspects, ALAN's impact on the endocrine has been highlighted. One most critical example is melatonin, which is directly regulated by light, and ALAN exposure can suppress melatonin levels (Fonken and Nelson, 2014; Reiter et al., 2007). A myriad of experimental studies has demonstrated that melatonin can modulate immunological disturbance through suppression of type 2 immune response, a critical biological process of atopic diseases (Gurram and Zhu, 2019; Marseglia et al., 2014). Besides, the antioxidant function of melatonin can also improve the pathological damage related to atopic diseases (Marseglia et al., 2014). More importantly, results from two clinical trials exhibited a significant improvement of AD severity after melatonin administration (Chang et al., 2016; Taghavi Ardakani et al., 2018). However, in these two clinical trials, the sleep quality did not improve correlated to the AD severity. In our study, we also found no significant role of sleep quality in the relation between ALAN and atopic diseases. In fact, exposure to higher dose of ALAN may greatly affected the melatonin secretion but affected less on the sleep quality since the major regulator of melatonin level is light/dark cycle while sleep quality can be largely affected by various factors (Claustrat and Leston, 2015). This research is a population-based observational study and it may of great significance to fully and precisely investigate ALAN exposure, melatonin secretion, sleep patterns, and atopic diseases in a well-designed intervention study, where more individual information about housing and behaviour factors, and real-time ALAN doses can be measured.

Some limitations cannot be ignored. First, due to the hometown address of individual was only collected at the scale of county/district and considering the scope of students' activities, the ALAN exposure was averaged at county/district scale. In further study, more detailed individual address information could be collected through new technologies (e.g. GNSS location, electronic map position). Then, estimate the average value of ALAN in a certain buffer area around the specific home address (e.g. block, building) as the exposure intensity can make the measurement of individual exposure more accurate. Second, the findings may not be generalized to the non-student population or the general population of a wider age range. Third, the retrospective study design may introduce recall bias and confounding bias, although we considered a series of potential confounders. Meanwhile, the designing of current research failed to quantify real-time ALAN exposure, indoor housing environment from individual levels. Also, future attention should be paid on the impact of allergen or microbiome confounders. However, there are some strengths in our study as well. We evaluated the effect of ALAN exposure during the 6 years before the participants' enrolment to the university, which corresponded to their adolescent period (13–18 years old). Significant urbanization took place in China during the past decade, making the exposure very representative for urban health studies. Besides, potential confounders for model adjustment were defined based on prior knowledge, facilitating the hypothesis-driven rather than data-driven findings.

5. Conclusion

Collectively, this study described the association between ALAN exposure and risk of atopic diseases for the first time. A possible adverse effect of ALAN on the risk of atopic diseases should be noticed. Reducing ALAN exposure among adolescents may be useful for the prevention of atopic diseases. More studies are warranted to further validate a causal effect and elucidate the underlying mechanism of the association, especially pathways involving circadian misalignment and endocrine.

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Ethical statement

This study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving study participants were approved by the institutional research ethics boards of Xiangya Hospital, Central South University, China (#201709993). Written informed consent was obtained from all participants or their custodians if aged under 18 before the investigation.

Author contributions

All authors participated in the field survey and data collection. Z.T.

drafted the manuscript. M.S. and S.L. analyzed the data. M.S. and X.C. designed the study. J.S., J.T., X.W., S.S., X.K., and B.W were study site coordinators. M.S., J.S., and X.C. obtained the funding. All authors participated in the field survey and data collection, critically revised the manuscript, and gave final approval to the version submitted for publication.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.113932.

References

Burbank, A.J., Sood, A.K., Kesic, M.J., Peden, D.B., Hernandez, M.L., 2017. Environmental determinants of allergy and asthma in early life. J. Allergy Clin. Immunol. 140, 1–12.

Chang, Y.S., Lin, M.H., Lee, J.H., Lee, P.L., Dai, Y.S., Chu, K.H., Sun, C., Lin, Y.T., Wang, L.C., Yu, H.H., Yang, Y.H., Chen, C.A., Wan, K.S., Chiang, B.L., 2016. Melatonin supplementation for children with atopic dermatitis and sleep disturbance: a randomized clinical trial. JAMA Pediatr. 170, 35–42.

Chen, Y., Wu, F., Liu, X., Parvez, F., LoIacono, N.J., Gibson, E.A., Kioumourtzoglou, M.A., Levy, D., Shahriar, H., Uddin, M.N., Islam, T., Lomax, A., Saxena, R., Sanchez, T., Santiago, D., Ellis, T., Ahsan, H., Wasserman, G.A., Graziano, J.H., 2019. Early life and adolescent arsenic exposure from drinking water and blood pressure in adolescence. Environ. Res. 178, 108681.

Claustrat, B., Leston, J., 2015. Melatonin: physiological effects in humans. Neurochirurgie 61, 77–84.

Dietch, J.R., Taylor, D.J., Sethi, K., Kelly, K., Bramoweth, A.D., Roane, B.M., 2016. Psychometric evaluation of the PSQI in U.S. College students. J. Clin. Sleep Med. 12, 1121–1129.

Eichenfield, L.F., Tom, W.L., Chamlin, S.L., Feldman, S.R., Hanifin, J.M., Simpson, E.L., Berger, T.G., Bergman, J.N., Cohen, D.E., Cooper, K.D., Cordoro, K.M., Davis, D.M., Krol, A., Margolis, D.J., Paller, A.S., Schwarzenberger, K., Silverman, R.A., Williams, H.C., Elmets, C.A., Block, J., Harrod, C.G., Smith Begolka, W., Sidbury, R., 2014. Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis. J. Am. Acad. Dermatol. 70, 338–351.

Fonken, L.K., Nelson, R.J., 2014. The effects of light at night on circadian clocks and metabolism. Endocr. Rev. 35, 648–670.

Franklin, M., Yin, X., McConnell, R., Fruin, S., 2020. Association of the built environment with childhood psychosocial stress. JAMA Netw. Open 3, e2017634.

Garcia-Saenz, A., Sánchez de Miguel, A., Espinosa, A., Valentin, A., Aragonés, N., Llorca, J., Amiano, P., Martín Sánchez, V., Guevara, M., Capelo, R., Tardón, A., Peiró-Perez, R., Jiménez-Moleón, J.J., Roca-Barceló, A., Pérez-Gómez, B., Dierssen-Sotos, T., Fernández-Villa, T., Moreno-Iribas, C., Moreno, V., García-Pérez, J., Castaño-Vinyals, G., Pollán, M., Aubé, M., Kogevinas, M., 2018. Evaluating the association between artificial light-at-night exposure and breast and prostate cancer risk in Spain (MCC-Spain study). Environ. Health Perspect. 126, 047011.

Gurram, R.K., Zhu, J., 2019. Orchestration between ILC2s and Th2 cells in shaping type 2 immune responses. Cell. Mol. Immunol. 16, 225–235.

Han, H., Roan, F., Ziegler, S.F., 2017. The atopic march: current insights into skin barrier dysfunction and epithelial cell-derived cytokines. Immunol. Rev. 278, 116–130.

Helbich, M., Browning, M., Huss, A., 2020. Outdoor light at night, air pollution and depressive symptoms: a cross-sectional study in The Netherlands. Sci. Total Environ. 744, 140914.

Hölker, F., Moss, T., Griefahn, B., Kloas, W., Voigt, C., Henckel, D., Hänel, A., Kappeler, P., Voelker, S., Schwope, A., Franke, S., Uhrlandt, D., Fischer, J., Klenke, R., Wolter, C., 2010. The dark side of light: a transdisciplinary research agenda for light pollution policy. Ecol. Soc. 15, 13.

Hussein, A.A.A., Bloem, E., Fodor, I., Baz, E.S., Tadros, M.M., Soliman, M.F.M., El-Shenawy, N.S., Koene, J.M., 2020. Slowly seeing the light: an integrative review on ecological light pollution as a potential threat for mollusks. Environ. Sci. Pollut. Res. Int. 28, 5036–5048.

Jing, D., Li, J., Tao, J., Wang, X., Shan, S., Kang, X., Wu, B., Zhang, Y., Xiao, Y., Chen, X., Shen, M., 2020. Associations of second-hand smoke exposure with hand eczema and atopic dermatitis among college students in China. Sci. Rep. 10, 17400.

Justiz Vaillant, A.A., Modi, P., Jan, A., 2021. Atopy, StatPearls. StatPearls Publishing, Copyright © 2021, StatPearls Publishing LLC., Treasure Island (FL).

Kemp, A.S., Ponsonby, A.L., Pezic, A., Cochrane, J.A., Dwyer, T., Jones, G., 2013. The influence of sun exposure in childhood and adolescence on atopic disease at adolescence. Pediatr. Allergy Immunol. 24, 493–500.

Lambrecht, B.N., Hammad, H., 2017. The immunology of the allergy epidemic and the hygiene hypothesis. Nat. Immunol. 18, 1076–1083.

Li, Y., Su, J., Luo, D., Duan, Y., Huang, Z., He, M., Tao, J., Xiao, S., Xiao, Y., Chen, X., Shen, M., 2021. Processed food and atopic dermatitis: a pooled analysis of three cross-sectional studies in Chinese adults. Front. Nutr. 8, 754663.

Liao, L.B., Weiss, S., Mills, S., Hauss, B., 2013. Suomi NPP VIIRS day-night band on-orbit performance. J. Geophys. Res. Atmos. 118, 12705–12718.

Liu, P., Wang, B., Zhao, X., Duan, X., Huang, N., Chen, Y., L, W., 2014. Research on inhalation rate of Chinese adults. J. Environ. Health 31, 953–956.

Mahdavinia, M., Greenfield, L.R., Moore, D., Botha, M., Engen, P., Gray, C., Lunjani, N., Hlela, C., Basera, W., Hobane, L., Watkins, A., Mankahla, A., Gaunt, B., Facey-Thomas, H., Landay, A., Keshavarzian, A., Levin, M.E., 2021. House dust microbiota and atopic dermatitis; effect of urbanization. Pediatr. Allergy Immunol. 32, 1006–1012.

Marco, E.M., Adriani, W., Ruocco, L.A., Canese, R., Sadile, A.G., Laviola, G., 2011. Neurobehavioral adaptations to methylphenidate: the issue of early adolescent exposure. Neurosci. Biobehav. Rev. 35, 1722–1739.

Marseglia, L., D'Angelo, G., Manti, S., Salpietro, C., Arrigo, T., Barberi, I., Reiter, R.J., Gitto, E., 2014. Melatonin and atopy: role in atopic dermatitis and asthma. Int. J. Mol. Sci. 15, 13482–13493.

Milligan, K.L., Matsui, E., Sharma, H., 2016. Asthma in urban children: epidemiology, environmental risk factors, and the public health domain. Curr. Allergy Asthma Rep. 16, 33.

Ministry of Environmental Protection of China, 2017. National Standard for Environmental Protection, Technical Guideline for Population Exposure Assessment of Environmental Pollutant. Ministry of Environmental Protection of China.

Murrison, L.B., Brandt, E.B., Myers, J.B., Hershey, G.K.K., 2019. Environmental exposures and mechanisms in allergy and asthma development. J. Clin. Invest. 129, 1504–1515.

Nagin, D.S., Odgers, C.L., 2010. Group-based trajectory modeling in clinical research. Annu. Rev. Clin. Psychol. 6, 109–138.

Obayashi, K., Saeki, K., Iwamoto, J., Ikada, Y., Kurumatani, N., 2013. Exposure to light at night and risk of depression in the elderly. J. Affect. Disord. 151, 331–336.

Okada, H., Kuhn, C., Feillet, H., Bach, J.F., 2010. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. Clin. Exp. Immunol. 160, 1–9.

Paksarian, D., Rudolph, K.E., Stapp, E.K., Dunster, G.P., He, J., Mennitt, D., Hattar, S., Casey, J.A., James, P., Merikangas, K.R., 2020. Association of outdoor artificial light at night with mental disorders and sleep patterns among US adolescents. JAMA Psychiatr. 77, 1266–1275.

Paller, A.S., Spergel, J.M., Mina-Osorio, P., Irvine, A.D., 2019. The atopic march and atopic multimorbidity: many trajectories, many pathways. J. Allergy Clin. Immunol. 143, 46–55.

Prasad, A., Gray, C.B., Ross, A., Kano, M., 2016. Metrics in urban health: current developments and future prospects. Annu. Rev. Publ. Health 37, 113–133.

Reiter, R.J., Tan, D.X., Korkmaz, A., Erren, T.C., Piekarski, C., Tamura, H., Manchester, L. C., 2007. Light at night, chronodisruption, melatonin suppression, and cancer risk: a review. Crit. Rev. Oncog. 13, 303–328.

Sanders, D., Frago, E., Kehoe, R., Patterson, C., Gaston, K.J., 2020. A meta-analysis of biological impacts of artificial light at night. Nat. Ecol. Evol.
Schikowski, T., 2022. Indoor and outdoor pollution as risk factor for allergic diseases of the skin and lungs. In: Traidl-Hoffmann, C., Zuberbier, T., Werfel, T. (Eds.), Allergic Diseases – from Basic Mechanisms to Comprehensive Management and Prevention. Springer International Publishing, Cham, pp. 359–366.
Shahar, G., Henrich, C.C., 2019. Role of adolescent exposure to rockets in the links between personality vulnerability and psychopathology. Dev. Psychopathol. 31, 1367–1380.
Shen, M., Gu, X., Li, S., Yu, Y., Zou, B., Chen, X., 2021. Exposure to black carbon is associated with symptoms of depression: a retrospective cohort study in college students. Environ. Int. 157, 106870.
Shen, M., Xiao, Y., Su, J., Zhao, S., Li, J., Tao, J., Kang, X., Wu, B., Shan, S., Wang, X., Chen, X., 2020. Prevalence and patient-reported outcomes of noncommunicable skin diseases among college students in China. JAAD Int. 1, 23–30.
Shi, K., Yu, B., Huang, Y., Hu, Y., Yin, B., Chen, Z., Chen, L., Wu, J., 2014. Evaluating the ability of NPP-VIIRS nighttime light data to estimate the gross domestic product and the electric power consumption of China at multiple scales: a comparison with DMSP-OLS data. Rem. Sens. 6, 1705–1724.
Sun, S.Z., Cao, W.N., Ge, Y., Ran, J.J., Sun, F., Zeng, Q., Guo, M.D., Huang, J.X., Lee, R.S. Y., Tian, L.W., Wellenius, G.A., 2021. Outdoor light at night and risk of coronary heart disease among older adults: a prospective cohort study. Eur. Heart J. 42, 822–830.
Taghavi Ardakani, A., Farrehi, M., Sharif, M.R., Ostadmohammadi, V., Mirhosseini, N., Kheirkhah, D., Moosavi, S.G.A., Behnejad, M., Reiter, R.J., Asemi, Z., 2018. The effects of melatonin administration on disease severity and sleep quality in children with atopic dermatitis: a randomized, double-blinded, placebo-controlled trial. Pediatr. Allergy Immunol. 29, 834–840.
Tamhane, A.R., Westfall, A.O., Burkholder, G.A., Cutter, G.R., 2016. Prevalence odds ratio versus prevalence ratio: choice comes with consequences. Stat. Med. 35, 5730–5735.
Tang, X., Kong, L., Zhu, J., Wang, Z., Li, J., Wu, H., Wu, Q., Chen, H., Zhu, L., Wang, W., Liu, B., Wang, Q., Chen, D., Pan, Y., Song, T., Li, F., Zheng, H., Jia, G., Lu, M., Wu, L., Carmichael, G.R., 2020. A Six-Year Long High-Resolution Air Quality Reanalysis Dataset over China from 2013 to 2018 (Monthly and Annual Version). Science Data Bank.
Touitou, Y., Reinberg, A., Touitou, D., 2017. Association between light at night, melatonin secretion, sleep deprivation, and the internal clock: health impacts and mechanisms of circadian disruption. Life Sci. 173, 94–106.
Weidinger, S., Beck, L.A., Bieber, T., Kabashima, K., Irvine, A.D., 2018. Atopic dermatitis. Nat. Rev. Dis. Prim. 4, 1.
Weidinger, S., Novak, N., 2016. Atopic dermatitis. Lancet 387, 1109–1122.
Weinberg, E.G., 2000. Urbanization and childhood asthma: an African perspective. J. Allergy Clin. Immunol. 105, 224–231.
Wesemann, D.R., Nagler, C.R., 2016. The microbiome, timing, and barrier function in the context of allergic disease. Immunity 44, 728–738.
Witkowski, J.A., Parish, L.C., 2004. The assessment of acne: an evaluation of grading and lesion counting in the measurement of acne. Clin. Dermatol. 22, 394–397.
Wu, H.M., Zhao, C.C., Xie, Q.M., Xu, J., Fei, G.H., 2020. TLR2-Melatonin feedback loop regulates the activation of NLRP3 inflammasome in murine allergic airway inflammation. Front. Immunol. 11, 172.
Xiao, Y., Huang, X., Jing, D., Huang, Y., Chen, L., Zhang, X., Zhao, S., Zhang, M., Luo, Z., Su, J., Kuang, Y., Li, J., Zhu, W., Zhang, J., Chen, X., Shen, M., 2019. The prevalence of atopic dermatitis and chronic spontaneous urticaria are associated with parental socioeconomic status in adolescents in China. Acta Derm. Venereol. 99, 321–326.
Yang, L., Fu, J., Zhou, Y., 2020. Research progress in atopic march. Front. Immunol. 11, 1907.
Yu, Q., Yu, X., Zhong, X., Ma, Y., Wu, Y., Bian, T., Huang, M., Zeng, X., 2020. Melatonin modulates airway smooth muscle cell phenotype by targeting the STAT3/Akt/GSK-3β pathway in experimental asthma. Cell Tissue Res. 380, 129–142.
Zhang, D., Jones, R.R., James, P., Kitahara, C.M., Xiao, Q., 2021. Associations between Artificial Light at Night and Risk for Thyroid Cancer: A Large US Cohort Study. Cancer.
Zhao, M.H., Cui, L., Zhang, C.Y., 2020. Host-guest recognition coupled with triple signal amplification endows an electrochemiluminescent biosensor with enhanced sensitivity. Chem Commun (Camb) 56, 2971–2974.
Zhong, C., Franklin, M., Wiemels, J., McKean-Cowdin, R., Chung, N.T., Benbow, J., Wang, S.S., Lacey Jr., J.V., Longcore, T., 2020. Outdoor artificial light at night and risk of non-Hodgkin lymphoma among women in the California Teachers Study cohort. Cancer Epidemiol. 69, 101811.

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Associations between drinking water disinfection byproducts and menstrual cycle characteristics: A cross-sectional study among women attending an infertility clinic

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ABSTRACT

Disinfection byproducts (DBPs) have been shown to alter ovarian steroidogenesis and cause estrous cyclicity disturbance and prolongation in experimental studies, however human studies are lacking. We aimed to evaluate the cross-sectional associations between drinking water DBPs and menstrual cycle characteristics. A total of 1078 women attending an infertility clinic in Wuhan, China were included between December 2018 and January 2020. Characteristics of menstrual cycle were collected by questionnaires. Concentrations of dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) were measured in urine as biomarkers of drinking water DBPs. Multivariate logistic and linear regression models were used to evaluate the associations between urinary DCAA and TCAA concentrations and menstrual cycle characteristics. Higher urinary DCAA concentrations were associated with increased odds ratios (ORs) of irregular menstrual cycle (OR = 1.80; 95% CI: 0.97, 3.33 for the highest vs. lowest quartile; P for trend = 0.05) and long menstrual cycle (OR = 1.62; 95% CI: 0.97, 2.70 for the highest vs. lowest quartile; P for trend = 0.06), as well as prolonged variation in cycle length (β = 1.27 days; 95% CI: -0.11, 2.66 for the highest vs. lowest quartile; P for trend = 0.04). Higher urinary TCAA concentrations were associated with prolonged bleeding duration (β = 0.23 days; 95% CI: -0.06, 0.51 for the highest vs. lowest quartile; P for trend = 0.07). These results suggest that exposure to drinking water DBPs is associated with menstrual cycle disturbances. These findings are warranted to confirm in other studies.

1. Introduction

Menstruation reflects ovarian function among women of reproductive age and also can be considered as the surrogates of female fecundity and women's health across the lifespan (Harlow and Ephross, 1995; Diaz et al., 2006). Menstrual dysfunction such as irregular and long cycles has been associated with increased risks of reduced fecundity (Jensen et al., 1999; McLain et al., 2012; Mumford et al., 2012), ovarian cancer (Cirillo et al., 2016), type 2 diabetes mellitus (Solomon et al., 2001), cardiovascular disease (Solomon et al., 2002), mental health problems (Yu et al., 2017), and premature mortality (Wang et al., 2020). The determinants of menstrual dysfunction have been an increasing clinic and public concern but are not largely elucidated (Rowland et al., 2002; Newton and Philhower, 2003; Nam et al., 2017; Sakai and Ohashi, 2021). Increasing evidence suggests that environmental risk factors including exposure to reproductive toxicants may contribute to

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menstrual dysfunction (Ding et al., 2020; Hammer et al., 2020).

Disinfection byproducts (DBPs) are a group of widespread contaminants in drinking water that are formed when disinfectants (e.g., chloramine, ozone, chlorine dioxide, and chlorine) react with organic and inorganic matters during the process of public drinking water treatment (Nieuwenhuijsen et al., 2000; Han et al., 2021). Among >700 identified DBPs, haloacetic acids (HAAs) are one of the common classes in chlorinated drinking water (Zhang et al., 2009). As non-volatile DBPs, human exposure to HAAs primarily occurs through ingestion of drinking water (Nieuwenhuijsen et al., 2009). Dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA), the two most abundant compounds of the HAAs class, have been proposed as candidate biomarkers for chronic ingestion of DBPs in drinking water (Weisel et al., 1999; Wang et al., 2014; Zhang et al., 2021).

Epidemiological studies have documented that exposure to DBPs is associated with adverse reproductive and developmental outcomes including intrauterine growth retardation (Hinckley et al., 2005), small for gestational age (Levallois et al., 2016; Mashau et al., 2018; Sun et al., 2020), low birth weight (Hinckley et al., 2005; Zhou et al., 2012; Smith et al., 2016; Yang et al., 2019), and preterm delivery (Rivera-Nunez and Wright, 2013), as well as reduced semen quality (Zeng et al., 2014), while others showed null associations (Luben et al., 2007; Ileka-Priouzeau et al., 2015; Kogevinas et al., 2016; Mashau et al., 2019). However, few human studies have focused on the associations between DBP exposures and female reproductive health. To our knowledge, only two human studies have examined the associations of exposure to drinking water DBPs with time to pregnancy and menstrual cycle function, relying on monitoring DBP concentrations in the tap water system and/or combining with individual water-use activities as surrogates of exposures (Windham et al., 2003; MacLehose et al., 2008). Nevertheless, increasing experimental studies have demonstrated that DBPs, particularly HAAs, can interfere with estrous cyclicity (Balchak et al., 2000), alter ovarian steroidogenesis (Goldman and Murr, 2002, 2003; Goldman et al., 2007), inhibit follicle growth (Jeong et al., 2016), reduce the population of primordial follicles (Bodensteiner et al., 2004), and disrupt oocyte maturation (Jiao et al., 2021).

In the present study, we measured urinary DCAA and TCAA concentrations among women attending an infertility clinic in Wuhan, China. The purpose of this study was to investigate whether urinary concentrations of DCAA and TCAA were associated with menstrual cycle characteristics. This study is the first, to our knowledge, to utilize urinary biomarkers of exposure to drinking water DBPs to examine the associations with menstrual cycle function.

2. Materials and methods

2.1. Study design and population

To explore the effects of lifestyle factors and environmental chemical exposures on human reproductive health, the Tongji Reproductive and Environmental (TREE) study was established in December 2018 and is ongoing at the Reproductive Medicine Center of Tongji hospital in Wuhan, China. This cohort study recruited couples who aged at least 20 years and planned to seek the treatment of assisted reproductive technology (ART). The subjects were followed to examine pregnancy outcomes. To date, the TREE study has recruited 2057 women and 2045 men. Each participant was required to provide biological samples (e.g., urine, blood, follicular fluid, and semen) and complete a face-to-face questionnaire. The questionnaire included demographic characteristics, lifestyle factors, reproductive and medical history, and water-use activities. Passive smoking was defined as exposure to secondhand smoke in the workplace or at home (Hu et al., 2019).

The present study was restricted to women who were enrolled in the TREE study from December 2018 to January 2020 and had the available measurements of urinary DCAA and TCAA concentrations (n = 1138). Of these, women were excluded in the current study if they reported a history of occupational exposure to certain synthetic materials (e.g., paints and glues) (n = 39), as these chemicals may contain 1,1,1-trichloroethane, trichloroethylene, and perchloroethylene that can be metabolized into TCAA and introduce misclassification of exposure (Zeng et al., 2014). Also, women were excluded if they had amenorrhoea (n = 1), or if they did not provide full information on menstrual cycle characteristics (n = 20). Finally, there were 1078 women available for the current analysis. This study protocol was approved by the Tongji Medical College Ethics Committee and each participant was given informed consent before participation.

2.2. Urine collection and analysis

We used a 50-mL polypropylene container to collect a single spot urine sample from each participant at the time of enrollment. All the urine samples were divided into aliquots and stored at −20 °C for analysis. Urinary concentrations of DCAA and TCAA were measured by liquid-liquid extraction and a gas chromatography (GC). Detailed methods for sample preparation, instrumental analysis, and quality controls have been provided previously (Xie et al., 2011; Deng et al., 2019). In short, a 5-mL urine sample was extracted with methyl-tert-butyl-ether (MTBE) containing 1,2-dipropyl bromide as the internal standard. Then, 1-mL acidic methanol was added into organic extraction to convert DCAA and TCAA into methyl esters at 50 °C for 2 h. After that, saturated sodium bicarbonate was used to neutralize the derivatization reaction. The target analytes were detected by the GC coupled with an electron capture detector (ECD). The limits of detection (LODs) of DCAA and TCAA were 1.00 μg/L and 0.50 μg/L, respectively. The targets in urine samples below their LODs were assigned by $LOD/\sqrt{2}$. Each analysis run included one blank and two quality control samples. The coefficients of variation (inter-day and intra-day variation) were < 10.00%, and the spiked recoveries for DCAA and TCAA were 93.90%–116.16% and 92.00%–118.19%, respectively. Specific gravity (SG) was measured by a handheld refractometer to correct for the variation in urine diluteness (Wang et al., 2019b).

2.3. Menstrual cycle characteristics

The information on menstrual cycle characteristics was obtained by face-to-face interviews under trained research staff using a uniform questionnaire according to the standards recommended by the Chinese Medical Association (Zhang et al., 2020). The following questions were asked: “Generally speaking, was your menstrual cycle regular in the past 12 months?”, if the answer is “Yes”, then ask, “What was your average menstrual cycle length (in days)?”; If the answer is “No”, then ask, “What were the shortest and longest menstrual cycle lengths (in days)?”. Variation in cycle length was calculated as the difference between the longest and shortest menstrual cycles (Windham et al., 2003). An irregular cycle was defined as the variation in cycle length for no less than 7 days (Buck et al., 2011; Lyngso et al., 2014). A short cycle was defined as ≤ 24 days, whereas a long cycle was ≥32 days according to previous studies (Kolstad et al., 1999; Small et al., 2007). The bleeding duration was assessed by asking “On average, how many days does your menstrual bleeding take?”. The amount of menstrual bleeding was given as three choices of light, moderate, and heavy. Less than 20 mL of bleeding amount is “light” that is defined as hypomenorrhea; 20–80 mL of bleeding amount is “moderate”; more than 80 mL of bleeding amount is “heavy” that is defined as menorrhagia. Dysmenorrhea was defined as a positive answer to the question “Did you experience menstrual cramps during your menstrual flow?’’.

2.4. Statistical analysis

All the statistical analyses were performed using R software (version 3.6.2). Descriptive statistics were calculated for the distribution of demographic and menstrual cycle characteristics and urinary DCAA and

TCAA concentrations. Parametric or nonparametric methods were appropriately used to estimate the differences in demographic characteristics between the study population with and without endocrinopathy. Urinary concentrations of DCAA and TCAA were standardized by SG using the following formula: $P_s = P[(1.018–1)/(SG_c\text{-}1)]$, where 1.018 is the mean SG concentration of all the participants, *P* represents the measured concentration, and P_s is the SG-adjusted concentration (μg/L). The concentrations of SG-adjusted DCAA and TCAA were natural logarithm (ln)-transformed to reduce the influence of outliers.

The associations of SG-adjusted urinary DCAA and TCAA concentrations with categorical menstrual cycle outcomes including irregular cycles, long cycles, hypomenorrhea, and dysmenorrhea were assessed separately by multivariate logistic regression models, in which regular cycles (variations in cycle length <7 days), normal cycles (cycle length between 25 and 31 days), normal volume (moderate amount of menstrual bleeding), and non-dysmenorrhea were treated as the references, respectively. Odds ratios (ORs) and 95% confidence intervals (CIs) for each outcome were estimated. Given the limited sample sizes of short cycle (n = 34) and menorrhagia (n = 13), we did not include the two outcomes in the analysis. The associations of SG-adjusted urinary DCAA and TCAA concentrations with continuous menstrual cycle outcomes including bleeding duration, cycle length, and variation in cycle length were performed by multivariate linear regression models. The SG-adjusted urinary DCAA and TCAA concentrations were categorized into quartiles, and the lowest quartiles were considered as the reference levels. Trend tests across increasing quartiles of exposures were conducted by entering the quartiles of SG-adjusted urinary DCAA and TCAA concentrations as ordinal integer values (1–4) in the regression models. All the analyses were conducted in both women with and without endocrinopathy (hyperprolactinemia, polycystic ovary syndrome, thyroid disorders, and diabetes mellitus, n = 206). The distribution of endocrinopathies by cycle length categories is displayed in Table S1.

Potential confounders were selected based on prior studies (Windham et al., 2003; Buck et al., 2011; Lyngso et al., 2014; Mena et al., 2019). Covariates retained in the final multivariable models were age at recruitment (continuous), age at menarche (continuous), body mass index (BMI, continuous), alcohol use (yes vs. no), physical activity (occasionally and often vs. never), parity (nulliparous vs. parous), and passive smoking status (yes vs. no). In addition, season of recruitment (spring: March to May; summer: June to August; autumn: September to November; winter: December to February) was also included in the final models based on the "change-in-estimate" method with >10% change in the estimated effects between SG-adjusted urinary DCAA and TCAA concentrations and menstrual cycle characteristics (Maldonado and Greenland, 1993). P-value < 0.05 was defined as statistically significant and P-value < 0.10 was considered as statistically suggestive (Kambakamba et al., 2016).

3. Results

3.1. Participant demographic and menstrual cycle characteristics and distribution of urinary DBPs concentrations

Demographic and menstrual cycle characteristics of the study population are shown in Table 1. There were no significant differences between the entire population and subgroup population in demographic characteristics except for menstrual cycle length categories and variation and regularity in cycle length. The mean (SD) age at recruitment and BMI for the entire study population were 30.9 (4.6) years and 22.1 (3.2) kg/m^2, respectively. Among the participants, 82.2% were nulliparous, 94.4% were non-smokers, 77.3% were non-drinkers, 74.7% reported drinking less than 1200 mL of water per day, 72.3% used boiled water, and 89.7% used filtered water. For the menstrual cycle characteristics, the mean (SD) age at menarche, bleeding duration, and variation in cycle length were 13.3 (1.2) years, 5.9 (1.5) days, and 4.2 (12.5) days, respectively. The majority of women (84.8%) had regular

Table 1
Demographic and menstrual cycle characteristics among women with and without endocrinopathy [mean ± SD or n (%)].

Characteristics	Entire population (n = 1078)[a]	Subgroup population (n = 872)[b]	P-Value
Demographic characteristics			
Age at recruitment (years)	30.9 ± 4.6	31.1 ± 4.8	0.38
Body mass index (kg/m^2)	22.1 ± 3.1	21.9 ± 3.0	0.33
Parity			
Nulliparous	886 (82.2)	698 (80.0)	0.23
Parous	192 (17.8)	174 (20.0)	
Smoking status			
Never	1017 (94.4)	820 (94.0)	0.95
Ever	51 (4.7)	43 (4.9)	
Current	10 (0.9)	9 (1.1)	
Alcohol use			
No	833 (77.3)	673 (77.2)	0.96
Yes	245 (22.7)	199 (22.8)	
Educational level			
Less than high school	417 (38.7)	348 (40.0)	0.82
Junior and senior high school	253 (23.5)	205 (23.5)	
College and above	408 (37.8)	319 (36.5)	
Household income (yuan/month)			
≤5000	537 (49.8)	449 (51.5)	0.76
5000–10,000	349 (32.4)	273 (31.3)	
≥10,000	192 (17.8)	150 (17.2)	
Passive smoking			
No	562 (52.1)	454 (52.1)	1.00
Yes	516 (47.9)	418 (47.9)	
Physical activity			
Never	443 (41.1)	365 (41.9)	0.89
Occasionally	449 (41.7)	363 (41.6)	
Often	186 (17.2)	144 (16.5)	
Total tap-water consumption (mL/day)			
<1200	799 (74.7)	647 (74.2)	0.94
≥1200	270 (25.3)	217 (24.8)	
Use of boiled water			
No	299 (27.7)	239 (27.4)	0.87
Yes	779 (72.3)	633 (72.6)	
Use of filtered water			
No	111 (10.3)	90 (10.3)	1.00
Yes	967 (89.7)	782 (89.7)	
Season of recruitment			
Spring	459 (42.6)	370 (42.4)	0.99
Summer	440 (40.8)	355 (40.7)	
Autumn	119 (11.0)	95 (10.9)	
Winter	60 (5.6)	52 (5.9)	
Menstrual cycle parameters			
Age at menarche (years)	13.3 ± 1.2	13.3 ± 1.2	0.89
Cycle length (days)	29.5 ± 4.1	29.2 ± 3.5	0.27
Normal cycle (25–31 days)	781 (72.4)	683 (78.3)	<0.01
Short cycle (≤24 days)	34 (3.2)	29 (3.3)	
Long cycle (≥32 days)	263 (24.4)	160 (18.4)	
Variation in cycle length	4.2 ± 12.5	2.4 ± 7.4	0.01
Regular cycle (<7 days)	914 (84.8)	777 (89.1)	<0.01
Irregular cycle (≥7 days)	164 (15.2)	95 (10.9)	
Bleeding duration	5.9 ± 1.5	5.9 ± 1.6	0.77
Amount of menstrual bleeding			
Hypomenorrhea	67 (6.2)	52 (6.0)	0.97
Medium	998 (92.6)	810 (92.9)	
Menorrhagia	13 (1.2)	10 (1.1)	
Dysmenorrhea			
No	829 (76.9)	670 (76.8)	0.97
Yes	249 (23.1)	202 (23.2)	

[a] The entire population including endocrinopathy, and a total of 9 women had missing information on total tap-water consumption.

[b] The subgroup population excluding endocrinopathy, and a total of 8 women had missing information on total tap-water consumption.

menstrual cycles, with an average cycle length of 29.5 days. The prevalence of long cycles, irregular cycles, hypomenorrhea, and dysmenorrhea were 24.4%, 15.2%, 6.2%, and 23.1%, respectively. Urinary DCAA and TCAA were both detected in >99% of the study participants, with the geometric mean (interquartile range) SG-adjusted urinary DCAA and TCAA concentrations were 4.58 (3.67–5.62) μg/L and 5.07 (3.61–6.89) μg/L, respectively (Table 2).

3.2. Associations between urinary DCAA and TCAA concentrations and menstrual cycle outcomes

The associations of SG-adjusted urinary DCAA and TCAA concentrations with categorical menstrual cycle outcomes among women without endocrinopathy are displayed in Table 3. After adjustment for potential confounders, elevated quartiles of urinary DCAA concentrations were monotonically associated with increased risks of irregular cycles and long cycles (P for trend = 0.05 and 0.06, respectively). Compared women in the lowest quartile of urinary DCAA concentrations, women in the highest quartile had increased risks of irregular cycles by 80% (OR = 1.80; 95% CI: 0.97, 3.33) and long cycles by 62% (OR = 1.62; 95% CI: 0.97, 2.70), respectively. We did not find any statistically significant associations between urinary DCAA or TCAA concentrations and other categorical menstrual cycle outcomes.

The associations of SG-adjusted urinary DCAA and TCAA concentrations with continuous menstrual cycle outcomes among women without endocrinopathy are presented in Table 4. After adjustment for potential confounders, elevated quartiles of urinary DCAA concentrations were monotonically and positively associated with variation of cycle length (P for trend = 0.04). Compared with women in the lowest quartile of urinary DCAA, women in the highest quartiles had an increase of 1.27 days (95% CI: -0.11, 2.66) in variation of cycle length. In addition, elevated quartiles of urinary TCAA concentrations were associated with prolonged bleeding duration in a monotonic dose-response manner (P for trend = 0.07), and there was an increase in bleeding duration of 0.23 days (95% CI: -0.06, 0.51) for the highest vs. lowest quartiles. We did not find any statistically significant associations between urinary DCAA or TCAA concentrations and other continuous menstrual cycle outcomes. When including women with endocrinopathy, the associations of urinary DCAA with increased risks of irregular cycles and long cycles and prolonged variation of cycle length, as well as the associations of urinary TCAA with prolonged bleeding duration persisted (see Tables S2–S3).

4. Discussion

To our knowledge, this is by far the first study to use urinary DCAA and TCAA as potential biomarkers to examine the associations between exposure to drinking water DBPs and menstrual cycle characteristics in humans. We found monotonic dose-response associations of elevated urinary DCAA levels with increased risks of long cycles and irregular cycles, as well as prolonged variation in cycle length. Moreover, there was a monotonic dose-response relationship between elevated urinary TCAA levels and prolonged bleeding duration.

In support of our findings, accumulating experimental evidence has demonstrated that exposure to DBPs can interfere with estrous cycle and disrupt ovarian function. Sprague-Dawley rats gavaged by high doses of dibromoacetic acid (DBAA, 270 and/or 90 mg/kg/day), one type of HAAs, can cause estrous cyclicity disturbance and prolongation including both prolonged diestrus and estrus episodes (Balchak et al., 2000; Goldman and Murr, 2003). Moreover, exposure to DBAA (5 or 50 mg/kg) via drinking water in Dutch-belted rabbits has been observed to reduce the population of primordial follicles and total healthy follicles (Bodensteiner et al., 2004). *In vitro* studies have also reported that exposure to HAAs (chloroacetic acid: 0.25–1.00 mM; bromoacetic acid and iodoacetic acid: 2–15 μM) can inhibit follicle growth and development (Jeong et al., 2016) and disrupt oocyte maturation (Jiao et al., 2021), which may cause menstrual cycle irregularity and prolongation (Baerwald et al., 2012; Zhou et al., 2017; Younis et al., 2020). It is noted that the doses used in experimental studies were much higher than the levels of urinary HAAs detected in this study.

To our knowledge, only one epidemiological study to date has evaluated the potential associations between exposure to drinking water DBPs and menstrual cycle characteristics. Among 403 premenopausal women from the Women's Reproductive Health Study, Windham et al. (2003) found that elevated total trihalomethane (THM) levels in tap water (>60 μg/L) were associated with decreases in follicular phase length and mean cycle length, as well as reductions in the odds of long follicular phases and long cycles, whereas little associations with cycle variability or bleeding duration were estimated. Our results of positive associations between urinary HAAs and menstrual cycle characteristics were inconsistent with this study. The discrepancy between the two studies may be partially attributed to the different methods of exposure assessment. The previous study used the monitoring data of THMs measured in tap water and combined with water-use activities to assign the individual's exposures. While in the current study we measured urinary concentrations of DCAA and TCAA as internal biomarkers, of which TCAA has been demonstrated to be significantly correlated with ingestion of exposure to THMs and TCAA in drinking water (Kim et al., 1999; Costet et al., 2012; Parvez et al., 2019). A previous study found a weak correlation between DCAA and total THMs in tap water served by a large water plant in Wuhan (Xie et al., 2010). Several studies have also observed weak to strong correlations of HAAs with THMs, haloketones, haloacetonitriles, and haloaldehydes in drinking water from China (Wei et al., 2010; Wang et al., 2019a; Yu et al., 2019). Therefore, the observed results in our study may have reflected a mixed effect of ingestion of exposure to DBPs, rather than the effects of HAAs alone. Additionally, the regional variability in DBP concentrations of tap water systems may contribute to the differences between studies. The monitoring concentrations of THMs and HAAs in water systems of Wuhan (average: 36.8 μg/L and 19.5 μg/L, respectively) (Li et al., 2021) were slightly lower than those reported in the United States (mean range: 43.4–56.9 μg/L and 13.7–31.8 μg/L, respectively) (Hinckley et al., 2005). Although there are opposite directions, the two studies suggest that menstrual cycle may be vulnerable to exposure to drinking water DBPs. More studies are warranted to explore the effects of exposure to DBPs on menstrual cycle characteristics.

The etiologic mechanisms underlying the associations between exposure to DBPs and menstrual dysfunction remain unclear. One

Table 2
Distribution of urinary TCAA and DCAA concentrations for the study population (n = 1078).

				Percentiles			
Variables	LOD	Percent > LOD (%)	Geometric mean	Median	5th	25th	75th	95th
Unadjusted (μg/L)								
DCAA	1.00	99.10	4.14	4.36	1.70	3.13	5.71	8.64
TCAA	0.50	99.90	4.58	4.72	1.55	2.99	6.99	13.01
SG-adjusted (μg/L)								
DCAA	–	–	4.58	4.46	2.62	3.67	5.62	8.88
TCAA	–	–	5.07	4.93	2.40	3.61	6.89	11.45

DCAA: dichloroacetic acid; TCAA: trichloroacetic acid; SG: specific gravity; LOD: limit of detection.

Table 3
Adjusted ORs (95% CI) for associations of SG-adjusted urinary DCAA and TCAA concentrations with irregular cycle, long cycle, hypomenorrhea, and dysmenorrhea among women without endocrinopathy.

Exposure	Irregular cycle			Long cycle			Hypomenorrhea			Dysmenorrhe		
	n[a]	n[b]	OR (95%CI)	n[a]	n[b]	OR (95%CI)	n[a]	n[b]	OR (95%CI)	n[a]	n[b]	OR (95%CI)
DCAA (μg/L)												
Q1 (≤3.67)	197	20	Ref	178	31	Ref	205	11	Ref	169	48	Ref
Q2 (3.67–4.46)	194	22	1.12 (0.58, 2.14)	170	39	1.33 (0.79, 2.25)	199	13	1.07 (0.46, 2.49)	172	44	0.91 (0.57, 1.46)
Q3 (4.46–5.62)	187	23	1.28 (0.67, 2.44)	162	43	1.54 (0.92, 2.60)	191	18	1.54 (0.69, 3.44)	161	49	1.08 (0.68, 1.71)
Q4 (>5.62)	199	30	1.80 (0.97, 3.33)	173	47	1.62 (0.97, 2.70)	215	10	0.65 (0.26, 1.60)	168	61	1.30 (0.83, 2.03)
P for trend			0.05			0.06			0.55			0.18
TCAA (μg/L)												
Q1 (≤3.61)	195	20	Ref	167	37	Ref	200	14	Ref	163	52	Ref
Q2 (3.61–4.93)	184	23	1.26 (0.66, 2.38)	171	32	0.84 (0.49, 1.41)	193	11	0.76 (0.33, 1.75)	155	52	1.05 (0.67, 1.64)
Q3 (4.93–6.89)	195	24	1.25 (0.66, 2.37)	169	39	1.07 (0.64, 1.77)	199	17	1.07 (0.51, 2.27)	171	48	0.87 (0.56, 1.37)
Q4 (>6.89)	203	28	1.38 (0.74, 2.55)	176	52	1.36 (0.84, 2.21)	218	10	0.58 (0.25, 1.37)	181	50	0.87 (0.55, 1.36)
P for trend			0.34			0.12			0.38			0.41

DCAA: dichloroacetic acid; TCAA: trichloroacetic acid; SG: specific gravity; OR: odd ratio.
[a] Number of subjects in each quartile with normal menstrual cycle characteristics.
[b] Number of subjects in each quartile with abnormal menstrual cycle characteristics. Models were adjusted for age, BMI, age at menarche, parity, season of recruitment, alcohol use, physical activity, and passive smoking status.

Table 4
Adjusted regression coefficients (βs) (95% CI) for associations of SG-adjusted urinary DCAA and TCAA concentrations with bleeding duration, cycle length, and variation in cycle length (in days) among women without endocrinopathy.

Exposure	Bleeding duration (n = 872)	Cycle length[a] (n = 629)	Variation in cycle length (n = 872)
DCAA (μg/L)			
Q1 (≤3.67)	Ref	Ref	Ref
Q2 (3.67–4.46)	0.00 (−0.30, 0.29)	0.39 (−0.38, 1.17)	0.05 (−1.35, 1.45)
Q3 (4.46–5.62)	0.12 (−0.18, 0.41)	0.45 (−0.33, 1.24)	0.72 (−0.69, 2.13)
Q4 (>5.62)	0.18 (−0.11, 0.47)	0.28 (−0.50, 1.06)	1.27 (−0.11, 2.66)
P for trend	0.16	0.47	0.04
TCAA (μg/L)			
Q1 (≤3.61)	Ref	Ref	Ref
Q2 (3.61–4.93)	0.05 (−0.24, 0.35)	0.42 (−0.35, 1.19)	−0.13 (−1.53, 1.28)
Q3 (4.93–6.89)	0.22 (−0.07, 0.51)	0.15 (−0.61, 0.91)	0.11 (−1.28, 1.50)
Q4 (>6.89)	0.23 (−0.06, 0.51)	−0.02 (−0.80, 0.77)	0.66 (−0.72, 2.04)
P for trend	0.07	0.87	0.31

DCAA: dichloroacetic acid; TCAA: trichloroacetic acid; SG: specific gravity.
[a] Women with self-reported regular menstrual cycles were included. Models were adjusted for age, BMI, age at menarche, parity, season of recruitment, alcohol use, physical activity, and passive smoking status.

potential mechanism is that DBPs disrupt the hypothalamic-hypophyseal-ovarian axis by exhibiting estrogenic and antiestrogenic properties (Kim et al., 2020). Existing evidence from *in vivo* and *in vitro* studies has demonstrated that exposure to DBPs can alter estrogen, progesterone, and luteinizing hormone levels (Balchak et al., 2000; Goldman and Murr, 2002, 2003). The altered hormone levels can lead to abnormal follicle development and estrous cyclicity disturbance (Balchak et al., 2000; Jeong et al., 2016). Another potential involved mechanism is the alteration in the hypothalamic-pituitary-thyroid regulatory axis induced by exposure to DBPs. *In vitro* and *in vivo* assays, Xia et al. (2018) found that exposure to HAAs could disrupt thyroid endocrine system, as manifested by decreased triiodothyronine and increased thyrotropin and thyrotropin-releasing hormone levels. Thyroid dysfunction among women has been found to be associated with abnormal menstrual patterns, as characterized by disrupted ovulation function and irregular menstrual cycles (Poppe and Velkeniers, 2004; Poppe, 2021).

In this study population, the prevalence of irregular cycles and long cycles were 15.2% and 24.4%, respectively. Our results were similar to a previous study conducted in preconception Chinese women (n = 950), in which the corresponding prevalence rates were both 20.1% (Zhou et al., 2017). The mean cycle length (29.5 days) and bleeding duration (5.9 days) in our study were also comparable to a larger study (n = 391, 320) among rural women of reproductive age in Henan Province (mean cycle length: 28.5 days and bleeding duration: 4.8 days) (Zhang et al., 2017). A number of epidemiological studies have shown that long and irregular cycles are associated with increased risks of ovarian cancer, type 2 diabetes mellitus, coronary heart disease, and premature mortality, as well as lower fecundity (Solomon et al., 2001; Solomon et al., 2002; Gast et al., 2010; Mumford et al., 2012; Cirillo et al., 2016; Wang et al., 2020). These findings combined with our results suggest that exposure to drinking water DBPs may have long-term adverse effects on women's health across the lifespan.

The strengths of our study included large sample size, multiple potential confounders, and the use of urinary biomarkers measured. However, our study also has several shortcomings. First, our study was conducted in an infertility clinic with the cross-sectional design. This may limit the generalizability of our findings to other populations without fertility concerns and to establish the causal inference. However, the parameters of menstrual dysfunction such as the prevalence of irregular cycles and long cycles in this study were comparable to the general female population in China. In addition, our findings were robust when including or excluding women with endocrinopathy, suggesting that endocrinopathy did not substantially affect the magnitude of associations between exposure to DBPs and menstrual cycle characteristics in this study population. Second, the menstrual cycle characteristics were self-reported and the information was obtained retrospectively, which may lead to misclassification for those categorized outcomes such as menstrual bleeding amount. However, several studies have reported that self-reported retrospective menstrual cycle

length was highly related to prospectively measured cycle length (Steiner et al., 2001; Creinin et al., 2004). In addition, the collected information on menstrual cycle characteristics in our study may be accurate since the participants who tried to conceive and might be more concerned about their menstrual cycles (Zhou et al., 2017). Finally, although the urinary biomarkers measured in this study can enhance the exposure assessment, a single spot urine measure still can result in misclassification of exposure. High intra- and inter-individual variability in urinary concentrations of DCAA and TCAA have been reported in previous studies (Smith et al., 2013; Wang et al., 2014) due to their short half-lives in the body. Moreover, it remains unclear whether urinary DCAA and TCAA can reflect other DBP exposures such as brominated and iodinated HAAs and other exposure pathways such as inhalation and dermal absorption of THMs. Future studies with prospective design among the general female population and multiple exposure measurements of DBP biomarkers are needed to confirm our findings.

5. Conclusion

Among women seeking infertility treatments, we observed evidence of associations of urinary DCAA with increased risks of long cycles and irregular cycles and prolonged variation in cycle length, as well as urinary TCAA with prolonged bleeding duration. Our findings suggest that menstrual function may be affected by exposure to drinking water DBPs. Given that human beings are ubiquitously exposed to drinking water DBPs and menstrual function plays an important role in female fecundity, our results may have important clinical and public health relevance that contributes to the knowledge of exposure to environmental chemicals associated with menstrual dysfunction.

Declaration of competing interest

The authors declare they have no actual or potential competing financial interests.

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Appendix A. Supplementary data

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References

Baerwald, A.R., Adams, G.P., Pierson, R.A., 2012. Ovarian antral folliculogenesis during the human menstrual cycle: a review. Hum. Reprod. Update 18, 73–91.
Balchak, S.K., Hedge, J.M., Murr, A.S., Mole, M.L., Goldman, J.M., 2000. Influence of the drinking water disinfection by-product dibromoacetic acid on rat estrous cyclicity and ovarian follicular steroid release in vitro. Reprod. Toxicol. 14, 533–539.
Bodensteiner, K.J., Sawyer, H.R., Moeller, C.L., Kane, C.M., Pau, K.Y., Klinefelter, G.R., Veeramachaneni, D.N., 2004. Chronic exposure to dibromoacetic acid, a water disinfection byproduct, diminishes primordial follicle populations in the rabbit. Toxicol. Sci. 80, 83–91.
Buck, L.G., Rios, L.I., McLain, A., Cooney, M.A., Kostyniak, P.J., Sundaram, R., 2011. Persistent organochlorine pollutants and menstrual cycle characteristics. Chemosphere 85, 1742–1748.
Cirillo, P.M., Wang, E.T., Cedars, M.I., Chen, L.M., Cohn, B.A., 2016. Irregular menses predicts ovarian cancer: prospective evidence from the child health and development studies. Int. J. Cancer 139, 1009–1017.
Costet, N., Garlantezec, R., Monfort, C., Rouget, F., Gagniere, B., Chevrier, C., Cordier, S., 2012. Environmental and urinary markers of prenatal exposure to drinking water disinfection by-products, fetal growth, and duration of gestation in the PELAGIE birth cohort (Brittany, France, 2002-2006). Am. J. Epidemiol. 175, 263–275.
Creinin, M.D., Keverline, S., Meyn, L.A., 2004. How regular is regular? An analysis of menstrual cycle regularity. Contraception 70, 289–292.
Deng, Y.L., Yang, P., Cao, W.C., Wang, Y.X., Liu, C., Chen, Y.J., Huang, L.L., Lu, W.Q., Wang, L.Q., Zeng, Q., 2019. Urinary biomarker of late pregnancy exposure to drinking water disinfection by-products and ultrasound measures of fetal growth in Wuhan, China. Environ. Res. 170, 128–133.
Diaz, A., Laufer, M.R., Breech, L.L., 2006. Menstruation in girls and adolescents: using the menstrual cycle as a vital sign. Pediatrics 118, 2245–2250.
Ding, N., Harlow, S.D., Randolph, J.J., Loch-Caruso, R., Park, S.K., 2020. Perfluoroalkyl and polyfluoroalkyl substances (PFAS) and their effects on the ovary. Hum. Reprod. Update 26, 724–752.
Gast, G.C., Grobbee, D.E., Smit, H.A., Bueno-de-Mesquita, H.B., Samsioe, G.N., van der Schouw, Y.T., 2010. Menstrual cycle characteristics and risk of coronary heart disease and type 2 diabetes. Fertil. Steril. 94, 2379–2381.
Goldman, J.M., Murr, A.S., 2002. Alterations in ovarian follicular progesterone secretion by elevated exposures to the drinking water disinfection by-product dibromoacetic acid: examination of the potential site(s) of impact along the steroidogenic pathway. Toxicology 171, 83–93.
Goldman, J.M., Murr, A.S., 2003. Dibromoacetic acid-induced elevations in circulating estradiol: effects in both cycling and ovariectomized/steroid-primed female rats. Reprod. Toxicol. 17, 585–592.
Goldman, J.M., Murr, A.S., Buckalew, A.R., Ferrell, J.M., Cooper, R.L., 2007. Moderating influence of the drinking water disinfection by-product dibromoacetic acid on a dithiocarbamate-induced suppression of the luteinizing hormone surge in female rats. Reprod. Toxicol. 23, 541–549.
Hammer, K.C., Veiga, A., Mahalingaiah, S., 2020. Environmental toxicant exposure and menstrual cycle length. Curr. Opin. Endocrinol. Diabetes Obes. 27, 373–379.
Han, J., Zhang, X., Li, W., Jiang, J., 2021. Low chlorine impurity might be beneficial in chlorine dioxide disinfection. Water Res. 188, 116520.
Harlow, S.D., Ephross, S.A., 1995. Epidemiology of menstruation and its relevance to women's health. Epidemiol. Rev. 17, 265–286.
Hinckley, A.F., Bachand, A.M., Reif, J.S., 2005. Late pregnancy exposures to disinfection by-products and growth-related birth outcomes. Environ. Health Perspect. 113, 1808–1813.
Hu, J., Zhao, H., Braun, J.M., Zheng, T., Zhang, B., Xia, W., Zhang, W., Li, J., Zhou, Y., Li, H., Li, J., Zhou, A., Zhang, Y., Buka, S.L., Liu, S., Peng, Y., Wu, C., Jiang, M., Huo, W., Zhu, Y., Shi, K., Li, Y., Cai, Z., Xu, S., 2019. Associations of trimester-specific exposure to bisphenols with size at birth: a Chinese prenatal cohort study. Environ. Health Perspect. 127, 107001.
Ileka-Priouzeau, S., Campagna, C., Legay, C., Deonandan, R., Rodriguez, M.J., Levallois, P., 2015. Women exposure during pregnancy to haloacetaldehydes and haloacetonitriles in drinking water and risk of small-for-gestational-age neonate. Environ. Res. 137, 338–348.
Jensen, T.K., Scheike, T., Keiding, N., Schaumburg, I., Grandjean, P., 1999. Fecundability in relation to body mass and menstrual cycle patterns. Epidemiology 10, 422–428.
Jeong, C.H., Gao, L., Dettro, T., Wagner, E.D., Ricke, W.A., Plewa, M.J., Flaws, J.A., 2016. Monohaloacetic acid drinking water disinfection by-products inhibit follicle growth and steroidogenesis in mouse ovarian antral follicles in vitro. Reprod. Toxicol. 62, 71–76.
Jiao, X., Gonsioroski, A., Flaws, J.A., Qiao, H., 2021. Iodoacetic acid disrupts mouse oocyte maturation by inducing oxidative stress and spindle abnormalities. Environ. Pollut. 268, 115601.
Kambakamba, P., Linecker, M., Alvarez, F.A., Samaras, P., Reiner, C.S., Raptis, D.A., Kron, P., de Santibanes, E., Petrowsky, H., Clavien, P.A., Lesurtel, M., 2016. Short chemotherapy-free interval improves oncological outcome in patients undergoing two-stage hepatectomy for Colorectal Liver metastases. Ann. Surg Oncol. 23, 3915–3923.
Kim, D.H., Park, C.G., Kim, Y.J., 2020. Characterizing the potential estrogenic and androgenic activities of two disinfection byproducts, mono-haloacetic acids and haloacetamides, using in vitro bioassays. Chemosphere 242, 125198.
Kim, H., Haltmeier, P., Klotz, J.B., Weisel, C.P., 1999. Evaluation of biomarkers of environmental exposures: urinary haloacetic acids associated with ingestion of chlorinated drinking water. Environ. Res. 80, 187–195.
Kogevinas, M., Bustamante, M., Gracia-Lavedan, E., Ballester, F., Cordier, S., Costet, N., Espinosa, A., Grazuleviciene, R., Danileviciute, A., Ibarluzea, J., Karadanelli, M., Krasner, S., Patelarou, E., Stephanou, E., Tardon, A., Toledano, M.B., Wright, J., Villanueva, C.M., Nieuwenhuijsen, M., 2016. Drinking water disinfection by-products, genetic polymorphisms, and birth outcomes in a European mother-child cohort Study. Epidemiology 27, 903–911.
Kolstad, H.A., Bonde, J.P., Hjollund, N.H., Jensen, T.K., Henriksen, T.B., Ernst, E., Giwercman, A., Skakkebaek, N.E., Olsen, J., 1999. Menstrual cycle pattern and fertility: a prospective follow-up study of pregnancy and early embryonal loss in 295 couples who were planning their first pregnancy. Fertil. Steril. 71, 490–496.
Levallois, P., Giguère, Y., Nguile-Makao, M., Rodriguez, M., Campagna, C., Tardif, R., Bureau, A., 2016. Disinfection by-products exposure and intra-uterine growth restriction: do genetic polymorphisms of CYP2E1or deletion of GSTM1 or GSTT1 modify the association? Environ. Int. 92–93, 220–231.
Li, Z., Song, G., Bi, Y., Gao, W., He, A., Lu, Y., Wang, Y., Jiang, G., 2021. Occurrence and distribution of disinfection byproducts in domestic wastewater effluent, tap water, and surface water during the SARS-CoV-2 pandemic in China. Environ. Sci. Technol. 55, 4103–4114.
Luben, T.J., Olshan, A.F., Herring, A.H., Jeffay, S., Strader, L., Buus, R.M., Chan, R.L., Savitz, D.A., Singer, P.C., Weinberg, H.S., Perreault, S.D., 2007. The healthy men study: an evaluation of exposure to disinfection by-products in tap water and sperm quality. Environ. Health Perspect. 115, 1169–1176.

Lyngso, J., Ramlau-Hansen, C.H., Hoyer, B.B., Stovring, H., Bonde, J.P., Jonsson, B.A., Lindh, C.H., Pedersen, H.S., Ludwicki, J.K., Zviezdai, V., Toft, G., 2014. Menstrual cycle characteristics in fertile women from Greenland, Poland and Ukraine exposed to perfluorinated chemicals: a cross-sectional study. Hum. Reprod. 29, 359–367.
MacLehose, R.F., Savitz, D.A., Herring, A.H., Hartmann, K.E., Singer, P.C., Weinberg, H. S., 2008. Drinking water disinfection by-products and time to pregnancy. Epidemiology 19, 451–458.
Maldonado, G., Greenland, S., 1993. Simulation study of confounder-selection strategies. Am. J. Epidemiol. 138, 923–936.
Mashau, F., Ncube, E.J., Voyi, K., 2018. Drinking water disinfection by-products exposure and health effects on pregnancy outcomes: a systematic review. J. Water Health 16, 181–196.
Mashau, F., Ncube, E.J., Voyi, K., 2019. Maternal urinary levels of trichloroacetic acid and association with adverse pregnancy outcomes. J. Water Health 17, 884–895.
McLain, A.C., Lum, K.J., Sundaram, R., 2012. A joint mixed effects dispersion model for menstrual cycle length and time-to-pregnancy. Biometrics 68, 648–656.
Mena, G.P., Mielke, G.I., Brown, W.J., 2019. The effect of physical activity on reproductive health outcomes in young women: a systematic review and meta-analysis. Hum. Reprod. Update 25, 541–563.
Mumford, S.L., Steiner, A.Z., Pollack, A.Z., Perkins, N.J., Filiberto, A.C., Albert, P.S., Mattison, D.R., Wactawski-Wende, J., Schisterman, E.F., 2012. The utility of menstrual cycle length as an indicator of cumulative hormonal exposure. J. Clin. Endocrinol. Metab. 97, E1871–E1879.
Nam, G.E., Han, K., Lee, G., 2017. Association between sleep duration and menstrual cycle irregularity in Korean female adolescents. Sleep Med. 35, 62–66.
Newton, T.L., Philhower, C.L., 2003. Socioemotional correlates of self-reported menstrual cycle irregularity in premenopausal women. Psychosom. Med. 65, 1065–1069.
Nieuwenhuijsen, M.J., Martinez, D., Grellier, J., Bennett, J., Best, N., Iszatt, N., Vrijheid, M., Toledano, M.B., 2009. Chlorination disinfection by-products in drinking water and congenital anomalies: review and meta-analyses. Environ. Health Perspect. 117, 1486–1493.
Nieuwenhuijsen, M.J., Toledano, M.B., Elliott, P., 2000. Uptake of chlorination disinfection by-products; a review and a discussion of its implications for exposure assessment in epidemiological studies. J. Expo. Anal. Environ. Epidemiol. 10, 586–599.
Parvez, S., Ashby, J.L., Kimura, S.Y., Richardson, S.D., 2019. Exposure characterization of haloacetic acids in humans for exposure and risk assessment applications: an exploratory study. Int. J. Environ. Res. Publ. Health 16.
Poppe, K., 2021. Management of endocrine disease: thyroid and female infertility: more questions than answers? Eur. J. Endocrinol. 184, R123–R135.
Poppe, K., Velkeniers, B., 2004. Female infertility and the thyroid. Best Pract. Res. Clin. Endocrinol. Metabol. 18, 153–165.
Rivera-Nunez, Z., Wright, J.M., 2013. Association of brominated trihalomethane and haloacetic acid exposure with fetal growth and preterm delivery in Massachusetts. J. Occup. Environ. Med. 55, 1125–1134.
Rowland, A.S., Baird, D.D., Long, S., Wegienka, G., Harlow, S.D., Alavanja, M., Sandler, D.P., 2002. Influence of medical conditions and lifestyle factors on the menstrual cycle. Epidemiology 13, 668–674.
Sakai, H., Ohashi, K., 2021. Effects of past environmental tobacco smoke exposure on the menstrual cycle and menstrual phase-related symptoms: a cross-sectional study. J. Obstet. Gynaecol. Res. 47, 243–253.
Small, C.M., Manatunga, A.K., Marcus, M., 2007. Validity of self-reported menstrual cycle length. Ann. Epidemiol. 17, 163–170.
Smith, R.B., Edwards, S.C., Best, N., Wright, J., Nieuwenhuijsen, M.J., Toledano, M.B., 2016. Birth weight, ethnicity, and exposure to trihalomethanes and haloacetic acids in drinking water during pregnancy in the born in Bradford cohort. Environ. Health Perspect. 124, 681–689.
Smith, R.B., Nieuwenhuijsen, M.J., Wright, J., Raynor, P., Cocker, J., Jones, K., Kappaostopoulou-Karadanelli, M., Toledano, M.B., 2013. Validation of trichloroacetic acid exposure via drinking water during pregnancy using a urinary TCAA biomarker. Environ. Res. 126, 145–151.
Solomon, C.G., Hu, F.B., Dunaif, A., Rich-Edwards, J., Willett, W.C., Hunter, D.J., Colditz, G.A., Speizer, F.E., Manson, J.E., 2001. Long or highly irregular menstrual cycles as a marker for risk of type 2 diabetes mellitus. JAMA 286, 2421–2426.
Solomon, C.G., Hu, F.B., Dunaif, A., Rich-Edwards, J.E., Stampfer, M.J., Willett, W.C., Speizer, F.E., Manson, J.E., 2002. Menstrual cycle irregularity and risk for future cardiovascular disease. J. Clin. Endocrinol. Metab. 87, 2013–2017.
Steiner, M.J., Hertz-Picciotto, I., Taylor, D., Schoenbach, V., Wheeless, A., 2001. Retrospective vs. prospective coital frequency and menstrual cycle length in a contraceptive effectiveness trial. Ann. Epidemiol. 11, 428–433.
Sun, Y., Wang, Y., Liu, C., Chen, Y., Lu, W., Messerlian, C., 2020. Trimester-specific blood trihalomethane and urinary haloacetic acid concentrations and adverse birth outcomes: identifying windows of vulnerability during pregnancy. Environ. Health Perspect. 128, 107001.
Wang, Y.X., Arvizu, M., Rich-Edwards, J.W., Stuart, J.J., Manson, J.E., Missmer, S.A., Pan, A., Chavarro, J.E., 2020. Menstrual cycle regularity and length across the reproductive lifespan and risk of premature mortality: prospective cohort study. BMJ 371, m3464.
Wang, L., Chen, Y., Chen, S., Long, L., Bu, Y., Xu, H., Chen, B., Krasner, S., 2019a. A one-year long survey of temporal disinfection byproducts variations in a consumer's tap and their removals by a point-of-use facility. Water Res. 159, 203–213.
Wang, Y.X., Liu, C., Chen, Y.J., Duan, P., Wang, Q., Chen, C., Sun, Y., Huang, L.L., Wang, L., Chen, C., Li, J., Ai, S.H., Huang, Z., Sun, L., Wan, Z.Z., Pan, A., Meng, T.Q., Lu, W.Q., 2019b. Profiles, variability and predictors of concentrations of blood trihalomethanes and urinary haloacetic acids along pregnancy among 1760 Chinese women. Environ. Res. 172, 665–674.
Wang, Y.X., Zeng, Q., Wang, L., Huang, Y.H., Lu, Z.W., Wang, P., He, M.J., Huang, X., Lu, W.Q., 2014. Temporal variability in urinary levels of drinking water disinfection byproducts dichloroacetic acid and trichloroacetic acid among men. Environ. Res. 135, 126–132.
Wei, J., Ye, B., Wang, W., Yang, L., Tao, J., Hang, Z., 2010. Spatial and temporal evaluations of disinfection by-products in drinking water distribution systems in Beijing, China. Sci. Total Environ. 408, 4600–4606.
Weisel, C.P., Kim, H., Haltmeier, P., Klotz, J.B., 1999. Exposure estimates to disinfection by-products of chlorinated drinking water. Environ. Health Perspect. 107, 103–110.
Windham, G.C., Waller, K., Anderson, M., Fenster, L., Mendola, P., Swan, S., 2003. Chlorination by-products in drinking water and menstrual cycle function. Environ. Health Perspect. 111, 935–941. A409.
Xia, Y., Mo, Y., Yang, Q., Yu, Y., Jiang, M., Wei, S., Lu, D., Wu, H., Lu, G., Zou, Y., Zhang, Z., Wei, X., 2018. Iodoacetic acid disrupting the thyroid endocrine system in vitro and in vivo. Environ. Sci. Technol. 52, 7545–7552.
Xie, S.H., Li, Y.F., Tan, Y.F., Zheng, D., Liu, A.L., Xie, H., Lu, W.Q., 2011. Urinary trichloroacetic acid levels and semen quality: a hospital-based cross-sectional study in Wuhan, China. Environ. Res. 111, 295–300.
Xie, S.H., Tan, Y.F., Zhou, W.S., Liu, A.L., Lu, W.Q., 2010. Occurrence of disinfection by-products in drinking water served by a large water plant in Wuhan. J. Environ. Health 27, 5–7.
Yang, P., Cao, W., Zhou, B., Zheng, T., Deng, Y., Luo, Q., Miao, Y., Chen, D., Zeng, Q., Lu, W., 2019. Urinary biomarker of prenatal exposure to disinfection byproducts, maternal genetic polymorphisms in CYP2E1 and GSTZ1, and birth outcomes. Environ. Sci. Technol. 53, 12026–12034.
Younis, J.S., Iskander, R., Fauser, B., Izhaki, I., 2020. Does an association exist between menstrual cycle length within the normal range and ovarian reserve biomarkers during the reproductive years? A systematic review and meta-analysis. Hum. Reprod. Update 26, 904–928.
Yu, M., Han, K., Nam, G.E., 2017. The association between mental health problems and menstrual cycle irregularity among adolescent Korean girls. J. Affect. Disord. 210, 43–48.
Yu, Y., Ma, X., Chen, R., Li, G., Tao, H., Shi, B., 2019. The occurrence and transformation behaviors of disinfection byproducts in drinking water distribution systems in rural areas of eastern China. Chemosphere 228, 101–109.
Zeng, Q., Wang, Y.X., Xie, S.H., Xu, L., Chen, Y.Z., Li, M., Yue, J., Li, Y.F., Liu, A.L., Lu, W. Q., 2014. Drinking-water disinfection by-products and semen quality: a cross-sectional study in China. Environ. Health Perspect. 122, 741–746.
Zhang, Q., Wang, Y.Y., Zhang, Y., Zhang, H.G., Yang, Y., He, Y., Xu, J.H., Zhao, J., Peng, Z.Q., Ma, X., 2017. The influence of age at menarche, menstrual cycle length and bleeding duration on time to pregnancy: a large prospective cohort study among rural Chinese women. BJOG 124, 1654–1662.
Zhang, S.H., Guo, A.J., Wei, N., Zhang, R., Niu, Y.J., 2021. Associations of urinary dichloroacetic acid and trichloroacetic acid exposure with platelet indices: exploring the mediating role of blood pressure in the general population. J. Hazard Mater. 402, 123452.
Zhang, W., Gabos, S., Schopflocher, D., Li, X.F., Gati, W.P., Hrudey, S.E., 2009. Reliability of using urinary and blood trichloroacetic acid as a biomarker of exposure to chlorinated drinking water disinfection byproducts. Biomarkers 14, 355–365.
Zhang, Y., Ji, L., Hu, Y., Tse, L.A., Wang, Y., Qin, K., Ding, G., Zhou, Y., Yu, X., Ouyang, F., Zhang, J., Gao, Y., Tian, Y., 2020. Exposure to organophosphate pesticides and menstrual cycle characteristics in Chinese preconceptional women. Am. J. Epidemiol. 189, 375–383.
Zhou, W., Zhang, L., Tong, C., Fang, F., Zhao, S., Tian, Y., Tao, Y., Zhang, J., 2017. Plasma perfluoroalkyl and polyfluoroalkyl substances concentration and menstrual cycle characteristics in preconception women. Environ. Health Perspect. 125, 67012.
Zhou, W.S., Xu, L., Xie, S.H., Li, Y.L., Li, L., Zeng, Q., Du, Y.K., Lu, W.Q., 2012. Decreased birth weight in relation to maternal urinary trichloroacetic acid levels. Sci. Total Environ. 416, 105–110.

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A systematic review on solid fuel combustion exposure and respiratory health in adults in Europe, USA, Canada, Australia and New Zealand

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ABSTRACT

Epidemiological studies performed in low- and middle-income countries have shown a positive association between solid fuel burning exposure and adverse health effects, including respiratory effects in adults. However, the evidence is less clear in other countries. We performed a systematic review of epidemiological studies conducted in Europe, North America (Canada and USA only), Australia and New Zealand on the association between outdoor and indoor exposure to solid fuel (biomass and coal) combustion and respiratory outcomes in adults.

We identified 34 articles. The epidemiological evidence is still limited. Positive associations were found between indoor coal, wood and combined solid fuel combustion exposure and lung cancer risk, although based on a limited number of studies. A significant association was found between indoor solid fuel exposure and COPD risk. Inconsistent results were found considering indoor coal, wood and mixed solid fuel burning exposure and other respiratory outcomes (i.e. lower respiratory infections, upper respiratory infections and other upper respiratory tract diseases, asthma and respiratory symptoms).

Inconsistent results were found considering the relationship between the exposure to outdoor wood burning exposure and overall respiratory mortality, asthma, COPD and respiratory symptoms in adults. The available epidemiological evidence between outdoor exposure to residential coal burning and respiratory outcomes suggests an increased risk of adverse respiratory effects. The studies considering the impact of the introduction of measures in order to reduce solid fuel burning on air quality and health showed an improvement in air quality resulting in a reduction of adverse respiratory effects.

The identified epidemiological studies have several limitations. Additional and better conducted epidemiological studies are needed to establish whether exposure occurring indoors and outdoors to solid fuel combustion pollutants is associated with adverse respiratory outcomes in adults.

1. Introduction

The combustion of solid fuels (i.e. wood, coal, charcoal, crop waste, dung) represents one of the major sources of household air pollution responsible for death and disability in the world, particularly among the poorest and marginalized populations, including women and children (Collaborators, 2018). Several air pollutants are emitted by solid fuel burning, including particulate matter (PM) of varying sizes, carbon monoxide (CO), volatile and semi-volatile organic compounds, and several others (Naeher et al., 2007). In addition, combustion of coal releases sulphur oxides, heavy metals such as arsenic, and fluorine. The emissions of air pollutants generated by solid fuel burning depend on several factors, including the type of solid fuel used, household ventilation, the device used and the presence of a chimney. In low- and middle-income countries (LMICs), burning solid fuels in poor housing conditions results in very high indoor air pollutant levels. The epidemiological evidence deriving from studies conducted in LMICs show that exposure to household air pollution is associated with a wide variety **of child and adult diseases,** including respiratory conditions such as acute lower respiratory infections (LRIs) (e.g. pneumonia), chronic obstructive pulmonary disease (COPD), lung cancer, stroke and cardiovascular diseases (Fatmi and Coggon, 2016; Hystad et al., 2019;

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Kurmi et al., 2010, 2012a,b; Po et al., 2011; Nigel). In adults, most of the studies conducted in LMICs show a significant association between indoor solid fuel burning exposure and lung cancer (Kurmi et al., 2012b), COPD and chronic bronchitis risk (Kurmi et al., 2010; Po et al., 2011). In 2010, IARC classified biomass use (primarily wood) as a group 2A carcinogen due to limited epidemiological evidence (IARC Working Group, 2010) and concluded that indoor emissions from household combustion of coal are carcinogenic to humans (group 1) mainly based on studies conducted in China.

The use of solid fuels for heating has been increasing in high-income countries (HICs). This has resulted in a growing interest in evaluating the effects on human health. The combustion of solid fuels in HICs produces lower levels of indoor air pollutants thanks to the use of more sophisticated solid fuel energy technologies and better exhaust extraction, not necessarily available in LMICs (Guercio et al., 2021). Therefore, the studies deriving from LMICs are not directly comparable to those conducted in HICs.

We previously reported that indoor wood burning exposure was not associated with an increased risk of asthma (RR 0.90, 95% CI 0.77–1.05), wheeze (RR 0.96, 95% CI 0.85–1.09), and cough (RR 1.02, 95% CI 0.92–1.15), in studies conducted among children in Europe, Canada, USA, Australia and New Zealand (Guercio et al., 2021). A slight non-significant increased risk was found for LRIs and upper respiratory infections (URIs). Results from epidemiological studies that evaluated the relationship between the exposure to outdoor emissions derived from indoor combustion of solid fuels were too limited to allow firm conclusions.

The aim of the present work was to perform a systematic review of the epidemiological studies conducted in Europe, North America, New Zealand and Australia on the relationship between indoor exposure to solid fuel (biomass and coal) burning and respiratory health in adults. In order to reduce the likelihood of heterogeneity we considered as Europe those European countries with fairly similar cultural and lifestyle habits. Likewise, we considered as North America only the USA and Canada. Furthermore, we systematically reviewed the epidemiological evidence on outdoor air pollution generated by residential indoor solid fuel combustion and its association with respiratory heath in adults. This is relevant because residential combustion of solid fuels contributes to outdoor air pollution too, particularly during the heating season and in areas where this is the main source of heating used by households.

2. Materials and methods

Our systematic literature review followed the Preferred Reporting and Items for Systematic Review and Meta-Analysis (PRISMA) guidelines for reporting (Liberati et al., 2009).

2.1. Search methods

The literature search was performed in November 2020 using the Embase and PubMed databases. The search string (Supplementary Table S1) combined terms for exposure to solid fuel combustion (e.g. wood burning, fireplace, heating stove, etc.), respiratory effects (e.g. asthma, respiratory diseases, respiratory infections, etc.) and for the target population (i.e. adults ≥19 years old), restricted by language (only studies published in English), species (not humans excluded).

2.2. Eligibility criteria

The study eligibility criteria were formulated, in accordance with the Population Exposure Comparison Outcome Study Design (PECOS) approach, as follows: (1) participants: adults (≥19 years old); (2) exposure: indoor and outdoor air pollution produced by residential solid fuel combustion including biomass (mainly wood) and coal. In particular, we defined an exposure to indoor solid fuels when household use of solid fuels for heating and/or cooking was reported. The exposure to outdoor solid fuels refers to studies conducted in subjects living in areas where residential solid fuel combustion was very common and it represented one of the major contributors to outdoor air pollution; in these studies the exposure assessment was carried out by means of measurement of outdoor air quality (either air pollutant levels or solid fuel marker levels, such as levoglucosan for wood, were measured);(3) comparator: unexposed subjects or subjects exposed to lower levels, or to cleaner fuels (e.g. electricity); (4) outcome: respiratory diseases including asthma, LRI, URI, lung cancer as well as respiratory symptoms (i.e. cough and wheeze) and respiratory parameter changes; (5) study design: cohort, case-control, cross-sectional, time-series, ecological study. Only studies conducted in North America (USA and Canada), Europe, Australia and New Zealand were included. We excluded studies conducted in subjects with underlying health conditions (n = 9). Further, studies were excluded if they included all ages participants (n = 8). Only studies considering all ages participants were included if the outcomes considered were respiratory mortality and COPD (Adeloye et al., 2015) because of their occurrence in adult population mostly (n = 4).

We excluded studies in which: it was not clear whether the source of air pollution was residential combustion of biomass or coal; the exposure to solid fuels was considered together with exposure to other fuels (e.g. gas, oil); the relationship between solid fuel exposure and allergen levels in the house was considered; skin allergy or unspecified allergy or when skin and respiratory allergic symptoms where considered together; or the air pollution was caused by wildfires. No studies were excluded *a priori* for weakness of design or data quality.

2.3. Selection process

After removing duplicates, two authors (VG and AD) independently assessed the titles and abstracts of all retrieved articles for the above eligibility criteria. If the title and abstract were not sufficiently informative, the full text was screened to verify whether it met the inclusion criteria. Subsequently, these two authors searched the reference lists of all included papers and previous reviews on the topic to identify further studies.

2.4. Data extraction

A standardized data extraction form in an Excel spreadsheet was designed to record all potentially relevant information from the selected papers. The template was discussed and piloted by the two reviewers using three selected papers. Data extraction was undertaken independently by two authors (VG and AD), who reported the following information: last name of the first author, year of publication, the name of the study, country where the study was conducted, study design, enrolment period and the date of follow up, sample size, sex and age, type of exposure (indoor or outdoor exposure, fuel and device used and whether for heating or cooking purposes) and exposure assessment, outcome and outcome assessment, exposure categories, subgroup analyses, risk estimate, and adjustment for confounding variables.

2.5. Risk of bias appraisal

Two researchers (VG and AD) independently assessed the methodological aspects of each study using a new tool of risk of bias (RoB) based on non-randomized studies modelled on RoB In Non-randomized Studies of Interventions (ROBINS-I) instrument adapted to environmental exposures (Morgan et al., 2019). This tool consists of seven domains: confounding, selection bias, exposure assessment, departures from exposure, missing data, measurement of outcomes and selective reported results. Each item can be judged as having low, moderate, serious or high RoB l (Morgan et al., 2019). For the item of potential confounders that were accounted for in the analysis, four critical potential confounders (temperature, seasonality, day-of-the-week, long-term

trends) were considered for time-series and case-cross over studies. If all these confounders were included, the item was classified as having low RoB; for the other study designs sex, age, education, and smoking were considered to be the most important potential confounders; low RoB was given to the studies adjusting for all confounders.

3. Results

3.1. Study selection

The literature search yielded 21562 publications: 10774 obtained from PubMed and 10778 from Embase (Fig. 1). After removing 7594 duplicate publications, we screened the abstracts and titles of 13968 records, selecting 204 articles for full-text screening; 5 additional publications were identified by scanning the reference lists of the retrieved articles. Out of these 204 studies, 170 were excluded because they did not meet our inclusion criteria for at least one of the following reasons: the publications were not epidemiological studies, they were conducted in LMICs, the exposure or outcome assessed was not relevant for this study, if they reported the association between indoor pollutant levels and respiratory outcomes without information on the source. Therefore, 34 publications on residential solid fuel combustion were selected for data extraction: 18 of them assessed exposure to indoor pollution (2 of them were included in a pooled analysis) (Table 1), 16 assessed exposure to outdoor pollution (Table 2).

3.2. Exposure to indoor solid fuel burning and respiratory outcomes in adults

3.2.1. Characteristics of the epidemiological studies

Six studies assessing the impact of indoor solid fuel exposure were conducted in Europe (Spain (Orozco-Levi et al., 2006), Finland (Kilpeläinen et al., 2001), Poland (Piekarska et al., 2018), Serbia (Stanković et al., 2011), Germany (Nowak et al., 1996), Sweden (Thorn et al., 2001) 1 in each). Of the remaining studies, 4 were conducted in USA (Nguyen et al., 2010; Sood et al., 2010; Triche et al., 2005; Wu et al., 1985) and 4 in Canada (Kim and Hanley, 2002; Loeb et al., 2009; Pahwa et al., 2017; Ramanakumar et al., 2007). Two studies were multicentric: 1 conducted in Europe and North America (Hosgood et al., 2010) and the other one mainly in Europe (Malats et al., 2000). Two additional studies, included in the multicentric study conducted in Europe and North America, were performed in the USA (Sloan et al., 2012) and Europe (Lissowska et al., 2005). The study designs were as follows: case-control (9 studies) (Orozco-Levi et al., 2006; Wu et al., 1985; Kim and Hanley, 2002; Loeb et al., 2009; Ramanakumar et al., 2007; Hosgood et al., 2010; Malats et al., 2000; Sloan et al., 2012; Lissowska et al., 2005), cross-sectional (6) (Kilpeläinen et al., 2001; Piekarska et al., 2018; Stanković et al., 2011; Nowak et al., 1996; Sood et al., 2010; Pahwa et al., 2017), nested case-control (2) (Thorn et al., 2001; Nguyen et al., 2010), cohort (1) (Triche et al., 2005).

The characteristics of the indoor exposure were as follows: 10 studies

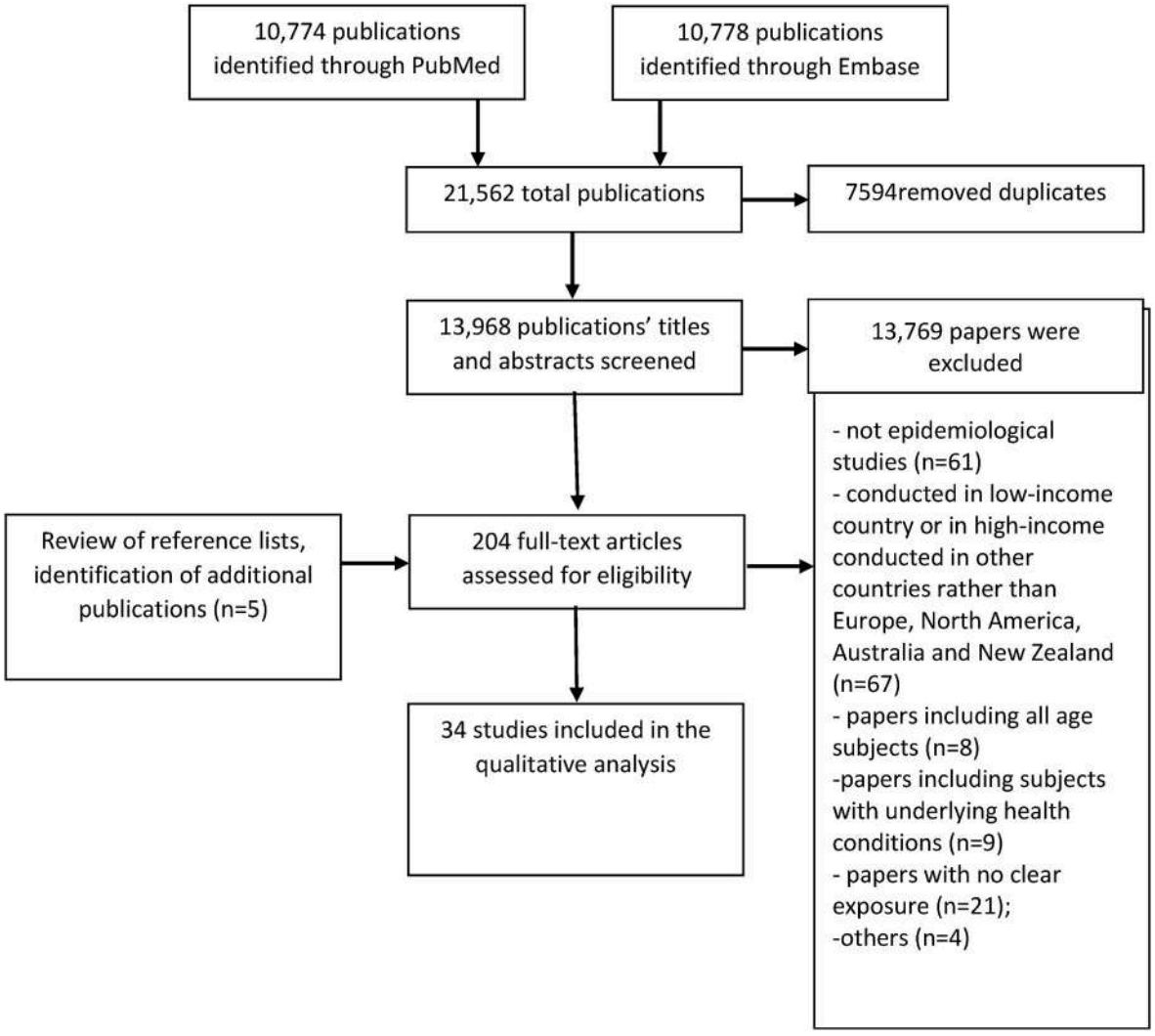

Fig. 1. Flow chart.

Table 1
Characteristics of the epidemiological studies on exposure to indoor solid fuels and respiratory outcomes in adults included in the systematic review.

First Author; Publication year; Study Name; Country	Enrolment period/FU	N. CA/n. CO/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
Case-control studies								
Wu et al., 1985 USA	PB; 1981–1982	149 CA with ADC and 71 with SCC of the lung/220 CO; W; <76 years (mean age at diagnosis of ADC CA 59.7 and 59.5 years for CO)	Interviews on the telephone with the use of a structured questionnaire.	Patients with no history of cancer (other than non-melanoma skin cancer) diagnosed with primary ADC or SCC of the lung were identified by the University of Southern California/ Los Angeles County Cancer Surveillance Program, the population-based tumour registry for Los Angeles County. Controls recruited with the same criteria as cases.	RR (95% CI)			Matched neighbourhood control for each case and with the date of birth (±5 years of birth date). The control had to fulfil all the criteria for cases matched
			Heating or cooking with coal burned in a stove or fireplace during childhood and teenage years (before age of 16 years)	SCC	1.9 (0.5, 6.5)	Exposed vs not exposed		
				ADC	2.3 (1.0, 5.5)	Exposed vs not exposed		
				ADC (143 case-control pairs).	3.2 (0.9, 11.S)	Exposed vs not exposed	Non-smokers	
					1.5 (0.6, 3.5)	Not Exposed ex-smokers vs not exposed non smokers	Ex-smokers	
					4.3 (1.0, 17.8)	Exposed ex-smokers vs not exposed non smokers		
					6.3 (3.0, 13.3)	Not Exposed current smokers vs not exposed non smokers	Current smokers	
					9.5 (2.1, 41.9)	Exposed current smokers vs not exposed non smokers		
Malats et al., 2000 Multicentric study (Sweden, Germany, France, Italy, Russia, Romania, Poland, and Brazil)	HB; NR	122 CA/121 CO Non-smokers including occasional smokers (smokers of up to 400 cigarettes in their lifetime) and non-smokers (never smokers); M/W; 49–70 years	Personal interview by a standard Questionnaire.	Cases were recruited among patients with histologically or cytologically confirmed diagnosis of primary lung cancer in hospitals. Controls were selected either among patients from the same hospital as the cases or, in Sweden and Germany, from healthy individuals identified from population registries. All Controls were	OR (95% CI)	Wood (>20 years): 38,8% CA and 22,3% CO; Coal (>17 years): 9,1% CA and 18,2% CO	Stratified analyses by Glutathione S-transferase (GST) polymorphism showed that GSTM1*2 individuals exposed to >20 years to indoor wood combustion had an increased lung cancer risk (OR 3.1, 95% CI, 0.9–9.9).	Age, sex and Center

(*continued on next page*)

Table 1 (*continued*)

First Author; Publication year; Study Name; Country	Enrolment period/FU	N. CA/n. CO/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
				tobacco-related free diseases, i.e., injuries; diseases of the musculoskeletal system; diseases of the genitourinary system; diseases of the digestive system such as hernia, diverticula, and fistula; chronic sinusitis; benign neoplasms; and malignant melanoma of the skin.				
			Wood combustion	Lung cancer	2.5 (1.0–6.2)	Exposed (>20 years) vs not exposed or <20 years		
			Coal combustion		0.4 (0.1–1.1)	Exposed (>17 years) vs not exposed or <17 years		
Kim, 2002 Canada (Quebec)	HB; March–Dec 1998	55 CA/55 CO; M/W; CA: median 50 (range 19–88) years; CO: median 48 (range 18–88) years	Administration of a standard written questionnaire. Woodstove	Patients enrolled from the hospitals. Diagnosis was made by endoscopic examination of nasal cavity. Both unilateral and bilateral polyposis included. Controls randomly selected from clinics with no nasal polyps or history of recurrent sinusitis or polyps. Nasal Polyps	OR (95% CI) 30.9 (6.9–135.6)	30–40% used a woodstove with most using it as a main heating source. 82% CA and 25% CO. exposed vs not exposed		Age, sex, allergy, aspirin intolerance, occupational exposure, smoking and pets. Percentage of cases and controls according to duration (years) and frequency (hours) of exposure is reported, but without ORs calculated.
Orozco-Levi et al., 2006 Spain	HB; 2001–2003	60 CA/60 CO; W (active and passive smokers included); >50 years (mean age CA 73 ± 8 years, CO 69 ± 9 years)	CA and CO were interviewed by trained clinicians using the American Thoracic Society questionnaire. The structured questionnaire included an additional set of questions, previously used in a Spanish population, regarding wood and charcoal smoke exposure (length, intensity, summer/winter differences, cooking and type of biomass fuel). Wood smoke exposure	Eligible hospitalized COPD cases were identified from records of the pulmonary outpatient clinic and pulmonary function test laboratory of the Hospital del Mar. Diagnosis of COPD was performed by assessment of both the signs and symptoms of chronic bronchitis and/or pulmonary emphysema, and functional criteria of chronic and irreversible airflow obstruction (FEV1/ forced vital capacity) < 70%, FEV1 < 80% predicted) and without asthma as assessed by clinical history and response to bronchodilators. Controls were selected from hospital and a diagnosis of COPD had to be absent. The subjects showed normal	OR (95% CI) 1.8 (0.6–6.0) (p = 0.33)	Wood and charcoal smoke in both cases and controls (82%). Mean exposure time was 16 years. Exposed vs not exposed		Age and smoking

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Table 1 (*continued*)

First Author; Publication year; Study Name; Country	Enrolment period/FU	N. CA/n. CO/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
				pulmonary function Tests. COPD hospitalization				
			Charcoal smoke exposure		1.5 (0.5–4.6) (p = 0.48)	Exposed vs not exposed		
			Wood or charcoal smoke exposure		4.5 (1.4–14.2) (p = 0.01)	Exposed vs not exposed		
					6.7 (2.1–21.5) (p = 0.00.2)	quartiles (>20 years vs 0–7 years)	Length (years)	Smoking (since age was highly correlated with years of exposure)
					1.1 (1.0–1.1) (p = 0.001)	Exposure length years (continuous)		
					1.4 (1.2–1.7) (p = 0.0001)	quartiles (>63 years vs 0–49 years)	Time from exposure (years)	No adjustment
					0.97 (0.94–1.00) (p = 0.02)	Time from exposure years (continuous)		Smoking (since age was highly correlated with years of exposure)
					23.3 (6.3–85.7) (p = 0)	tertile (12–24 h/day vs 0–6 h/day)	Exposure intensity in summer in tertiles	smoking (since age was highly correlated with years of exposure)
					1.4 (1.2–1.7) (p = 0.0001)	Exposure intensity in summer h/ day (continuous)		No adjustment
					8.1 (3.0–22.3) (p. 0)	tertile (12–24 h/day-1 vs 0–7 h/day)	Exposure intensity in winter in tertiles	smoking (since age was highly correlated with years of exposure)
					1.1 (1.0–1.2) (p = 0.002)	Exposure intensity in winter h/day (continuous)		No adjustment
Ramanakumar, 2006 Canada (Montreal)	1996–2001,	1202 CA/1541 CO; M/W; 35–75 years	Interviews were conducted for all subjects by trained and bilingual (English and French) interviewers using a structured questionnaire. Traditional heating sources (stoves or fireplace)	Cases were ascertained in the 18 largest hospitals located in the metropolitan area. Cases were ascertained through hospital tumour registries or through active monitoring of pathology department records. Only histologically confirmed cases diagnosed were included. Controls were randomly sampled from the population based electoral lists. No specified the type of fuel used for heating. Traditional cooking included both wood and gas. Lung cancer	OR (95% CI) 0.8 (0.6, 1.1)	Exposed vs not exposed Exposed (%) M 48.8 (CA) 51.1 (CO); W 62.0 (CA) 43.5 (CO).		Age, ethnic group, family income, smoking (three variables), place of birth (North America, other), type of interview (self or proxy), years of schooling, and exposure to at least one of the occupational hazards (asbestos, silica, chromium compounds, and environmental tobacco smoke).
					2.0 (1.4, 2.8) 0.8 (0.7, 1.2)		W Age <20 years, M	

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First Author; Publication year; Study Name; Country	Enrolment period/FU	N. CA/n. CO/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
					2.0 (1.4, 2.9)		Age <20 years, W	
					0.6 (0.5, 1.9)		Age ≥20 years, M	
					2.4 (1.5, 3.7)		Age ≥20 years, M	
					1.0 (0.6, 1.6)		Age at onset of cancer <60 years, M	age, ethnic group, family income, smoking (three variables), place of birth (North America, other), type of interview (self or proxy), years of schooling, and exposure to at least one of the occupational hazards (asbestos, silica, chromium compounds, and environmental tobacco smoke).
					0.7 (0.6, 1.0)		Age at onset of cancer ≥60 years, M	
					1.1 (0.6, 1.9)		Age at onset of cancer <60 years, W	
					2.9 (1.9, 4.4)		Age at onset of cancer ≥60 years, W	
					0.8 (0.6, 1.1)		Smoking status Medium/high M	
					2.1 (1.4, 3.3)		Smoking status Medium/high W	
					0.7 (0.5, 1.1)		Smoking status non-smokers/ low (nonsmokers, smokers of <10 cigarettes/day for a duration of <10 years, and former smokers who had quit >10 years ago. M	
					1.7 (1.0, 2.9)		Smoking status non-smokers/ low W	
					1.2 (0.6, 2.3)	Exposed (%) M, 3.5 (CA) 2.5 (CO); W 6.9 (CA) 7.4 (CO)	Only traditional heating sources M	
					1.8 (1.0, 3.2)		Only traditional heating sources W	
			Both traditional heating and traditional cooking		0.7 (0.5, 1.0)	Exposed (%): M 44.7 (CA) 47.9 (CO); W 54.3 (CA) 36.1 (CO)	M	

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First Author; Publication year; Study Name; Country	Enrolment period/FU	N. CA/n. CO/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
					2.5 (1.5, 3.6)	Consistent findings by smoking status with an increased risk in W but not In M	W	
Loeb et al., 2009 Canada	PB; 2002–2005	717 CA/867 CO; M/W; ≥65 years	Trained interviewers used structured questionnaires to collect data from cases (in the emergency department or on medical units) and controls (interviewed by telephone at home).	Cases enrolled from Hospitals who presented to emergency departments. Cases presented at least two of the following symptoms and signs: temperature higher than 381C, productive cough, chest pain, shortness of breath, or crackles on auscultation. They also had to have a new opacity on a chest radiograph interpreted by a radiologist as being compatible with pneumonia. Controls were selected on a contemporaneous basis with cases. Control participants were excluded if they had been diagnosed with pneumonia in the previous 12 months or had any other active infection.	OR (95% CI)	Exposed CA: 19.6%, CO:26.2% Exposed vs not exposed		Age and residence
			Use of a fireplace in previous 12 months	Community-acquired pneumonia	0.69 (0.54–0.87)			
Hosgood et al., 2010 (Collaborators, 2018) Multicentric study (1study from Europe, 3 studies from North America (about 80%), 3 studies from Asia) *International Lung Cancer Consortium (ILCCO) Central and Eastern Europe (CEE), Europe; University of California–Los Angeles (California), USA; New England Lung Cancer*	7 case-control (HB and PB); 1985–2008	4184 CA/5155 CO; M/W; All ages	Each study developed their own questionnaire; details of these surveys and the methods used to administer them have been reported in each study. The New England and California studies provided information on the main heating and cooking fuel for individuals during both childhood and adulthood. The Toronto, Central and Eastern Europe (CEE)	Cases and controls enrolment specific for each study. Lung cancer	OR (95% CI) 1.26 (1.14–1.39)			age (≤60, >60 years), sex, education (low, medium/high), race/ethnicity (white, Asian, other), study center, and smoking status (ever, never)

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First Author; Publication year; Study Name; Country	Enrolment period/FU	N. CA/n. CO/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
Study (New England), USA; Samuel Lunenfeld Research Institute (Toronto), Canada.			studies provided information on the main heating and cooking fuel for multiple homes throughout the lifetime of the participants. All solid fuels Predominant Wood use		1.21 (1.06–1.38) p 0.06	Predominant users vs subjects who used nonsolid fuels (gas, oil, electric),	All subjects	
					1.19 (1.02–1.39)		M	
					1.19 (0.94–1.51)		W	
					1.22 (1.05–1.42)		Ever-smokers	
					1.01 (0.74–1.37)		Never-smokers	
					1.43 (0.97–2.11),		Lifetime	
					1.05 (0.78–1.40)		PB	
					1.24 (1.05–1.46)		HB	
			Predominant coal use		1.15 (1.02–1.30)		All subjects	
Sloan et al., 2012 (Naeher et al., 2007) USA *New England Lung Cancer Study (NELCS)*	PB; 2005–2007	277 CA, 251 CO; M/W; 30–74 years	All in-person interviews were conducted by trained interviewers using a structured questionnaire. =	Incident cases of lung cancer were identified using the Tumour Registry. Controls were randomly selected from a commercial database and frequency matched to lung cancer cases within 5-year age group and gender	OR (95% CI)			Age, sex, second-hand smoke and pack years of smoking
			Number of winters primarily heating with wood and/or coal	Lung cancer	0.60 (0.38–0.95) (p = 0.03)	Ever vs never		
			Number of winters primarily heating with wood and/or coal (subjects who had used wood or coal as the primary heating)		1.07 (1.01,1.12) (p = 0.02)	For a winter increase	age <18 years	
					2.43 (1.26, 4.67) (p = 0.008)	≥25% (10+ winters) vs <25% (1–9 winters)	age <18 years	
					1.0 (0.98,1.02) (p = 0.93)	For a winter increase	age >18 years	
					1.0 (0.98,1.01) (p = 0.57)	For a winter increase	All ages	
Lissowaska, 2015 (Naeher et al., 2007)	HB and PB; 1998–2002	2861 CA/3118 CO;	In-person, structured interviews were	Incident cases of lung cancer identified through the main	OR (95% CI)	CA exposed 83%, not exposed 17%;		Controls were frequency matched to

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First Author; Publication year; Study Name; Country	Enrolment period/FU	N. CA/n. CO/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
Eastern/Central Europe (the Czech Republic, Hungary, Poland, Romania, Russia, Slovenia and the United Kingdom *The IARC International Multicentre Case-Control Study*		M/W; 20–79 years	conducted. For each residence of more than 1 year, subjects were asked about the principal fuel types used for heating and cooking in each home.	hospitals in participating centers. Only cytologically or histologically confirmed cases were eligible for study. HB controls were selected and used from a prespecified list of persons with diseases that excluded other cancers or tobacco-related diseases. PB controls were selected from residents study areas for those centers.		CO Exposed 81%, not exposed 81%		cases by geographic area (15 centers), 5-year age group and sex. center, age, sex, education, and tobacco pack-years.
			Cooking or heating with Traditional solid fuels (coal and biomass, mainly wood)	Lung cancer	1.22 (1.04, 1.44)	Ever users Vs never users (users of nonsolid fuels gas, kerosene and electricity).		
			Solid fuels for heating only		1.08 (0.84, 1.38)			
			Solid fuels for cooking only		1.37 (0.90, 2.09)			
			Cooking fuels		1.13 (0.94, 1.38)		Ever coal/ never wood	
					1.23 (1.00, 1.52)		Ever wood/ never coal	
					0.98 (0.74, 1.29)		Ever both	
					1.52 (1.23, 1.82) p trend <0.0001		% of lifetime used solid fuel for cooking (>50 years)	
			Heating fuels		1.08 (0.89, 1.31)		Ever coal/ never wood	
					1.31 (1.06, 1.61)		Ever wood/ never coal	
					1.04 (0.82, 1.35)		Ever both	
					1.07 (0.82, 1.39) P trend 0.42		% of lifetime used solid fuel for cooking (>50 years)	Additionally adjusted for duration of solid-fuel use for cooking.
			Cooking fuels		1.06 (0.64, 1.76)		% of lifetime used solid fuel for cooking (>50 years) and tobacco smoking status (never smokers)	
					1.65 (1.36, 2.01)		% of lifetime used solid fuel for cooking (>50 years) and tobacco smoking status (ever smokers)	
					1.73 (1.15, 2.61)		% of lifetime used solid fuel for cooking (>50 years)	

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First Author; Publication year; Study Name; Country	Enrolment period/FU	N. CA/n. CO/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
							and tobacco smoking status (Light smokers (_20 pack-years)	
					1.60 (1.22, 2.03)		% of lifetime used solid fuel for cooking (>50 years) and tobacco smoking status (Heavy smokers (>20 pack-years)	
							No significant differences by country, gender, education, smoking status, years since tobacco quitting, and histological subtype.	
					1.16 (1.00, 1.34)	Former vs never users	switching from solid cooking fuels to nonsolid cooking fuels. The risk decreased significantly with time since switching to modern fuels.	
					1.05 (0.89, 1.25)		Period solid fuel used for cooking Only childhood (<20 years of age)	
					1.32 (1.11, 1.57)		Only adult (_20 years of age)	
					1.23 (1.02, 1.49)		Childhood and some adult	
					1.81 (1.36, 2.41)		Whole life	
Nested case-control studies								
Thorn et al., 2001 Sweden	Nested case-control; 1980–1994	174 CA/870 CO; M/W; 20–50 years	Mailed questionnaire. The classification of the subjects' exposures in the home environment was based on self-reporting of certain exposures.	Mailed questionnaire. 'Physician-diagnosed' asthma was defined as a positive response to the question about physician-diagnosed asthma in both the screening and the case-referent questionnaire, combined with a report of a diagnosis year after 16 years of age. The controls consisted of a random subsample from the original population sample.	OR (95% CI)	Exposed to Woodstove CA: 34.1%, CO: 25%; Exposed to open fireplace CA: 48.9% CO: 49.8%.		Age, sex, smoking habits, and atopy

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First Author; Publication year; Study Name; Country	Enrolment period/FU	N. CA/n. CO/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
			Woodstove	Asthma	0.9 (0.7–1.3)			
					1.1 (0.6–2.1)		M	
					2.2 (1.4–3.5)		W	
			Open fireplace, tiled stove or iron stove	Asthma	1.7 (1.2–2.5)			
					1.1 (0.6–1.9)		M	
					0.8 (0.5–1.3)		W	
Nguyen et al., 2010 USA *The National Asthma Survey—New York State (NAS-NYS)*	nested case-control within the cross-sectional 2002–2003	1025 CA/2,290 CO; M/W; ≥18 years	Questionnaire administered with a telephone interview, designed by the National Centre for Environmental Health Fireplace/wood-burning stove	Questionnaire administered with a telephone interview, designed by the National Centre for Environmental Health. Current Asthma	OR (95% CI) 0.6 (0.5–0.8)	Exposed vs Not exposed	NA	age, sex, race/ ethnicity, geographic region, education, percent of the poverty threshold
				Asthma hospital admissions	0.4 (0–4.8)			
Longitudinal studies								
Triche et al., 2005 USA (Connecticut and Virginia)	Prospective cohort; 1993–1996/ 1994–1995 and 1995–1996 FU	888; W (mothers delivering babies, any household smoking excluded)	At enrolment, approximately 3–5 months after delivery, a standardized questionnaire in home was administered by a trained research assistant. For one year, women were contacted by telephone approximately every 2 weeks to obtain information on heating source use during that period. At home interview, the research assistant placed the monitors in the main living area of the home and instructed respondents on their use.	Standardized questionnaire administered in home by a trained research assistant. Respondents recorded daily respiratory symptom information on a calendar provided to them at the initial interview. For one year, women were contacted by telephone approximately every 2 weeks to obtain information on presence of respiratory symptoms on each day during the period.	RR (95% CI)	Among the 888 W, 219 (25%) used fireplaces, 25 (3%) gas space heaters, 160 (18%) kerosene heaters, and 155 (17%) wood stoves at least once during the heating season.		number of children in household, multifamily dwelling, history of allergies, education, race, gas stove use, state of residence, and other source use variables (Fireplace Use, Gas Space Heater Use, Kerosene Heater Use, Wood Stove Use).
			Fireplace Use	Wheezing	1.07 (0.97–1.18)	For each 1-h per day increase in source use		
				Chest tightness	1.05 (0.99–1.12)			
				Laryngitis	1.02 (0.94–1.10)			
				Phlegm	1.04 (0.99–1.09)			
				Cough	1.05 (1.01–1.09)			
				Runny/stuffy nose	0.99 (0.95–1.04)			
				Sore throat	1.04 (1.00–1.08)			
				SO_2	Median (range) ppb Not users 0.3			

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First Author; Publication year; Study Name; Country	Enrolment period/FU	N. CA/n. CO/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
					(range 0–90.5), woodstove users 0.4 (range 0–42.8);			
				NO_2	Median (range) ppb Not users 13.5 (range 0–147.8), woodstove users 9.3 (range 0–166.4);			
			Woodstove use	Wheezing	0.97 (0.91–1.04)	For each 1-h per day increase in source use		
				Chest tightness	1.01 (0.98–1.03)			
				Laryngitis	1.00 (0.97–1.02)			
				Phlegm	1.00 (0.99–1.02)			
				Cough	1.01 (0.99–1.02)			
				Runny/stuffy nose	1.01 (0.99–1.02)			
				Sore throat	1.00 (0.99–1.02)			
			Monitors were exposed in the home for 2 weeks, corresponding to the first 2-week symptom reporting period, and then mailed back to our laboratory. NO2 and SO2 levels were assessed.	SO_2	Median (range) ppb Not users 0.3 (range 0–84.8), woodstove users 0.3 (range 0–90.5);			
				NO_2	Median (range) ppb not users 13.1 (range 0–166.4); users 11.2 (Range 0–137.3).			
Cross-sectional studies								
Nowak et al., 1996 Germany	1990–1992	4500 (Hamburg) and 4990 (Erfurt) subjects born between 1945 and 1971; M/W; 20–44 years	Mailed Questions on housing conditions were based on those used in the Children's Health Study performed at the Harvard School of Public Health.	In Stage I, the screening questionnaire standardized within the EC Respiratory Health Survey was mailed. Stage II comprised a detailed questionnaire, spirometry measurements, methacholine or bronchodilator inhalation tests, skin-testing, and determination of total and specific IgE. This questionnaire, with 71 items, had been developed from pre-existing	OR (95% CI)			

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First Author; Publication year; Study Name; Country	Enrolment period/FU	N. CA/n. CO/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
				questionnaires: questions relating to respiratory symptoms and medical history were taken from the International Union Against Tuberculosis and Lung Disease (IUATLD) questionnaire; those on smoking from the American Thoracic Society (ATS) questionnaire; and those on occupation and social status from the Office of Population Censuses and Surveys. Methacholine challenge After measuring baseline values and the airway response to the diluent, increasing concentrations of standard methacholine were given.				
			Heating open coal, wood or wood fire	Bronchial hyperresponsiveness (PD20 $\leq$ 2.0 mg or positive response to a bronchodilator test)	1.25 (0.89–1.76) (p = 0.2021)			Region, sex, heating by open gas fire
			Cooking open coal, wood or wood fire		4.21 (1.05–16.89) (p = 0.0427)			Region, sex, Cooking with gas
Kilpelainen, 2001 Finland	Winter season 1995–1996	10,667; M/W; 18-25 (mean age 20.9) years	Postal self-administered questionnaire Wood-stove heating	Validated questions on the occurrence of physician-diagnosed asthma, allergic rhinitis and/or conjunctivitis, atopic dermatitis and self-reported wheezing during lifetime.	OR (95% CI)	8.5% wood stove Heating. Exposed vs not exposed (i.e. any other heating system, e.g. electric heating, central heating, under-floor or roof radiation heating at age 0–6 years and no wood stove heating at age 0–18 years).	Stratified analyses available according to dwelling type, rural, urban or farm. Subjects who had lived on a farm, wood stove heating was related to significantly higher prevalence of atopic dermatitis (OR 1.44, P = 0.049)	sex, parental atopy, socioeconomic status of the family, number of older siblings in the family, residential environment at age 0–6 years (farm, non-farm rural, urban), passive smoking at age 0–2 years, furred pets in the home at age 0–2 years and day care outside the home at age 0–2 years.
				Allergic rhinitis and/or conjunctivitis	0.96 (0.77–1.20)			
				Asthma	0.99 (0.65–1.53)			
				Wheezing (defined as the occurrence of attacks of shortness of breath with wheezing, apart from respiratory infections)	1.01 (0.71–1.42)			
Sood,2010 New Mexico	Cross-sectional	1861; M/W; ever		Pre- and post-bronchodilator		27.7% exposed		age, sex, Hispanic

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First Author; Publication year; Study Name; Country	Enrolment period/FU	N. CA/n. CO/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
(USA) *Lovelace Smokers Cohort*	from a cohort study; 2001–2007	smokers (former or current smokers with at least 10 pack-years of smoking history); 40–75 years	Self-reported exposure via questionnaire.	spirometry were obtained on all subjects by registered respiratory therapists, strictly adhering to the 1994 American Thoracic Society (ATS) guidelines. COPD was defined by measurements of percent lung function (FEV1 measurement), presence of airflow obstruction (Airflow obstruction was defined by a postbronchodilator FEV1/FVC ratio of less than 70%, as defined by the GOLD criteria), and chronic bronchitis (Participants with self-reported cough productive of phlegm for at least 3 months per year for at least 2 consecutive years).		72.3% not exposed Exposed vs not exposed		ethnicity, obesity (body mass index >30 kg/m^2), educational status (at least high school or not), heavy smoking history (pack-years. 40 or not), current cigarette smoke exposure.
			Woodsmoke	FEV1% Predicted	PE (SE) −0.03 (0.01) p = 0.001			
				Airflow Obstruction	OR (95% CI) 1.96 (1.52–2.52) p = 0.001			
				Chronic Bronchitis	OR (95% CI) 1.64 (1.31–2.06) p = 0.001			
			Wood smoke only (n = 197)	FEV1% Predicted	PE (SE) −0.03 (0.01) p = 0.001			
				Airflow Obstruction	OR (95% CI) 1.70 (1.15–2.49) p = 0.007			
				Chronic Bronchitis	OR (95% CI) 2.12 (1.41–3.18) p = 0.001			
			Additive effect of Cigarette smoke and wood smoke (n = 318)	FEV1% Predicted	PE (SE) −0.06 (0.01), p = 0.001		Stratified analyses showed that WS exposure was associated with a risk of respiratory disease, particularly among current smokers, non-Hispanic whites, and men. Wood smoke exposure interacted in a multiplicative	

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First Author; Publication year; Study Name; Country	Enrolment period/FU	N. CA/n. CO/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
							manner with aberrant promoter methylation of the p16 or GATA4 genes on lower percent predicted FEV1.	
				Airflow Obstruction	OR (95% CI) 2.71 (1.89–3.89), p = 0.001			
				Chronic Bronchitis	OR (95% CI) 5.74 (4.05–8.13), p = 0.001			
Stanković et al., 2011 Serbia	March–Sept 2008	1082 never-smoking (living in areas with low concentrations of outdoor air pollutants and who were not professionally exposed to air pollution); M/W; 20–40 years	Interview questionnaire administered by trained physicians	Trained physicians filled out the questionnaires during an interview with the women. The questionnaire was adapted from the American Thoracic Society questionnaires validated for Serbian language.	OR (95% CI)	Exposed vs not exposed		Age, education, family history of respiratory illnesses and annual average of SO_2 and black smoke, second-hand smoke, Home dampness, keeping of pets
			Use of biomass fuels	Cough	1.36 (1.07–1.74)			
				Phlegm	0.94 (0.79–1.12)			
				Blocked/runny nose	0.96 (0.82–1.12)			
				Wheezing	0.97 (0.77–1.23)			
				Shortness of breath	1.40 (1.12–1.75)			
				Asthma	0.90 (0.70–1.16)			
				Bronchitis	0.91 (0.71–1.15)			
				Allergic rhinitis	0.94 (0.63–1.48)			
				Sinusitis	0.88 (0.67–1.11)			
				Pneumonia	0.99 (0.80–1.22)			
			Concentrations of outdoor air pollutants (SO2 and black smoke) were measured for 24 hours/day in the women's living area during the period 2004–2008.	SO_2	(mean concentration) $\mu g/m^3$ 10.01 (2004) 4.05 (2008)			
				BS	1.22 (2004) 0.45 (2008)			
Pahwa et al., 2017 Canada *First Nations Lung Health Project (FNLHP)*	Cross-sectional (within a Prospective cohort. Reported data at	720; M/W; All ages (mean age 34.83 ± 14.47 years)	Interviewer-administered surveys	Self-reported physician-diagnosis, as determined from the Interviewer-administered surveys	OR (95% CI)	Exposed (%) 3.7 not exposed 7.9. Exposed vs not exposed		Age, sex, body mass index

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Table 1 (*continued*)

First Author; Publication year; Study Name; Country	Enrolment period/FU	N. CA/n. CO/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
	baseline) 2012–2013							
			Wood stove or wood to heat house	Chronic bronchitis	0.46 (0.06, 3.59)			
Piekarska et al., 2018 Poland *The project Epidemiology of Allergic Disorders in Poland (ECAP)*	2006–2008	9386; M/W; 20–44 years	Exposure assessment was done by a questionnaire-based survey carried out by using the computer-assisted personal interviewing technique and personal digital assistant devices, based on European Community Respiratory Health Survey (ECRHS) questionnaire translated in Polish.	Phase 1 was a questionnaire-based survey carried out by using the computer-assisted personal interviewing technique and personal digital assistant devices, based on ECRHS questionnaire. Phase 2 was a complementary clinical assessment.	OR (95% CI)	Exposed vs not exposed		No adjustments
			solid-fuel–fired furnaces (coal, coke, or wood)	Allergic rhinitis	1.92 (1.07–3.46)			
				Nonallergic rhinitis	2.02 (1.29–3.18)			
				Seasonal allergic rhinitis	1.07 (0.44–2.62)			

Abbreviation: M: men; W: women: CA: cases; CO: controls; FU: follow-up; ADC: adenocarcinoma; SCC: squamous cell carcinoma; HB: hospital based; PB: population based; NR: not reported; OR: odds ratio; RR = relative risk; CI: confidence interval; SE: standard error; PE: point etimate; NA: not applicable; COPD: chronic obstructive pulmonary disease; FEV: Forced expiratory volume; FVC: forced vital capacity; SO_2: sulfur dioxide; NO_2: nitrogen dioxide.
[1]Included studies from North America and Europe only.
[2]Study Included in the pooled case-control study (Hosgood et al., 2010).

assessed the exposure to indoor pollution produced by the combustion of wood; four studies assessed indoor exposure to residential coal combustion, 8 studies considered exposure to mixed solid fuels, 1 study considered the exposure to charcoal, 3 studies assessed the device used and 2 studies considered exposure to woodsmoke and fireplace together. The exposure was assessed in almost all studies through questionnaires. Regarding the outcomes, studies considering indoor exposure to solid fuels assessed both respiratory diseases and symptoms. More precisely, there were 4 studies for asthma (Kilpeläinen et al., 2001; Stanković et al., 2011; Thorn et al., 2001; Nguyen et al., 2010), 6 studies for lung cancer (Wu et al., 1985; Ramanakumar et al., 2007; Hosgood et al., 2010; Malats et al., 2000; Sloan et al., 2012; Lissowska et al., 2005), 4 for LRIs (Stanković et al., 2011; Sood et al., 2010; Loeb et al., 2009; Pahwa et al., 2012), 5 studies for URIs and other respiratory tract diseases (Kilpeläinen et al., 2001; Piekarska et al., 2018; Stanković et al., 2011; Triche et al., 2005; Kim and Hanley, 2002), and 2 COPD (Orozco-Levi et al., 2006; Sood et al., 2010). Four studies assessed respiratory symptoms (Kilpeläinen et al., 2001; Piekarska et al., 2018; Stanković et al., 2011; Nowak et al., 1996) (Table 1).

In the following sections we present results for selected respiratory outcomes: lung cancer, LRIs, URIs and other respiratory tract diseases, COPD, asthma, and respiratory symptoms.

3.2.2. *Lung cancer*

Four papers, published between 1985 and 2012, considered the association between solid fuel burning exposure and lung cancer risk (Wu et al., 1985; Ramanakumar et al., 2007; Hosgood et al., 2010; Malats et al., 2000). The study conducted by Hosgood et al. (2010) was a pooled analysis of case-control studies conducted in North America, Europe and Asia. Two additional studies (Sloan et al., 2012; Lissowska et al., 2005), included in the pooled case-control study (Hosgood et al., 2010), were also considered separately as they reported additional information compared to the pooled analysis.

Three case-control studies evaluated the association between indoor coal burning exposure and lung cancer risk (Wu et al., 1985; Hosgood et al., 2010; Malats et al., 2000). The study by Lissowaska et al. (Lissowska et al., 2005) was included in the pooled analysis (Hosgood et al., 2010). A small USA case-control study of 220 women affected by lung cancer and 220 controls found that exposure to burning coal, either for heating or cooking, during the majority of childhood and teenage years was associated with a moderate risk of lung cancer in adulthood (OR 2.3, 95% CI 1.0–5.5 for lung adenocarcinoma, RR 1.9, 95% CI 0.5–6.5 for lung squamous-cell carcinoma). After stratification by smoking, a significant association with lung adenoma carcinoma was found in both former and current smokers (RRs 4.3 and 9.5 respectively) but not in non-smokers (RR 3.2, 95% CI 0.9-11.8) (Wu et al., 1985). The pooled study which included 4 studies conducted in Europe and North America

Table 2
Characteristics of the epidemiological studies on outdoor exposure to solid fuels and respiratory outcomes in adults included in the systematic review.

First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
Ecological studies								
Brownin et al, 1990 USA	Ecological 1986–1987 (heating season)	255 exposed to high and170 exposed to low Woodsmoke (WS) M/W ≥15 years	Ambient monitoring data available for the previous heating season 1986–1987 and for the winter of the study. Ambient air measurements were taken in both areas during the study period, using Harvard samplers for PM_{10} and 1590 series nephelometers for light scattering. Considering ambient wood concentration from the previous year, a map that correlated location with wood smoke concentration was built. High wood smoke area was characterised by lower and elevation and the presence of valleys and creek drainages. The area with low wood smoke levels was characterised by higher elevation and the presence of ridges and hills. Mailed questionnaire on frequency of wood stove use.	An initial mailed questionnaire containing 38 questions and a follow-up questionnaire with 6 questions were developed from the standardized respiratory questionnaire of the American Thoracic Society's Epidemiology Standardization Project.	Prevalence ratio (95% CI)	Ratio of prevalence in high wood smoke to low wood smoke areas		No adjustments
		109 exposed to high WS; 70 exposed to low WS	Woodsmoke	Mild symptoms: Usually coughs with cold	1.09 (0.78, 1.53)		15–44 years	
		86exposed to high WS; 55 exposed to low WS		Mild symptoms: Usually coughs with cold	1.06 (0.73, 1.55)		45–64 years	
		60 exposed to high WS; 45 exposed to low WS		Mild symptoms: Usually coughs with cold	0.87 (0.53, 1.43)		≥65 years	
				Mild: Usually congested with cold	0.93 (0.66, 1.29)		15–44 years	
				Mild: Usually congested with cold	1.12 (0.77, 1.62)		45–64 years	
					0.76 (0.44, 1.31)		≥65 years	

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Table 2 (*continued*)

First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
				Mild: Usually congested with cold				
				Mild: Yearly coughs and congestion	0.95 (0.65, 1.40)		15–44 years	
				Mild: Yearly coughs and congestion	0.91 (0.56, 1.47)		45–64 years	
				Mild: Yearly coughs and congestion	0.53 (0.27, 1.31)		≥65 years	
				Moderate symptoms: History of asthma	1.35 (0.67, 2.71)		15–44 years	
				Moderate symptoms: History of asthma	0.98 (0.46, 2.05)		45–64 years	
				Moderate symptoms: History of asthma	0.72 (0.26, 1.99)		≥65 years	
				Moderate: Occasional wheeze with cold	1.12 (0.72, 1.75)		15–44 years	
				Moderate: Occasional wheeze with cold	1.24 (0.74, 2.06)		45–64 years	
				Moderate: Occasional wheeze with cold	0.88 (0.42, 1.82)		≥65 years	
				Severe symptoms: Occasional wheeze without cold	1.21 (0.62, 2.35)		15–44 years	
				Severe symptoms: Occasional wheeze without cold	0.83 (0.40, 1.71)		45–64 years	
				Severe symptoms: Occasional wheeze without cold	0.87 (0.38, 2.02)		≥65 years	
				Severe symptoms: Usually congested without cold	0.77 (0.39, 1.51)		15–44 years	
				Severe symptoms: Usually congested without cold	0.51 (0.24, 1.06)		45–64 years	
				Severe symptoms: Usually congested without cold	0.57 (0.24, 1.33)		≥65 years	
				Severe symptoms: Usually coughs without cold	1.38 (0.66, 2.86)		15–44 years	
				Severe symptoms:	0.57 (0.28, 1.14)		45–64 years	

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Table 2 (*continued*)

First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
				Usually coughs without cold				
				Severe symptoms: Usually coughs without cold	0.40 (0.18,0.89)		≥65 years	
			56 exposed to high WS; 48 exposed to low WS	Cold	1.13 (0.66, 1.93)	At second follow-up	15–44 years	
			49 exposed to high WS; 42 exposed to low WS	Cold	1,21 (0.59, 2,47)		45–64 years	
			33 exposed to high WS; 29 exposed to low WS	Cold	0.51 (0.51, 1.70)		≥65 years	
				Cough	1,25 (0.67, 2.31)		15–44 years	
				Cough	0.87 (0.43, 1.78)		45–64 years	
				Cough	0.62 (0.26, 1.48)		≥65 years	
				Congestion, wheezing	1.46 (0.71, 3.04)		15–44 years	
				Congestion, wheezing	1.69 (0.75, 3.80)		45–64 years	
				Congestion, wheezing	0.67 (0.24, 1.87)		≥65 years	
				Absence due to respiratory disease	1.30 (0.55, 3.09)		15–44 years	
				Absence due to respiratory disease	1.55 (0.58, 4.16)		45–64 years	
				Absence due to respiratory disease	0.33 (0.03, 3.20)		≥65 years	
				PM_{10} levels	Monthly average (November and December 1987 and January 1988 $\mu g/m^3$) 36,41, and 22 72, 53, and 41	Low wood smoke area High wood smoke area		
Hales, 2000 New Zealand, Christchurch (Area affected by air pollution deriving especially from household fires)	Time-series 1988-1993	300 000; M/W All ages	Hourly SO_2, NOx, Carbon monoxide and PM_{10} data, particulate measured by the beta-attenuation method, were available from a representative, centrally located city June 1988–December 1993.	Mortality data were extracted from a national database for the Christchurch Territorial Local Authority area, using ICD-9. Daily totals of respiratory mortality (ICD 460–519) and mortality in people over age 65 were extracted.	RR (SE)/(95% CI)			Time-related effects (Temperature)
			PM_{10}	Respiratory mortality	1.004 (0.0011)/ (1.001, 1.006)	An increase in PM_{10} of 10μg/m3 on the day prior to death	1-day lag	
				PM_{10} levels	daily average ($\mu g/m^3$) 28 (range 0–187)			

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First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
Clancy et al, 2002; Ireland (in 1990 the Irish government banned the marketing, sale, and distribution of bituminous coals within the city of Dublin).	Time-series 72 months before ban 1984–1990 and 72 months after ban 1990–1996	5042 deaths per year before ban/4639 deaths per year after ban M/W All ages	Mean daily air pollution (black smoke and SO_2) concentrations with measurements from six residential monitoring stations in the city of Dublin. Mean daily temperatures and mean daily relative humidity were obtained from Dublin airport. The change in mean air pollution and weather variables before and after the ban on coal sales were calculated and assessed significance by *t*-test of the means.	Daily age stratified numbers of Dublin city residents who died within the city for total non-trauma deaths (ICD9, respiratory deaths (ICD9 480–496 plus 507).	Percentage change (95% CI)	After compared with before the ban		Temperature, relative humidity, day of week, respiratory epidemics, and standardised cause-specific death rates in rest of Ireland.
			Coal	Respiratory mortality	−15·5 (−19·1, −11.6); p=<0.0001			
				BS levels	Average concentration (μg/m^3) −35.6 (<0·0001)	Changes before and after intervention		
				SO_2 levels	−11.3 (<0·0001)			
McGowan et al, 2002; New Zealand, Christchurch (Winter air pollution in Christchurch is dominated by particulate matter from solid fuel domestic heating. In this area more than 90% of particulate air pollution has been estimated to come from city's 47 000 wood burners and open fires that are used during the cold winters months)	Time-series 1988–1998	333 000 M/W ≥15 years	Pollution levels were obtained from monitoring site from June 1988 to Dec 1998. Concentration of air pollutants was provided in the form of 24- hour mean concentrations of the following pollutants: CO, $PM_{10,}$ SO_2, and NOx.	Hospital admission information was obtained from the New Zealand Health Information Service, diagnoses based on ICD 9: acute respiratory infections (460–466), other diseases of upper tract (470-8), Pneumonia/ influenza (ICD-480-7), chronic lung diseases (491–492, 494–496), asthma (493).	Percentage change (95% CI)	IQR PM10 level increase (14.8 μg/m^3)		Meteorological variables
			PM_{10}	Respiratory admissions	3.37 (2.34–4.40)		2-day lag	
				Respiratory admissions	3.39 (1.85–4.93)		15–64 years	
				Respiratory admissions	2.86 (1.23–4.49)		≥65 years	
				PM_{10} levels	Mean (SD) (range) (mg/m^3) 25.17 (25.49) (0–283)			
					OR (95% CI)			

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Table 2 (*continued*)

First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
Bennett et al, 2010 Australia (Tasmania) Tasmanian Longitudinal Health Study (TAHS) Study conducted in Launceston and Hobart, two relatively similar cities with a similar prevalence of wood heating, but different air quality outdoor. Launceston and the surrounding Tamar Valley, approximately 30% of households uses a wood burner or open fireplace as their primary form of home heating. Hobart, has a similar climate and similar prevalence of wood burner use. However, the topography and meteorology of the two regions is different and Hobart has substantially better air quality)	Ecological study 1968/ 2004–2005 (last survey)	1672 (601 participants in Launceston and 1071 participants in Hobart) M/W mean age 43.0 years, ±0.06	PM_{10} is measured daily in both Launceston and Hobart by the Environment Protection Authority (Department of Primary Industries, Parks, Water and Environment, Tasmania) using a Tapered Element Oscillating Microbalance and a Hi-Volume air sampler. Exposure was assigned based on the place of residence of each participant, with Launceston residents classed as 'exposed' and Hobart residents classed as 'unexposed.'	The postal survey was used to collect demographic data about participants and dichotomous responses to questions about self-reported respiratory symptoms experienced over the previous 12 months.		Exposed (residents in Launceston) vs not exposed (residents in Hobart)	In the Subgroup who regularly used a wood heater in their home (n = 629), there was a consistent trend towards greater symptom prevalence in Launceston residents who used wood burners compared to Hobart residents who used wood burners, although none was statistically significant.	Gender, atopy, history of allergic disease (eczema and hay fever) and current smoking status. Further adjustment for body mass index, socio-economic factors (including education level, occupation and number of children), past smoking history (those who once smoked but did not currently), hours of tobacco smoke exposure at home and/or at work, and presence of mould in the home did not significantly affect the results.
				Wheezy breathing Wheeze with breathlessness Wheeze without a cold Woken with chest tightness Woken with shortness of breath Usually cough Usually cough with phlegm Asthma attack	1.03 (0.75–1.42) 1.11 (0.67–1.83) 0.96 (0.55–1.65) 1.21 (0.86–1.71) 0.88 (0.55–1.40) 1.06 (0.75–1.48) 1.03 (0.71–1.49) 0.99 (0.63–1.54)			
				PM_{10} levels	Maximum concentration (95th percentile) $\mu g/m^3$ 116.5 (57.5) 73.0 (27)	Launceston Hobart		
Ostro et al, 2010 USA; *California Teachers Study (CTS)*	Prospective cohort 1995/2001-2007	7888 (living within 8 km of a monitor) 44 847 (living within 30 km of a monitor) W Mean age: 54 (30–80) years	Exposure to $PM_{2.5}$ and several of its constituents including K used as a biomarker of biomass burning was estimated. Monthly averages of exposure were created using pollution data from June 2002 through July 2007. Participants	Mortality data were obtained from the California Department of Health, the US Social Security Administration death master. Deaths were assigned codes based on the International Classification of Diseases, 10th Revision (ICD-	HR (95% CI)			Age, smoking status, total pack-years, BMI, marital status, alcohol consumption, second-hand smoke exposure at home, dietary fat, dietary fiber, dietary calories, physical activity, menopausal status, hormone replacement therapy use,

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First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
			whose residential addresses were within 8 and 30 km of a monitor collecting $PM_{2.5}$ constituent data were included.	10) for the following outcomes with pulmonary deaths (C34, J00–J98).				family history of myocardial infarction or stroke, blood pressure medication and aspirin use, and contextual variables (income, income inequality, education, population size, racial composition, unemployment).
			K form woodsmoke	Pulmonary mortality	1.22 (0.82–1.82)	for an IQR increase of 0.05 μg/m3)	per 8 km-buffer	
					1.24 (0.99–1.55)	For an IQR increase of 0.07 μg/m3)	per 30 km-buffer	
Bui et al, 2013 Australia (Tasmania) *Tasmanian Longitudinal Health Study (TAHS)*	Cohort 1968 (7 years old children invited)/ 2003–2005	1383 Mean age: non-asthmatic 42.6 (0.76), asthmatic 42.7 (0.82) years	Mailed questionnaire. Levels of ambient wood smoke in winter were defined by the question 'On a scale of 0–10, how much is the area where you live subjected to wood smoke in winter?'	Mailed questionnaire. Asthma severity rating used the National Asthma Council Australia classification for untreated, newly diagnosed asthma	OR (95% CI)	Per 1-unit increase		Sex, socioeconomic status and smoking status. Other potential confounders were examined by being added to the baseline model one by one, including family history of asthma, history of allergic diseases (hay fever and eczema), atopic status, presence of mould in the home, type of energy used for cooking and heating, and passive smoking. The confounding variable was retained in the model if its inclusion altered the point estimate by 10% or more.
			Levels of ambient wood smoke	Current asthma (in last 12 months)	1.01 (0.96–1.06) p = 0.55	Per 1-unit increase	Results from sensitivity analysis by omitting participants with remitted asthma from the control group did not materially differ to those from the main analysis.	
				Asthma severity (persistent asthma vs all	1.11 (1.02–1.20) p = 0.01			

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First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
				lower categories)				
Gan et al, 2013 Canada	Prospective cohort Jan1994 to Dec 1998/Jan 1999 to Dec 2002 (4 years of FU)	400 254 subjects who did not change their woodsmoke exposure status during the 5-year exposure period M/W 45-83 (average age 60 ± 11) years	Exposure to woodsmoke was assessed using mobile monitoring of ambient $PM_{2.5}$ and fixed-location measurements of a specific woodsmoke tracer, levoglucosan. Woodsmoke levels were divided into tertiles, representing low, medium, and high woodsmoke pollution areas. The woodsmoke exposures were assigned to study subjects through their residential postal codes.	COPD hospitalization cases and death cases were identified from the linked health databases using ICD-9 codes 490–492 and 496 or 10th Revision (ICD-10) codes J40–J44.	RR (95% CI)	Tertile: high vs low		Age, sex, SES, asthma, diabetes, CHD, HHD, Black carbon, $PM_{2.5}$, and NO_2 levels
			Woodsmoke	COPD hospitalization COPD mortality	1.15 (1.02–1.29) 0.81 (0.64–1.03)			
Johnson et al, 2013; Australia (Tasmania) During the late 1980s and early 1990s, wood stoves increased for home heating in Tasmania. During the 1990s, 66% of Launceston households used domestic wood stoves as the main source of heating, and emissions from these accounted for an estimated 85% of particulate air pollution in winter.	Time-series (Interventional); 1994–2000 (pre-intervention)/ 2000–2007 (post-intervention)	67 000 residents of central Launceston and 148 000 residents of central Hobart (at 2001 census) Launceston (Intervention: with air quality improvement) Hobart (control without any air quality improvements) M/W All ages	A comprehensive monitoring campaign was started in 1991 in Launceston. Daily monitoring of concentrations PM_{10} was conducted at five sites in 1991-93 with gravimetric sampling methods. Air quality and the 24h PM_{10} concentration was measured on a one in six day cycle from 1994 to 1997. Starting in May 1997 measurements were taken daily during winter, and starting in June 2001 measurements were taken daily all year. PM_{10} only reported Since 2001, however, concentrations of $PM_{2.5}$ have also been measured and these data show that the	Mortality data were obtained from Australian Bureau of Statistics. Causes of death were categorised according to the ICD coding into all cause (non-trauma), cardiovascular, and respiratory deaths (from 1994 to 2007). Hospital admissions data were provided by the Tasmania Department of Health and Human Services and were used to identify epidemics of respiratory infections by extracting the daily counts for admissions for pneumonia and flu (ICD9-9480–487 or 507; ICD-10 J10–18 and J69-70).	Percent decrease (95% CI)			Age structure, meteorological conditions, and secular mortality trends in Tasmania

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First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
			mean daily concentrations of $PM_{2.5}$ are highly correlated with the concentrations of PM_{10}, accounting for 50% of the annual average and 65% of the wintertime average					
			Intervention decreasing wood-stove use, Community education campaigns, enforcement of environmental regulations, and a wood heater replacement programme to reduce ambient pollution from residential wood stoves started in the winter of 2001. By the end of the programme, the prevalence of wood stoves in Launceston had fallen from 66% to 30% of all households.	Respiratory mortality	−8.5 (−23.2, 9.0) p = 0.32	Deaths per 1000 person years, before and after intervention	All year, M + W Launceston	
					−22.8 (−40.6, 0.3) p = 0.05		All year, M Launceston	
					1.0 (−18.9, 24.4) p = 0.96		All year, W Launceston	
					4.8 (−7.4,18.6) p = 0.50		All year, M + W Hobart	
					3.4 (−13.1, 24.4) p = 0.67		All year, M Hobart	
					−1.4 (−15.5, 15.1) p = 0.86		All year, W Hobart	
					−27.9 (−49.5, 3.1) p = 0.07		Wintertime, M + W Launceston	
					8.0 (−16.9, 40.4) p = 0.60		Wintertime, M + W Hobart	
					22.9 (7.6–38.3)		Winter	
				PM_{10} levels	Annual mean 23.7 μg/m3 18.4 (P < 0.001)		All year Before intervention (1997–2000) after the intervention (2001–07).	
					43.6 27.0 (p < 0.001)		Wintertime before intervention (1997–2000) after the intervention (2001–07).	

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Table 2 (*continued*)

First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
Meszaros et al, 2015 Australia (Tasmania including Hobart and Launceston city) which represents an area where domestic wood heating is extensively used	Time-series Jan 1992–Dec 2002	M/W All Ages	PM_{10} levels provided by the Department of Primary Industry, Parks, Water and Environment and meteorological variables from the Bureau of Meteorology.	Daily respiratory admissions from hospital records using the International ICD-9, coding 491–492.8 was used up to 1999 and ICD, Tenth Revision, Australian Modification (ICD10-AM) codes J41-J44.9 thereafter.	Regression coefficient (SE)			Temporal trends, weekly and seasonal variation,
			PM_{10} from wood-heaters	COPD hospital admissions	−0.00090 (0.00235) p = 0.70	Increase in PM_{10} of 10 μg/m3.		
				PM_{10} levels	Maximum daily level (mean levels) μg/m3 186 (50.7) 77 (16.5)		1992 2002 Hospitalisations for COPD showed a marked seasonal pattern (p < 0.001) with admissions peaking in winter. There was no evidence of a relationship between average daily PM10 levels and hospital admissions for acute exacerbations of COPD (p = 0.30).	
Ostro, 2015; USA *California Teachers Study (CTS)*	Prospective cohort; 1995/2001-2007	101 884 (current and former female teachers and administrators identified through the State Teachers Retirement System) W Mean age: 54 (30–80) years	Residential levels were provided by a chemical transport model that computed pollutant concentrations from >900 sources in California. Besides particle mass, monthly concentrations of 11 species and 8 sources or primary particles were generated at 4-km grids.	Record linkage conducted annually to mortality files administered by the California Department of Public Health. Deaths were assigned codes based on the International Classification of Diseases, 10th Revision (ICD-10) with pulmonary deaths (C34, J00–J98).	HR (95% CI)			Age, smoking status, smoking pack years, adult second-hand smoke exposure, BMI, marital status, alcohol consumption, physical activity, menopausal status and HT use combined, family history of heart disease, hypertension medication/ aspirin use, and dietary fat, fiber, and caloric intake
			$PM_{2.5}$ from Woodsmoke	Pulmonary mortality	1.02 (0.94–1.1) P = 0.73	For an IQR $PM_{2.5}$ increase of 1.3 μg/m^3		
			UFP from Woodsmoke		1.02 (0.95–1.1) P = 0.59	For an IQR UFP increase of 332 ng/m^3		
				$PM_{2.5}$ levels form woodsmoke	Mean/Media (25th-75th percentile) (μg/m3)			

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Table 2 (*continued*)

First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
				UFP levels from woodsmoke	1.4/0.9 (0.5–1.8) Mean/Media (25th-75th percentile) (ng/m^3) 310/205 (105–437)			
Yap and Garcia, 2015; USA (California) in 1992 adopted Rule 4901, which aimed at reducing emissions of CO and PM from residential woodburning fireplaces and heaters during the burn season (Nov–Feb) and establishing a public health education program to reduce wood-burning emissions. The rule was amended and enforced in 2003.	Time-series (Interventional); 2000–2006 (Study conducted burning season Nov–Feb, for the 3 years before and after the 2003 amendment for reducing air pollutants from wood-burning)	M/W ≥45 years	Data for daily ambient $PM_{2.5}$ and coarse particle concentrations were extracted from the California-based National Air Monitoring Stations/State and Local Air Monitoring Stations for the burn seasons (Nov–Feb) of 2000 through 2006. Particles were estimated using geographic information system (GIS) software and then estimated at ground level by modelling a pollutant surface using the Inverse Distance Weighting method and assigning the results from this interpolation to the centroid of each zip code.	Daily hospital admissions were obtained from the California Office of Statewide Health Planning and Development for the winter periods from 2000 to 2006. COPD hospital admissions included using ICD-9 codes 490–496.	Rate ratio (95% CI)			Temperature, relative humidity, average wind speed, calendar years, day of the week, calendar year, no-burn days, weekend, and poverty.
			$PM_{2.5}$	COPD hospital admissions	0.90 (0.78–1.95)		45–64 years	
					0.93 (0.83–1.04)		≥65 years	
					0.90 (0.74, 1.10)		Rural 45–64 years	
					0.91 (0.78, 1.07)		Rural ≥65 years	
					0.91 (0.78, 1.06)		Urban 45–64 years	
					0.95 (0.86, 1.06)		Urban ≥65 years	
				Wintertime $PM_{2.5}$	−3.79 $\mu g/m^3$	changes pre and post rule 4901 levels were reduced	ALL	Year and no-burn days
					-3.23 $\mu g/m^3$		RURAL	
					-5.65 $\mu g/m^3$		URBAN	
				Wintertime PM coarse	−1.61 $\mu g/m^3$		ALL	
					-1.37 $\mu g/m^3$		RURAL	
					-2.24 $\mu g/m^3$		URBAN	
Pindu et al, 2016; Estonia *RHINE III (Respiratory Health in Northern Europe)*	Cross-sectional; 2011–2012	905 M/W 39-63 (mean age 50) years	Emissions data was based on a residential wood combustion (RWC) database that consisted of data from buildings (heating system	Postal questionnaire on Respiratory Health and Cardiac Diseases	OR (95% CI)		No association between health symptoms and 2009–2012 particle exposure among respondents who had lived at the	Gender, age, Body Mass Index, education, smoking history in the last 10 years (current, ex–, never) and ETS, traffic PM_{10}

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Table 2 (*continued*)

First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
			and heated area in m2) and cadastral (cadastral coordinates) registries, which allowed to locate every household that used wood for heating. Household $PM_{2.5}$ emissions (g/s) were calculated according to the size (m^2) of each's heated area (modelled for 2009–2012). The RWC emission database was based on emission factors that have been measured in the Estonian Environmental Research Centre's stove testing laboratory and wood usage was acquired from the earlier questionnaire study in Tartu.				same address in Tartu since 2001	
			Wood Residential heating induced $PM_{2.5}$	Attack of cough	0.95 (0.72–1.29)	$PM_{2.5}$ IQR increase 1.5 $\mu g/m^3$		
				Wheeze without cold	1.14 (0.75–1.73)			
				Ever had asthma	1.16 (0.6–2.19)			
				Allergic rhinitis	0.63 (0.42–0.94)			
				Attack of breathlessness	0.97 (0.64–1.48)			
				Chest tightness	1.05 (0.72–1.51)			
				$PM_{2.5}$ levels	Average concentration (SD; maximum) $\mu g/m^3$ 2.3 (0.9; 4).			
Hansell et al, 2016; UK (England and Wales) Changes in air pollution concentrations in the UK are well documented as, uniquely, the UK had a comprehensive national air quality monitoring network	1971/2009 (mean age 38 (SD 22.9)) years in 1971	367 658 M/W	Land use regression techniques were used to model Black smoke and SO_2 annual concentrations in 1971, 1981 and 1991 at 1 km grids. Models were developed against concentration data from national monitoring station sites	Office of National Statistics. using the International ICD-9, coding 491–492.8 Respiratory mortality (excluding lung cancer): ICD-8460 to 493, 516, 518, 783; ICD-9460 to 519.9; ICD-10 chapter J. Respiratory mortality:	OR (95% CI)			Age, sex, social class, area-level deprivation, region, population density, area-level lung cancer risk as a proxy of tobacco smoking

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First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
running from the 1950s to the 1990s measuring black smoke and SO_2 arising from domestic and industrial coal and fossil fuel combustion, then major sources of emissions.			where operational days in the year exceeded 75%, which involved a total of 966 sites for Black smoke and 825 sites for SO_2. Land use regression techniques were used to model PM_{10} at 100 m grids in 2001. For 1971, individuals were assigned the annual average Black smoke and SO_2 concentration of the 1 km grid in which their residence was located. For other years (1981, 1991 and 2001) the pollutant surfaces (ie, regular grids) were intersected with ward boundaries and area-weighting was used to calculate the average values of each pollutant within each ward.	Respiratory Infections: ICD-8 460 to 466, 470 to 474 480 to 486; ICD9: 460 to 466, 470 to 478 480 to 488; ICD-10: J00 to J06, J09 to J18 J20 to J22; Respiratory mortality: COPD, ICD-8, 491 to 492 and 518; ICD-9490 to 492, 494 and 496. ICD-10 J40 to J44 and J47. Lung cancer: ICD-8 and ICD-9 162; ICD-10 C34 and C33.				
			Black smoke	Respiratory mortality	1.07 (1.04, 1.10)	Per 10 μg/m3 increase of black smoke exposure in 1971	Exposure in 1971 and mortality risk between 1972 and 2009. Consistent results by decades (i.e. 1972-1981; 1982–1991; 1992–2001; 2002–2009) and by year of exposure (i.e. in 1981 and 1991)	
					1.05 (1.01, 1.09)		Mortality risk in 2002–2009	
				Respiratory mortality: Respiratory Infections	1.06 (1.02, 1.11)		Exposure in 1971 and mortality risk in 1972–2009. Consistent results by decades and by year of exposure.	
				Respiratory mortality: COPD	1.07 (1.03, 1.11)		Exposure in 1971 and mortality risk in 1972–2009. Consistent results by decades and by year of exposure.	

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Table 2 (*continued*)

First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
				Lung cancer mortality	1.05 (1.02, 1.09)		Exposure in 1971 and mortality risk in 1972–2009. Consistent results by decades and by year of exposure.	Age, sex, social class, area-level deprivation, region, population density and lung cancer
			SO_2	Respiratory mortality	1.03 [1.01; 1.04]	per 10 μg/m^3 for SO2 exposure	SO_2 exposure in 1971. Consistent results by decades and by year of exposure	
				Respiratory mortality: Respiratory Infections	1.03 [1.01; 1.05]			
				Respiratory mortality: COPD	1.03 [1.01; 1.04]			
				Lung cancer mortality	1.04 [1.02; 1.05]			
				BS levels	Mean (SD); median (μg/m3) 42.7 (20.4); 41 16.2 (5.2); 16 11.8 (4.7); 12		1971 1981 1991	
				SO_2 levels	85.2 (36.8); 77 43.1 (12.1); 41.5 29.6 (6.5); 29.5		1971 1981 1991	
Madsen, 2017; Norway The dominant source of air pollution is traffic on weekdays and in wintertime wood burning at nights and on weekends	Time-stratified case-crossover; 1992–2001	Approximately 500 000 (48 713 deaths) M/W ≥50 years	A dispersion model was used to assess short-term air pollution for daily (24-h) averages and peak concentrations of NO_2 from exhaust and $PM_{2.5}$ from urban traffic and wood-burning at residential neighbourhood level for each individual.	All death certificates registered with Statistics Norway based on a unique personal identification number for all Norwegian inhabitants. The study was restricted to non-accidental deaths among included individuals using the ICD-9 and ICD-10 coding	Percent increase (95% CI)			
			$PM_{2.5}$ (urban traffic pollutants together with wood burning wintertime)	Respiratory mortality	3 (−1.0, 7.1)	10 μg/m^3 variation in $PM_{2.5}$ (lag 0–5)	24-h average	
				Respiratory mortality	2.3 (0.0, 4.7)	10 μg/m^3 variation in $PM_{2.5}$ (lag 0–5)	Peak	
				$PM_{2.5}$ levels	Annual mean (peak) μg/m^3 15.4 (20.6) 12.4 (19.5)	1992 2001		
Phillips et al, 2018 UK (England and Wales)	Ecological study May 1951–May 1952/1993-2012	3 535 136 deaths M/W 35–74 years	The Ministry of Fuel and Power published estimates of the quantities of	Mortality rates were calculated using population estimates provided by the	RR (95% CI)			Source of coal (industrial, carbonitation, electricity generation)

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Table 2 (*continued*)

First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
			different types of solid fuel burnt annually in each of the 1145 Local fuel overseer (LFO) areas between May 1951 and May 1952. Domestic (household) supplies included coal, the amounts used in each LFO based on the number of registered premises, and other solid fuels including coke, briquettes, ovoids and similar patent fuels where consumption was based on merchant's disposals.	Office for National Statistics from death certificate for each Local Government District during 1993–2012 using ICD9: 460–519, ICD10: J00–J99.				
			Domestic coal use	Respiratory mortality	1.127 (1.118, 1.137)	per SD increase in fuel usage		Social class, education, crowding, unemployment, industrial usage and fuel used in carbonisation and electricity generation
				Respiratory mortality	1.116 (1.107, 1.126)		Combined effects analysis of domestic usage and density	
				Respiratory mortality	1.189 (1.177, 1.201)			Social class, education, crowding, density, unemployment, effect of domestic usage allowing for 1951 and 2001 socioeconomic indicator and current $PM_{2.5}$
				Respiratory mortality	SMR (Fig2 in the paper) The associations were progressive and remained strong and statistically significant	4th vs 1st quartile of domestic fuel consumption (ex smokeless)		socioeconomic indicators in 1951 and 2001 and current $PM_{2.5}$
				COP mortality	RR (95% CI) 1.142 (1.122, 1.163)	per SD increase in fuel usage		
				Asthma mortality	RR (95% CI) 1.093 (1.032, 1.158)			
				Pneumonia mortality				

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First Author; Publication Year; Country; *Study Name*	Study Design; Enrolment period/FU	N. Ca/n. Co/ Sample Size; Sex; Age	Exposure Assessment; Exposure	Outcome Assessment; Outcome	Risk Estimates	Percentage of Exposed/not Exposed; Exposure Category	Stratified Analysis	Adjustments
					RR (95% CI) 1.227 (1.205, 1.25)			
				Tuberculosis mortality	RR (95% CI) 1.298 (1.167, 1.444)			
				Trachea, bronchus and lung cancer	RR (95% CI) 1.126 (1.115, 1.137)			
				Fuel consumption in England and Wales (1951)/ 1952	Total annual consumption (tonnes × 1000) 36 783			
				$PM_{2.5}$ levels	Mean (SD) 9.48 (1.67) μg/m3			

Abbreviation: M: men; W: women: CA: cases; CO: controls; FU: follow-up; IQR: interquartile range; K: potassium; HR: hazard ratio; OR: odds ratio; RR: relative risk; CI: confidence interval; SMR: standardized mortality ratio; BS: black smoke; BMI: body mass index; ICD: international classification of diseases; NOx: nitrogen oxides; COPD: chronic obstructive pulmonary disease, SO_2: sulphur dioxide; PM: particulate matter; SD: standard deviation; SE: standard error; UFP: ultrafine particulate.

showed a significant lung cancer increase among predominant coal burning users (OR 1.15, 95% CI 1.02–1.30) (Hosgood et al., 2010). The study by Lissowaska et al. (Lissowska et al., 2005), included in the pooled case-control study (Hosgood et al., 2010) and performed in six eastern and central European countries and in the UK, found a slightly non-significant increased lung cancer risk among users of coal for heating and cooking compared to users of modern nonsolid fuel (Lissowska et al., 2005).

Using coal for more than 17 years was inversely associated with lung cancer risk in a small multicentre study of 122 cases and 121 controls conducted in eight countries (OR 0.4, 95% CI 0.1–1.1) (Malats et al., 2000).

Two publications accounting for 5 studies (4 studies were included in the pooled case-control study) evaluated the association between indoor wood burning exposure and lung cancer risk (Hosgood et al., 2010; Malats et al., 2000). A pooled case-control study from the international Lung Cancer Consortium including about 4200 cases and 5200 controls showed that predominant wood users had a moderate increased risk of developing lung cancer (OR 1.21, 95% CI 1.06–1.38). However, stratified analysis by smoking status showed an increased risk in ever smokers (OR 1.22, 95% CI 1.05–1.42), but not in never smokers (OR 1.01, 95% CI 0.74–1.37) (Hosgood et al., 2010). Stratified analyses, performed using the study conducted in Central and Eastern Europe and UK, showed that lifetime use of wood was associated with a non-significant increased risk of lung cancer (Lissowska et al., 2005).

In a multicentric case–control study conducted in non-smokers by Malats et al. (2000), the use of wood for more than 20 years vs less than 20 years was associated with a significant increased risk of lung cancer (OR 2.5, 95% CI 1.0–6.2).

Three publications (one pooled case-control study) evaluated the association between indoor mixed/combined solid fuel (wood and coal) exposure and lung cancer risk (Ramanakumar et al., 2007; Hosgood et al., 2010; Sloan et al., 2012).

In a case-control study conducted in Canada (Ramanakumar et al., 2007), among 1205 cases and 1541 controls, no significant associations with exposure to traditional heating (stoves or fireplace) or cooking (wood and/or gas) were found in men (most ORs below 1.0). Conversely, in women significant associations were found with traditional heating and cooking. The most elevated risks associated with traditional heating were found in subjects ≥60 years at the age of onset (OR 2.9, 95% CI 1.9, 4.4) and in medium/high smokers (OR, 2.1, 95% CI 1.4, 3.3).

The pooled case-control study from the international Lung Cancer Consortium, found a significant increased lung risk among all solid fuel (coal and wood) users in European and North American studies compared to nonsolid fuel users (OR 1.26, 95%CI 1.14–1.39) (Hosgood et al., 2010).

The study by Sloan et al. conducted in the USA, included in the pooled case-control study (Hosgood et al., 2010), also found a significant increased risk among wood and coal users for heating for ≥10 winters (OR 2.43, 95% CI 1.26–4.67) compared to user for 1–9 winters. When timing of exposure was considered, stronger association was found among subjects exposed when less than 18-years-old (OR 1.07, 95% CI 1.01–1.12) compared to age >18 years (OR 1.00, 95% CI 0.98–1.01) (Sloan et al., 2012).

3.2.3. LRIs

Four studies evaluated the association between exposure to solid fuel combustion and LRIs (Stanković et al., 2011; Sood et al., 2010; Loeb et al., 2009; Pahwa et al., 2017).

In the study conducted in Serbia, the use of biomass fuels was not associated with an increased risk of both bronchitis and pneumonia (ORs 0.91, 95% CI 0.71–1.15 and 0.99, 95% CI 0.80–1.22 respectively) (Stanković et al., 2011).

A cross-sectional study conducted in Canada among 720 individuals found a non-significant inverse association between having a wood stove or heating the house with wood and chronic bronchitis (OR 0.46, 95% CI 0.06–3.59) (Pahwa et al., 2017).

A case-control study conducted in Canada found a significant inverse association between the use of a fireplace in the previous 12 months and pneumonia risk (OR 0.69, 95% CI 0.54–0.87) (Loeb et al., 2009).

A cross-sectional study performed in the USA including more than 1850 ever smokers, found a significant increased risk of chronic bronchitis among subjects exposed to woodsmoke (OR 1.64, 95% CI 1.31–2.06) (Sood et al., 2010).

3.2.4. URIs and other upper respiratory tract diseases

Two studies considered the association between solid fuel combustion exposure and URIs (Stanković et al., 2011; Triche et al., 2005).

A prospective cohort study conducted in USA among 888 women enrolled at hospitals after delivering babies, found that the use of indoor wood stoves was not associated with laryngitis or sore throat. Whereas,

each hour of fireplace use (fuel not specified) was marginally associated with sore throat (RR 1.04; 95% CI 1.00–1.08) (Triche et al., 2005).

In the study conducted in Serbia, the use of biomass fuels was not associated with an increased risk of sinusitis (OR 0.88, 95% CI 0.67–1.11) (Stanković et al., 2011).

In a case-control study conducted in Canada including 55 cases of nasal polyps and 55 matched controls, the use of an indoor wood stove was significantly associated with nasal polyp risk (OR 30.9, 95% CI6.9–135.6) (Kim and Hanley, 2002).

Three studies considered the association between exposure to solid fuel combustion and rhinitis (Kilpeläinen et al., 2001; Piekarska et al., 2018; Stanković et al., 2011).

A cross-sectional study among Finnish university students, found no association between wood stove heating at age 0–6 years and adulthood allergic rhinitis and/or conjunctivitis (Kilpeläinen et al., 2001).

The European Community Respiratory Health Survey (ECRHS), conducted a large study in Poland including 9386 adults (20–44 years). They found that indoor solid fuel stove use was significantly associated with an increased risk of allergic and nonallergic rhinitis (ORs 1.92, 95% CI 1.07–3.46 and 2.02, 95% CI 1.29–3.18 respectively) but not with seasonal allergic rhinitis (OR 1.07, 95% CI0.44–2.62) (Piekarska et al., 2018).

In the study conducted in Serbia, including more than 1000 never smoking women, the use of biomass fuels was not associated with an increased risk of allergic rhinitis (OR 0.94, 95% CI 0.63–1.48) (Stanković et al., 2011).

3.2.5. COPD

Two studies evaluated the association between indoor solid fuel combustion exposure and risk of COPD (Orozco-Levi et al., 2006; Sood et al., 2010).

In a case-control study conducted in Spain among women (60 cases and 60 controls), the use of wood or charcoal alone independently increased risk of COPD (OR 1.8 and 1.5 respectively), but the combination of both was strongly associated with COPD (OR 4.5, 95% CI 1.4–14.2). The association between length of exposure and COPD suggested a dose–response pattern ($p = 0.001$). Intensity (h/day) of exposure in both summer and winter was also related to COPD, with a higher risk among subjects exposed to solid fuel burning more hours per day (Orozco-Levi et al., 2006).

For the cross-sectional study, drawn from the Lovelace Smokers' Cohort, a predominantly female cohort of smokers in the USA, COPD outcomes (i.e. percent predicted FEV1, airflow obstruction, and chronic bronchitis) were investigated in relation to self-reported wood smoke exposure. Wood smoke exposure was associated with a lower percent predicted FEV1 (point estimate 20.03, Standard Error 0.01) and a higher prevalence of airflow obstruction and chronic bronchitis (ORs 1.96; 95% CI 1.52–2.52 and 1.64, 95% CI 1.31–2.06 respectively). These associations were stronger among current cigarette smokers, non-Hispanic whites, and men (Sood et al., 2010).

3.2.6. Asthma

Four studies evaluated the association between exposure to solid fuel combustion and asthma.

Only a nested case-control study conducted in Sweden including 174 cases and 870 controls found an increased risk associated with the presence of open fireplace, tiled stove or iron stove (OR 1.7, 95% CI 1.2–2.5) but not with a woodstove (Thorn et al., 2001).

A large cross-sectional study among more than 10,600 Finnish university students, found no association between wood stove heating at age 0–6 years and asthma at age 18–25 years and up to young adulthood (Kilpeläinen et al., 2001). A cross-sectional study conducted in USA found a significant inverse association between use of a fireplace or wood-burning stove and current asthma (Nguyen et al., 2010).

The study conducted in Serbia including more than 1000 never-smoking women showed that the exposure to indoor biomass fuels was not associated with an increased risk of asthma (Stanković et al., 2011).

3.2.7. Respiratory symptoms

Four studies considered the association between solid fuel combustion exposure and respiratory symptoms.

The cohort study conducted in USA among 888 pregnant women found a significant association between the indoor fireplace use and cough (OR 1.05, 95% CI 1.01–1.09) for each 1-h per day increase in source use. Null associations were found considering other outcomes (i.e. wheezing, runny/stuffy nose, phlegm, chest tightness) as well as exposure to woodstove (Triche et al., 2005).

In the study conducted in Serbia, the use of biomass fuels was associated with a significant increased risk of cough and shortness of breath (ORs 1.36, 95% CI 1.07–1.74 and1.40, 95% CI 1.12–1.75 respectively). Null association was found considering phlegm, wheezing and runny/stuffy nose (Stanković et al., 2011).

The cross-sectional study conducted in Germany showed that the exposure to cooking by open coal, wood or wood fire, but not heating, was significantly associated with bronchial hyperresponsiveness (OR 4.21, 95% CI 1.05–16.89) (Nowak et al., 1996).

A cross-sectional study conducted in Finland, found no association between wood stove heating at age 0–6 years and Wheezing at age 18–25 years and up to young adulthood (OR 1.01, 95% CI 0.71–1.42) (Kilpeläinen et al., 2001).

3.2.8. Risk of bias appraisal

In the Supplementary Table S2, we present an evaluation of risk of bias.

In four out of 7 domains (i.e. departure from exposure, missing data, outcome assessment and reported results) the RoB was judged to be low to moderate. Critical RoB was found in the exposure assessment domain in one of the studies because the exposure was assessed by questionnaire. Three studies were judged as having serious/moderate RoB because the authors collected more information through questionnaire regarding the exposure (e.g. duration, length). In the other 2 domains, i.e. confounding and selection bias, mixed results were found; the articles were judged as having from low to critical RoB. The classification of critical RoB in the confounding domain was related to a lack of any adjustment; serious RoB was attributed to studies with basic adjustments; moderate when 1 out of 4 of identified relevant confounding variables was missing.

3.3. Exposure to outdoor solid fuel burning and respiratory outcomes in adults

3.3.1. Characteristics of the epidemiological studies

In this section exposure to outdoor solid fuel burning refers to studies conducted in areas where residential solid fuel combustion is common. It represents the main source of ambient air pollution. Exposure was assessed by measuring either air pollutant levels or biomarkers of solid fuel combustion in ambient air.

Five studies assessing the impact of outdoor solid fuel exposure were conducted in Europe (Clancy et al., 2002; Hansell et al., 2016; Madsen et al., 2012; Phillips et al., 2018; Pindus et al., 2016) (2 in the UK (Hansell et al., 2016; Phillips et al., 2018) and 1 each in Norway (Madsen et al., 2012), Ireland (Clancy et al., 2002), Estonia (Pindus et al., 2016)). Four studies were conducted in USA (Browning et al., 1990; Ostro et al., 2010; Yap and Garcia, 2015) and 1 in Canada (Gan et al., 2013). Four studies were conducted in Australia (Madsen et al., 2012; Bennett et al., 2010; Bui et al., 2013; Johnston et al., 2013) and 2 studies in New Zealand (Hales et al., 2012; McGowan et al., 2002). The study designs were as follows: time-series (6 studies) (Clancy et al., 2002; Yap and Garcia, 2015; Johnston et al., 2013; Hales et al., 2012; McGowan et al., 2002; Mészáros et al., 2015), cohort (5) (Hansell et al., 2016; Ostro et al., 2010, 2015; Gan et al., 2013; Bui et al., 2013),

cross-sectional (1) (Pindus et al., 2016), case-crossover (1) (Madsen et al., 2012) and ecological (3) (Phillips et al., 2018; Browning et al., 1990; Bennett et al., 2010).

In most of the studies, the exposure was assessed by measuring the level of pollutants or biomarker of solid fuel combustion in the outdoor air that was generated by residential wood burning. More precisely: in 4 studies $PM_{2.5}$ concentrations deriving from residential wood burning were measured. In 3 out of 4 studies assessing PM_{10} concentrations, PM_{10} were measured in areas where residential wood burning accounts for about 80–90% of PM_{10} emissions. Two studies considered changes in the outcome occurrence following an intervention programme to reduce either wood or coal burning. One study measured potassium (K) levels, a biomarker of wood smoke. Finally, one study assessed the subjects' concern regarding ambient wood-smoke exposure using a questionnaire.

Three studies evaluated the exposure to coal fuel combustion. One study considered the health effects before and after an interventional programme to reduce coal combustion. One study evaluated long-term effects of black smoke (BS), sulphur dioxide (SO_2) and PM_{10} levels. Another study evaluated the long-term effect of coal burning used in 1 year.

Regarding the outcomes, studies considering outdoor exposure to solid fuels assessed both respiratory diseases and symptoms. More precisely, there were 8 studies for respiratory mortality (Clancy et al., 2002; Hansell et al., 2016; Madsen et al., 2012; Phillips et al., 2018; Ostro et al., 2010, 2015; Johnston et al., 2013; Hales et al., 2012), 2 lung cancer (Hansell et al., 2016; Phillips et al., 2018), 4 for COPD (Hansell et al., 2016; Yap and Garcia, 2015; Gan et al., 2013; Mészáros et al., 2015), 5 studies for asthma (Pindus et al., 2016; Browning et al., 1990; Bennett et al., 2010; Bui et al., 2013; Phillips et al., 2018), 5 for other respiratory diseases (Hansell et al., 2016; Phillips et al., 2018; Pindus et al., 2016; Browning et al., 1990; McGowan et al., 2002). Three studies assessed other respiratory symptoms (Pindus et al., 2016; Browning et al., 1990; Bennett et al., 2010) (Table 2).

In the following sections we present results for selected respiratory outcomes: overall respiratory mortality, lung cancer, COPD, asthma, other respiratory diseases and respiratory symptoms.

3.3.2. *Outdoor air pollutant levels*

Six studies assessed outdoor PM_{10} levels measured in areas where residential solid fuel combustion was very common (Browning et al., 1990; Bennett et al., 2010; Johnston et al., 2013; Hales et al., 2012; McGowan et al., 2002; Mészáros et al., 2015). The ecological study conducted in the USA in 1987–1988 by Browning et al. (1990) compared subjects living in a high wood smoke area (mean PM_{10} levels from 72 to 41 $\mu g/m^3$) to subjects living in a low wood smoke area (mean PM_{10} levels from 41 to 22 $\mu g/m^3$). Three studies were performed in the same area in Australia (Tasmania) (Browning et al., 1990; Bennett et al., 2010; Mészáros et al., 2015). The time series study performed by Meszaros et al. (Mészáros et al., 2015), showed a reduction in PM_{10} levels over time from 1992 to 2002, from 50.7 to 16.5 $\mu g/m^3$ (mean daily concentration). The ecological study performed by Bennett et al. (2010) compared subjects living in a polluted area because of high wood-burning stove use (PM_{10} levels 57.5 $\mu g/m^3$) to subjects living in a less polluted area (PM_{10} levels 27 $\mu g/m^3$).

The study by Johnson et al. (Johnston et al., 2013) showed a significant change in air quality following a intervention to decrease wood-stove use (a communication campaign). The annual mean concentration of PM_{10} before the intervention (1997–2000) was 23.7 $\mu g/m^3$. This was higher ($P < 0.001$) than the annual mean of 18.4 $\mu g/m^3$ after the intervention (2001–2007). There was also a significant ($P < 0.001$) decrease in the wintertime mean concentration of PM10 from 43.6 $\mu g/m^3$ before the intervention period to 27.0 $\mu g/m^3$ after the intervention. Two studies (Hales et al., 2012; McGowan et al., 2002) were conducted in New Zealand (study period 1988–1993 and 1988–1998 respectively) in an area dominated by PM from solid fuel domestic heating with annual mean PM_{10} levels of 28 and 25 $\mu g/m^3$ respectively (Hales et al., 2012; McGowan et al., 2002).

Three studies measured outdoor $PM_{2.5}$ levels (Pindus et al., 2016; Yap and Garcia, 2015; Ostro et al., 2015). The study conducted by Yap et al. examined the impact of 'Rule 4901', aimed at reducing residential wood burning, in the San Joaquin Valley Air Basin in California (Yap and Garcia, 2015). After implementation, reductions of 12% in $PM_{2.5}$ levels ($PM_{2.5}$ before 'rule 4910' (2000–2003) annual mean 30.76 (SD 622.88) $\mu g/m^3$, After 'rule 4910' (2003–2006) 26.10 (SD 616.56) $\mu g/m^3$), and 8% in $PM_{2.5\text{-}10}$ levels (before 'rule 4910' 19.02 (SD 616.91) $\mu g/m^3$, after 'rule 4910' 14.63 (SD 612.09) $\mu g/m^3$) were observed (Yap and Garcia, 2015). In the study conducted by Pindu et al. in Estonia from 2011 to 2012, low levels of $PM_{2.5}$ were detected (annual mean 2.3, SD 0.9 $\mu g/m^3$ (Pindus et al., 2016). In the cohort study performed by Ostro, annual mean PM2.5 levels from woodsmoke were estimated (Mean-Median (25th-75th percentile) 1.4–0.9 (0.5–1.8) μg/m3).

In the city of Dublin, Ireland, great improvements in air quality were observed after the introduction of domestic coal burning regulations in 1990 when the Irish Government banned the marketing, sale, and distribution of bituminous coals. Average annual BS concentrations declined by 35.6 $\mu g/m^3$ (70%) from 50.2 $\mu g/m^3$ between 1984 and 1990 to 14.6 between 1990 and 1996 $\mu g/m^3$ [34]. Similarly, in the longitudinal study conducted in England and Wales, median air pollution exposures to BS and SO_2 were twofold to threefold higher in 1971 compared with 1991. Ranges (10th–90th centiles) for BS were 18.5–70.5 $\mu g/m_3$ in 1971 and 3–19 $\mu g/m_3$ in 1991 (Hansell et al., 2016).

3.3.3. *Overall respiratory mortality*

Four papers, accounting for 3 studies, considered the association between woodsmoke exposure and respiratory mortality. Two papers considered the effect of coal burning on overall respiratory mortality. In addition, two studies aimed to investigate the effect of reducing household solid fuel combustion (1 on wood and 1 on coal) on air quality and health.

In a prospective cohort study performed in California-including more than 101,000 current and former female teachers and administrators-an interquartile range (IQR) increase of 1.3 $\mu g/m^3$ in $PM_{2.5}$, deriving from wood smoke, was not associated with an increased pulmonary mortality (HR 0.97, 95% CI 0.90–1.04). Similarly, an IQR increase of Ultrafine particulate (332 ng/m^3) was not associated with an increased pulmonary mortality (HR 0.95, 95% CI 0.89–1.02) (Ostro et al., 2015). A non-significant increased risk was found in the same cohort study considering exposure to K, as a marker of biomass burning exposure for participants whose residential addresses were both within 8 and 30 km of a monitor (HRs 1.22, 95% CI 0.82–1.82 and 1.24, 95% CI 0.99–1.55 respectively) (Ostro et al., 2010).

To address possible short-term impact on respiratory mortality by air pollution a time-stratified case-crossover study was conducted to estimate associations of wood burning and daily respiratory mortality during a period of 10 years among residents above 50 years of age in Oslo, Norway. An increase of 10 $\mu g/m^3$ in $PM_{2.5}$ exposure, deriving from urban traffic pollutants together with wood burning, was associated with a non-significant increased respiratory mortality (Madsen et al., 2012).

Another study, conducted in Christchurch, New Zealand, affected by air pollution deriving especially from household fires, found that an increase of 10 $\mu g/m^3$ in PM_{10} exposure was associated (after a lag of one day) with a moderate significant increase in respiratory mortality (risk estimate 1.004, 95% CI 1.001–1.006) (Hales et al., 2012).

An ecological study conducted across England and Wales, showed that a standard deviation increase in domestic coal use in 1951–1952 was significantly associated with respiratory mortality (RR 1.127, 95% CI 1.118–1.137) from 1993 to 2012 (Phillips et al., 2018).

Similarly, a large national study (England and Wales) evaluated long-term effects on mortality. Air pollution concentrations (BS and SO_2 arising mainly from domestic and industrial coal and fossil fuel from the 1950s to the 1990s in the UK) were estimated at residence for 1971,

1981, 1991 and PM_{10} in 2001 in relation to mortality up to 2009 in 367,658 members of the longitudinal survey (1% sample of the English Census). BS and SO_2 exposures were associated with mortality decades after exposure—BS exposure in 1971 was significantly associated with respiratory (OR 1.05,95% CI 1.01–1.09) mortality in 2002–2009 (per 10 $\mu g/m^3$ increase). Largest effect sizes were seen for more recent exposures (Hansell et al., 2016). Consistent results were found considering specific-cause mortality.

In the city of Dublin, Ireland, the great improvements in air quality resulted in a reduction in respiratory deaths by 15·5% (95% CI 12–19, p < 0·0001) (Clancy et al., 2002).

Johnston et al. conducted a time series study in Australia with the aim to assess the effect of reductions in air pollution from biomass smoke following community education campaigns, enforcement of environmental regulations, and a wood heater replacement programme on daily overall mortality (Johnston et al., 2013). The period of improved air quality following the intervention was associated with small non-significant reductions in annual respiratory mortality. Only in males the observed reductions in respiratory mortality were larger and significant (−22.8%, 95% CI −40.6%, 0.3%; p = 0.05) (Johnston et al., 2013).

3.3.4. Lung cancer

The 2 studies study conducted across England and Wales, showed an increased lung cancer mortality among people living in areas with high coal burning (Hansell et al., 2016; Phillips et al., 2018). In the study by Hansell et al., BS and SO_2 were associated with an increased lung cancer mortality risk after decades from the exposure (ORs 1.05, 95% CI 1.02–1.09 and 1.04, 95% CI 1.02–1.05 respectively) (Hansell et al., 2016). Similarly, in the study by Phillips et al., exposure to domestic coal burning in early life was associated with a significant increased mortality risk of trachea, bronchus and lung cancer in adulthood (RR 1.126, 95% CI 1.115, 1.137) (Phillips et al., 2018).

3.3.5. COPD

Three studies considered the association between woodsmoke exposure and hospital admission and mortality due to COPD (Yap and Garcia, 2015; Gan et al., 2013; Mészáros et al., 2015). One study considered the association between marker of exposure to coal combustion (BS and SO_2) and COPD mortality.

The study conducted by Yap et al. examined the impact of 'Rule 4901', aimed at reducing residential wood burning, on PM levels and COPD hospitalizations in the San Joaquin Valley Air Basin in California. Reductions in rates of hospital admissions for COPD were observed (10% and 7% in subjects 45–64 years old and over 65 years respectively), although the adjusted rate ratios were not statistically significant (Yap and Garcia, 2015).

The study conducted in Canada found a significant association between woodsmoke exposure and COPD hospitalization (RR 1.15,95% CI 1.02–1.29) but not COPD mortality (RR 0.81, 95% CI 0.64–1.03) for the highest vs the lowest level of exposure (Gan et al., 2013). A time-series study found a non-significant association between an increase of 10 $\mu g/m^3$ increase in exposure to PM_{10} from wood-heaters and COPD hospital admissions in Australia (p = 0.70) (Mészáros et al., 2015).

Exposure to BS and SO_2 in 1971 was significantly associated with an increased COPD mortality in following decades in the study conducted in England and Wales (ORs 1.07, 95% CI 1.03–1.11 and 1.03, 95% CI 1.01–1.04 respectively) (Hansell et al., 2016).

3.3.6. Asthma

Four studies evaluated the association between exposure to outdoor wood smoke and asthma. Two studies were performed in the same population, in Australian areas, in which winter air pollution is dominated by PM from solid fuel domestic heating (Pindus et al., 2016; Browning et al., 1990; Bennett et al., 2010; Bui et al., 2013). Only one study considered coal burning as an exposure (Phillips et al., 2018).

The cross-sectional study conducted within the framework of RHINE III in Estonia found a non-significant association between wood residential heating induced $PM_{2.5}$ and asthma (OR 1.16, 95% CI 0.60–2.19) (Pindus et al., 2016).

There was no association between a 1-unit increase in wood smoke exposure level and the prevalence of current asthma in the Tasmanian Longitudinal Health Study in Australia. A statistically significant but modest association was observed between wood smoke exposure and asthma severity (OR 1.11; 95% CI 1.02–1.20) (Bui et al., 2013). No association between wood smoke exposure and asthma attack was found in the same study with a longer follow up (OR 0.99, 95% CI 0.63–1.54) (Bennett et al., 2010).

A cross-sectional study conducted in USA found no positive association between ambient wood smoke exposure and asthma in subjects aged more than 45 years (OR 0.98, 95% CI 0.46–2.05 for 45–64 years old and OR 0.72, 95% CI 0.26–1.99 for ≥65 years old). A non-significant increase was found in subjects between 15 and 44 years old (OR, 1.35, 95% CI 0.67–2.71) (Browning et al., 1990).

In an ecological study conducted in England and Wales domestic coal consumption in 1951–1952 was significantly associated with asthma mortality (RR 1.093, 95% CI 1.032–1.158 for SD increase in domestic usage) after decades (Phillips et al., 2018).

3.3.7. Other respiratory diseases

An IQR increase of 14.8 $\mu g/m^3$ in PM_{10} levels was associated with higher hospital admissions for respiratory diseases in an area in New Zealand in which more than 90% of particulate air pollution has been estimated to come from the city's 47,000 wood burners and open fires that are used during the cold winters months (percentage increase 3.37, 95% CI 2.34–4.4) (McGowan et al., 2002).

A cross-sectional study conducted in USA found a non-significant association between ambient wood smoke exposure and absence due to respiratory diseases in subjects highly exposed to wood smoke compared to subjects exposed to low wood smoke (OR 1.30, 95% CI 0.55–3.09 for 45–64 years old and 1.55, 95% CI 0.58–4.16 for ≥65 years old) (Browning et al., 1990).The cross-sectional study conducted within the framework of RHINE III in Estonia found a significant inverse association between wood residential heating induced $PM_{2.5}$ and allergic rhinitis (OR 0.63, 95% CI 0.42–0.94) (Pindus et al., 2016).

BS and SO_2 were associated with a significant increased mortality risk due to respiratory infections in England and Wales (Hansell et al., 2016). In addition, domestic coal consumption in 1951–1952 was significantly associated with pneumonia mortality after decades (Phillips et al., 2018).

3.3.8. Respiratory symptoms

Three studies evaluated the association between exposure to ambient woodsmoke and respiratory symptoms in adults.

The study conducted in Estonia found no association between an increase of 2.3 $\mu g/m^3$ in $PM_{2.5}$ levels and respiratory symptoms considered (i.e. attack of cough, wheeze without cold, attack of breathlessness, chest tightness) (Pindus et al., 2016). Similarly, in the studies conducted by Browning et al. (1990) and Bennet et al. (Bennett et al., 2010), exposure to ambient woodsmoke was not significantly associated with any respiratory symptoms considered (e.g. cold, wheeze, cough).

3.3.9. Risk of bias appraisal

In the Supplementary Table S3, we present an evaluation of risk of bias.

In six out of 7 domains (excluding the measurement of exposure) the RoB was judged to be low in most of the studies. Serious RoB was found in the measurement of the outcome domain in 3 studies, because the authors used a non-standardised questionnaire for collecting health data. Moderate RoB was found in the exposure assessment domain in almost all studies. In the only article with critical RoB in this domain,

exposure was assessed by mailed questionnaire including a question regarding the subjective perception of living in an area impacted by wood smoke. The only study with critical RoB in the confounding domain did not adjust for any covariate.

4. Discussion

The epidemiological evidence on the association between indoor solid fuel combustion exposure and lung cancer risk in Europe, North America, Australia and New Zealand is still limited. Among the 4 studies considering the association between coal exposure and lung cancer (one study included in the pooled case-control study), the large multicentric study conducted in North America and Europe is the most informative and found a significant association (Hosgood et al., 2010). However, consistent results were shown in the other included studies. Among the 2 studies considering exposure to wood burning, a significant 20% increased risk was found among those who used wood for cooking or heating in the pooled case-control study (Hosgood et al., 2010). Exposure to mixed solid fuel burning was modestly associated with lung cancer risk, although this was based on just 3 studies.

The 2 studies considering the relationship between solid fuel combustion exposure and COPD risk found a significant increased association. Inconsistent and limited results were found considering different type of solid fuels and different LRIs (i.e. pneumonia and bronchitis), URIs (e.g. sore throat, sinusitis) and the upper respiratory tract diseases. A few epidemiological studies evaluated the association between indoor wood burning exposure and asthma showing inconsistent results. Limited and inconsistent results were found considering solid fuel exposure and respiratory symptoms.

Only a few studies evaluated the relationship between exposure to outdoor wood burning exposure and risk of respiratory outcomes in adults. There were inconsistent results for both overall respiratory mortality and specific-cause mortality, asthma, COPD, other respiratory diseases and respiratory symptoms. The available epidemiological evidence between outdoor coal burning and respiratory outcomes is very limited with only 3 studies considering such an association (Clancy et al., 2002; Hansell et al., 2016; Phillips et al., 2018). The study conducted by Philip showed that areas of the UK that had high domestic consumption of coal in 1951/1952 have raised mortality from a wide variety of causes, including respiratory diseases and certain cancers. Very few comparable data exist in the literature, particularly with such a long follow-up period. Similarly, the study by Hansell et al. (2016) showed that exposure to BS and SO_2 (mainly arising from domestic and industrial coal and fossil fuel from the 1950s to the 1990s in the UK, although no specific emission from domestic coal was considered) were associated with respiratory mortality and cause specific mortality decades after exposure. Some of the included studies evaluated the impact of the introduction of measures in order to reduce solid fuel burning on air quality and health. The studies consistently showed an improvement in air quality resulting in respiratory outcome reduction.

Previously available systematic reviews and meta-analyses on domestic use of solid fuel exposure included studies performed in LMICs where the use of unvented, inefficient, leaky and inexpensive stoves used in very poor housing conditions is most common and are therefore not directly comparable with this review. The use of solid fuel combustion in such conditions results in high indoor pollutant levels. In 2010, the IARC (IARC Working Group, 2010) classified coal as a carcinogenic to humans (group 1) and biomass use (mainly wood) as a group 2A carcinogen due to limited evidence. Most of the studies considered were conducted in China. A later systematic review and meta-analysis on the relationship between solid fuel use and lung cancer showed that coal smoke had a greater association with lung cancer (OR 1.82, 95% CI 1.60–2.06) than biomass smoke (OR 1.50, 95% CI 1.17–1.94). A greater risk was found in women (OR 1.81, 95% CI 1.54–2.12) compared to men. However, also in this case most of the included studies were conducted in China (Kurmi et al., 2012b).

Another systematic review and meta-analysis of epidemiological studies found positive associations between the use of solid fuels and COPD (OR = 2.80, 95% CI 1.85-4.0) and chronic bronchitis (OR = 2.32, 95% CI 1.92-2.80). Almost all studies were conducted in LMICs (Kurmi et al., 2010). An updated meta-analysis including 24 studies found that biomass smoke increased the risk of COPD diagnosis in women (OR 1.38, 95% CI 1.28-1.57) (Sana et al., 2018) as well as of chronic bronchitis.

Another meta-analysis found that biomass fuels was associated with chronic bronchitis and COPD in women but not with asthma (Po et al., 2011).

4.1. Strengths and Limitations

To our knowledge this is the first systematic review conducted on the association between solid fuel burning exposure and respiratory health in adults in Europe, North America, Australia and New Zealand. We included both indoor and outdoor exposure to all solid fuels, biomass, and coal, and all the respiratory outcomes in adults, including both respiratory diseases and respiratory symptoms. This review is therefore comprehensive. However, due to the limited available studies in addition to the heterogeneity of the type of solid fuels as well as the outcomes considered, we were not able to conduct a meta-analysis. Nevertheless, we believe that the inclusion of studies conducted in Europe, North America, Australia and New Zealand with fairly similar cultural and lifestyle habits and health status could reduce the likelihood of heterogeneity.

Most of the studies evaluating the association between indoor exposure to solid fuel burning and respiratory health in adults had either a case-control or cross-sectional design. Only 1 prospective cohort studies (Triche et al., 2005) and one nested case-control study within a cohort study (Thorn et al., 2001) were included. The 2 studies found a null association between wood combustion exposure and risk of asthma, URIs and respiratory outcomes. These results were consistent with case-control and cross-sectional studies which considered the same outcomes (Kilpeläinen et al., 2001; Stanković et al., 2011; Nowak et al., 1996; Nguyen et al., 2010).

In addition, sources of bias could affect the association between solid fuel combustion exposure and respiratory health in adults. Selection bias has been identified as an important bias of the available evidence mainly due to low response-rate in many of the included studies which could distort such an association. Also, some of the case-control studies used hospital-based controls which could result in a weaker association, whereas others used population-based controls. Nevertheless, the results were consistent between the control selection. The only exception is represented by the population-based case-control study conducted in Canada finding that the use of a fireplace in previous 12 months was inversely associated with pneumonia hospitalization (Loeb et al., 2009). Misclassification of the exposure might affect this result rather than the selection of the controls. In fact, the measurement of exposure to indoor solid fuels was identified as the main source of bias as the exposure was assessed by self-reported questionnaire without quantitative environmental measurements. As a result, misclassification of exposure status cannot be ruled out as self-reported exposure is subject to recall bias. In the study by Triche et al. (2005) indoor monitors for measuring NO_2 and SO_2 levels were placed in the main living area of some homes for 2 weeks. Median levels of SO_2 and NO_2 in monitoring periods with wood stove use did not differ as compared with periods with no use.

In addition, in case-control and cross-sectional studies, subjects with respiratory problems could reduce solid fuel use for cooking and/or heating resulting in an underestimation of the deleterious effects of solid fuel combustion. Furthermore, questionnaires varied across studies, so the degree of misclassification bias also may have varied (Hosgood et al., 2010). Likewise, in the studies in which the outcome was assessed by questionnaire, although some of them utilised standardised and validated questionnaires, misclassification of the ascertainment of the

outcome cannot be excluded. However, in most of the studies cases were ascertained through hospital registries or through active monitoring of pathology department records resulting in a low risk of bias.

Another limitation of the current evidence is that some of the included studies did not report the information on the type of solid fuel used for cooking or heating. Many of the studies evaluated the association between specific solid fuels (wood or coal) and respiratory health in adults. Nevertheless, the reported associations could be due to the exposure to mixed fuels. In the study by Oronzco et al. (Orozco-Levi et al., 2006), either the exposure to wood or charcoal alone increased the risk of COPD, but only the combination of both was statistically significant.

Despite heating with solid fuels in HICs being more common than cooking with solid fuels, some of the studies reported risk estimates for both heating and cooking with solid fuels. The multicentric case-control study in Eastern Europe and the UK by Lissowa et al. (Lissowska et al., 2005) suggested that the lung cancer risk was principally due to cooking. Similarly, cooking with solid fuel was associated with a significant increase of bronchial hyperresponsiveness (Nowak et al., 1996). This might be that the exposures from cooking and heating differ because of different conversion technologies. Conversely, the study conducted by Ramanakur et al. (Ramanakumar et al., 2007) showed a slightly higher lung cancer risk associated with heating with solid fuels compared to cooking with solid fuels. Another important limitation of the reviewed evidence is that in most studies the exposure to indoor solid fuels was reported as a binary exposure (exposed or not exposed). Only a few studies categorized the exposure to solid fuels into different categories considering the frequency and/or duration of use. In the study conducted by Triche et al. (2005) each hour increase of woodstove use was not associated with lower and upper respiratory symptoms. Conversely, the association between length of the exposure (both as a continuous or categorical variable) and COPD suggested a dose–response pattern in the study conducted by Oronczo et al. (Orozco-Levi et al., 2006). In addition, intensity of exposure both in summer and winter was also related to COPD. Subjects exposed to solid fuel burning for more than 12–24 h/day had an increased significant COPD risk compared to subjects exposed 0–6 h/day. Past or more recent exposure did not differ as risk factors for COPD (Orozco-Levi et al., 2006). In the multicentric case-control study, the risk of lung cancer increased with percentage of the lifetime that solid fuels were used for cooking (**p=< 0.0001**) and decreased with time since switching from solid fuels to modern fuels (p=0.001) (Lissowska et al., 2005). In the study by Malats et al. (2000) exposure to wood burning >20 years was associated to an increased lung cancer risk. On the contrary the exposure to coal burning >17 years was not associated with an increased lung cancer risk.

In addition, other important factors that can affect the indoor pollutant levels derived from solid fuel combustion are particular features of the house, such as ventilation, type of device used to burn solid fuels, time spent indoors and the presence or absence of a vent or chimney. Unfortunately, no information was available regarding these features of the homes.

Tobacco smoking represent the main risk factor for respiratory diseases. Eight studies adjusted for tobacco smoking (Orozco-Levi et al., 2006; Thorn et al., 2001; Sood et al., 2010; Kim and Hanley, 2002; Ramanakumar et al., 2007; Hosgood et al., 2010; Sloan et al., 2012; Lissowska et al., 2005). Five of them found an association with lung cancer (Ramanakumar et al., 2007; Hosgood et al., 2010), nasal polysps (Kim and Hanley, 2002), COPD (Orozco-Levi et al., 2006; Sood et al., 2010). The study conducted by Thorn found null association with asthma (Thorn et al., 2001). However, subgroup analyses by smoking status performed in 2 studies (Ramanakumar et al., 2007; Hosgood et al., 2010) showed higher risk of developing lung cancer risk in ever or medium/high smokers compared to no or no/low smokers showing that residual confounding for tobacco smoking cannot be excluded. Stratified analyses by smoking status were performed by Wu et al. (1985). A greater lung cancer risk was found among ex or current smokers. Nevertheless, coal burning use was significantly associated with an increased lung cancer risk among never smokers. However, it is difficult to understand whether the increased risk is due to residual confounding by smoking or to an interaction between solid fuel and smoking. In the study by Sood et al. (2010) the additive effect of cigarette smoke and wood smoke was evaluated, and a greater risk of COPD was found among subjects exposed to cigarette smoking together with wood burning.

Four studies included non-smokers only (Stanković et al., 2011; Nguyen et al., 2010; Triche et al., 2005; Malats et al., 2000). The exposure to indoor solid fuel combustion was not associated with an increased risk of respiratory diseases and symptoms. A significant increased risk was found with cough and shortness of breath (Stanković et al., 2011). In the study conducted by Malats et al. (2000), the exposure to wood burning, but not coal burning, was associated with an increased risk of lung cancer in non-smokers. However, occasional smokers (smokers of up to 400 cigarettes in their lifetime), were considered together with non-smokers. In addition to smoking status, another important confounding variable is represented by environmental tobacco smoke (ETS) exposure which is a recognised risk factor for both acute and chronic respiratory illness, although it is difficult to quantify the second-hand exposure and was generally not measured in the included studies. Three studies considering such covariates (Kilpeläinen et al., 2001; Stanković et al., 2011; Ramanakumar et al., 2007) found inconsistent results.

Among the other relevant confounding factors to be considered, socio-economic status (SES) represents an important covariate to be considered. Families with high SES tend to use clean fuels compared to solid fuels for heating. Some of the epidemiological studies adjusting for such a covariate, in addition to smoking status (Kilpeläinen et al., 2001; Stanković et al., 2011; Nguyen et al., 2010; Sood et al., 2010; Triche et al., 2005; Ramanakumar et al., 2007; Hosgood et al., 2010; Lissowska et al., 2005), found null or inverse association between solid fuel exposure and respiratory health in adults (Kilpeläinen et al., 2001; Nguyen et al., 2010; Triche et al., 2005), whereas other studies found positive associations (Stanković et al., 2011; Sood et al., 2010; Ramanakumar et al., 2007; Hosgood et al., 2010; Lissowska et al., 2005).

Because total exposure is a result of both indoor and outdoor exposure, we also included in this systematic review the epidemiological studies considering the association between outdoor exposure to solid fuel burning deriving from indoor combustion in community burning wood or coal for heating and cooking, of which we identified 16 studies.

As compared to studies evaluating the indoor exposure to solid fuel combustion in which the exposure was assessed by questionnaire, in the studies considering the exposure to outdoor solid fuel burning, pollutant levels were measured. Nevertheless, exposure misclassification can occur -all studies were judged to have RoB in the measurement of exposure domain-because the exposure to air pollutants deriving from solid fuel burning were attributed to participants based on their residential address. In addition, different air pollutants were measured with some studies assessing $PM_{2.5}$, others PM_{10} and others constituent of $PM_{2.5}$ such as K, used as a marker of wood burning exposure. A few studies measuring $PM_{2.5}$ levels, considered to be a better indicator of exposure to lower airways, found mixed results (Pindus et al., 2016; Ostro et al., 2015). In addition, different methods have been used to present results (such as a change in PM levels per 10 $\mu g/m^3$ or IQR change in level of particulate or tertile of exposure). These differences make it difficult to compare the results from various studies. Despite outdoor monitoring stations being used in the studies to measure air pollutant levels and we included studies conducted in area where residential solid fuel combustion is common and it represents the main source of ambient air pollution, the contribution from other outdoor sources such as fossil fuels, industrial sources or motor vehicle exhaust while commuting should be considered. Almost all studies addressed the relationship between outdoor pollutant levels from solid fuel burning and respiratory health and did not consider the role of other pollutants

and/or sources. The study by Pindu et al. (Pindus et al., 2016) considered the association between $PM_{2.5}$ level and several respiratory symptoms adjusting for the exposure to PM_{10} from traffic. A non-significant association was found. Also, a careful evaluation of indoor solid fuel exposure smoke exposures is needed to evaluate the impact of the exposure to solid fuels burning on health (Yap and Garcia, 2015). The studies measuring ambient air pollutant levels (i.e. BS, SO_2, PM_{10}, $PM_{2.5}$ and PM_{coarse}) showed a significant improvement in air quality before and after interventions with the aim to reduce solid fuel burning. Furthermore, the studies measuring ambient air pollutant levels during the study period showed a clear reduction in PM_{10}, BS and SO_2 levels over time.

Another limitation of the available evidence is that in most studies participants were enrolled and the diagnosis was confirmed from health insurance administrative databases; as a result, some individual important risk factors, such as active or passive smoking status and individual SES can be missed (Gan et al., 2013).

5. Conclusions

It is well known that solid fuel burning can emit very high level of air pollutants in the air and studies have shown that the reduction in solid fuel burning improves the air quality (Clancy et al., 2002; Yap and Garcia, 2015; Johnston et al., 2013).

However, the epidemiological evidence on the association between indoor and outdoor exposure to pollution from solid fuel combustion and respiratory health in adults in Europe, Canada, USA, Australia and New Zealand is still limited. Positive associations were found between indoor solid fuel combustion exposure and lung cancer risk. Significant association was found between indoor solid fuel exposure and COPD risk. Inconsistent and limited results were found considering different types of solid fuels and LRIs, URIs and other upper respiratory tract diseases, asthma and respiratory symptoms.

Only a few studies evaluated the relationship between exposure to outdoor wood burning exposure and risk of respiratory outcomes in adults with inconsistent results for overall respiratory mortality, asthma, COPD and respiratory symptoms. The available epidemiological evidence between outdoor coal burning exposure and respiratory outcomes suggests an increased risk in adverse respiratory effects. However, the evidence is very limited because only 3 studies considered such an association. Two out of the 3 studies included a big sample size with a long-term follow up (Phillips et al., 2018; Hansell, 2018) and they showed a positive association.

The studies considering the impact of the introduction of measures in order to reduce solid fuel burning on air quality and health showed an improvement in air quality resulting in a reduction of adverse respiratory effects.

The identified epidemiological studies have several limitations including the potential misclassification of exposure. Also, errors arising from the outcome being assessed by questionnaire-based self-reports only as well as residual confounding cannot be excluded. In addition, the information on a dose-response relationship is almost always missing, making it difficult to interpret the results. Additional and better conducted epidemiological studies are needed to establish whether both outdoor and indoor solid fuel combustion exposure is associated with adverse respiratory outcomes in adults. In particular, the exposure to solid fuel burning should be evaluated by more objective quantitative measurements of indoor air pollutants (i.e. $PM_{2.5}$) emitted from solid fuel combustion. In particular, individual exposure could be assessed as is becoming easy and cheaper due to the application of low-cost and advanced technology monitors (Steinle et al., 2015). In addition information on the devices used to burn solid fuels and duration/frequency of use as well as information on house characteristics such as ventilation should be considered. Adjustment for confounding variables considering the SES and second-hand smoking in home, and prospective study design are needed in order to establish a causal relationship between indoor exposure to solid fuels and respiratory outcomes in adults.

CRediT authorship contribution statement

Valentina Guercio: Conceptualization, Methodology, Data curation, Writing – original draft, preparation, Visualization, Supervision, Writing – review & editing. **Artemis Doutsi:** Data curation, Writing – review & editing. **Karen S. Exley:** Conceptualization, Writing – review & editing.

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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.2022.113926.

References

Adeloye, D., Chua, S., Lee, C., et al., 2015. Global and regional estimates of COPD prevalence: systematic review and meta-analysis. J. Glob. Health 5, 020415.
Bennett, C.M., Dharmage, S.C., Matheson, M., et al., 2010. Ambient wood smoke exposure and respiratory symptoms in Tasmania, Australia. Sci. Total Environ. 409, 294–299.
Browning, Kg Jm, Koenig, Q., Harvey, Checkoway, Timothy, V., Larson, V., William E. Pierson, 1990. A questionnaire study of respiratory health in areas of high and low ambient wood smoke pollution. Pediatr. Asthma Allergy Immunol. 4.
Bui, D.S., Burgess, J.A., Matheson, M.C., et al., 2013. Ambient wood smoke, traffic pollution and adult asthma prevalence and severity. Respirology 18, 1101–1107.
Clancy, L., Goodman, P., Sinclair, H., Dockery, D.W., 2002. Effect of air-pollution control on death rates in Dublin, Ireland: an intervention study. Lancet 360, 1210–1214.
Collaborators, G.B.D.R.F., 2018. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392, 1923–1994.
Fatmi, Z., Coggon, D., 2016. Coronary heart disease and household air pollution from use of solid fuel: a systematic review. Br. Med. Bull. 118, 91–109.
Gan, W.Q., FitzGerald, J.M., Carlsten, C., Sadatsafavi, M., Brauer, M., 2013. Associations of ambient air pollution with chronic obstructive pulmonary disease hospitalization and mortality. Am. J. Respir. Crit. Care Med. 187, 721–727.
Guercio, V., Pojum, I.C., Leonardi, G.S., et al., 2021. Exposure to indoor and outdoor air pollution from solid fuel combustion and respiratory outcomes in children in developed countries: a systematic review and meta-analysis. Sci. Total Environ. 755, 142187.
Hales, S., Blakely, T., Woodward, A., 2012. Air pollution and mortality in New Zealand: cohort study. J. Epidemiol. Community Health 66, 468–473.
Hansell, A., Ghosh, R.E., Blangiardo, M., et al., 2016. Historic air pollution exposure and long-term mortality risks in England and Wales: prospective longitudinal cohort study. Thorax 71, 330–338.
Hosgood 3rd, H.D., Boffetta, P., Greenland, S., et al., 2010. In-home coal and wood use and lung cancer risk: a pooled analysis of the International Lung Cancer Consortium. Environ. Health Perspect. 118, 1743–1747.
Hystad, P., Duong, M., Brauer, M., et al., 2019. Health effects of household solid fuel use: findings from 11 countries within the prospective urban and rural epidemiology study. Environ. Health Perspect. 127, 57003.
Johnston, F.H., Hanigan, I.C., Henderson, S.B., Morgan, G.G., 2013. Evaluation of interventions to reduce air pollution from biomass smoke on mortality in Launceston, Australia: retrospective analysis of daily mortality, 1994-2007. Bmj 346, e8446.
Kilpeläinen, M., Koskenvuo, M., Helenius, H., Terho, E., 2001. Wood stove heating, asthma and allergies. Respir. Med. 95, 911–916.
Kim, J., Hanley, J.A., 2002. The role of woodstoves in the etiology of nasal polyposis. Arch. Otolaryngol. Head Neck Surg. 128, 682–686.
Kurmi, O.P., Semple, S., Simkhada, P., Smith, W.C., Ayres, J.G., 2010. COPD and chronic bronchitis risk of indoor air pollution from solid fuel: a systematic review and meta-analysis. Thorax 65, 221–228.
Kurmi, O.P., Lam, K.B., Ayres, J.G., 2012a. Indoor air pollution and the lung in low- and medium-income countries. Eur. Respir. J. 40, 239–254.
Kurmi, O.P., Arya, P.H., Lam, K.B., Sorahan, T., Ayres, J.G., 2012b. Lung cancer risk and solid fuel smoke exposure: a systematic review and meta-analysis. Eur. Respir. J. 40, 1228–1237.

Liberati, A., Altman, D.G., Tetzlaff, J., et al., 2009. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. Bmj 339, b2700.
Lissowska, J., Bardin-Mikolajczak, A., Fletcher, T., et al., 2005. Lung cancer and indoor pollution from heating and cooking with solid fuels: the IARC international multicentre case-control study in Eastern/Central Europe and the United Kingdom. Am. J. Epidemiol. 162, 326–333.
Loeb, M., Neupane, B., Walter, S.D., et al., 2009. Environmental risk factors for community-acquired pneumonia hospitalization in older adults. J. Am. Geriatr. Soc. 57, 1036–1040.
Madsen, C., Rosland, P., Hoff, D.A., Nystad, W., Nafstad, P., Naess, O.E., 2012. The short-term effect of 24-h average and peak air pollution on mortality in Oslo, Norway. Eur. J. Epidemiol. 27, 717–727.
Malats, N., Camus-Radon, A.M., Nyberg, F., et al., 2000. Lung cancer risk in nonsmokers and GSTM1 and GSTT1 genetic polymorphism. Cancer Epidemiol. Biomarkers Prev. 9, 827–833.
McGowan, J.A., Hider, R.N., Chacko, E., Town, G.I., 2002. Particulate air pollution and hospital admissions in Christchurch, New Zealand. Aust. N. Z. J. Publ. Health 26, 23–29.
Mészáros, D., Markos, J., FitzGerald, D.G., Walters, E.H., Wood-Baker, R., 2015. An observational study of PM10 and hospital admissions for acute exacerbations of chronic respiratory disease in Tasmania, Australia 1992-2002. BMJ Open Respir. Res. 2, e000063.
Morgan, R.L., Beverly, B., Ghersi, D., et al., 2019. GRADE guidelines for environmental and occupational health: a new series of articles in Environment International. Environ. Int. 128, 11–12.
Naeher, L.P., Brauer, M., Lipsett, M., et al., 2007. Woodsmoke health effects: a review. Inhal. Toxicol. 19, 67–106.
Nguyen, T., Lurie, M., Gomez, M., Reddy, A., Pandya, K., Medvesky, M., 2010. The National Asthma Survey–New York State: association of the home environment with current asthma status. Publ. Health Rep. 125, 877–887.
IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Household use of solid fuels and high-temperature frying. IARC Monogr Eval Carcinog Risks Hum. 2010;95:1-430. PMID: 20701241; PMCID: PMC5046077.
Nigel. WHO Indoor Air Quality Guidelines: Household Fuel Combustion.
Nowak, D., Heinrich, J., Jörres, R., et al., 1996. Prevalence of respiratory symptoms, bronchial hyperresponsiveness and atopy among adults: west and east Germany. Eur. Respir. J. 9, 2541–2552.
Orozco-Levi, M., Garcia-Aymerich, J., Villar, J., Ramírez-Sarmiento, A., Antó, J.M., Gea, J., 2006. Wood smoke exposure and risk of chronic obstructive pulmonary disease. Eur. Respir. J. 27, 542–546.
Ostro, B., Lipsett, M., Reynolds, P., et al., 2010. Long-term exposure to constituents of fine particulate air pollution and mortality: results from the California Teachers Study. Environ. Health Perspect. 118, 363–369.
Ostro, B., Hu, J., Goldberg, D., et al., 2015. Associations of mortality with long-term exposures to fine and ultrafine particles, species and sources: results from the California Teachers Study Cohort. Environ. Health Perspect. 123, 549–556.
Pahwa, P., Karunanayake, C., Willson, P.J., et al., 2012. Prevalence of chronic bronchitis in farm and nonfarm rural residents in Saskatchewan. J. Occup. Environ. Med. 54, 1481–1490.
Pahwa, P., Karunanayake, C.P., Rennie, D.C., et al., 2017. Prevalence and associated risk factors of chronic bronchitis in First Nations people. BMC Pulm. Med. 17, 95.
Phillips, D.I.W., Osmond, C., Southall, H., Aucott, P., Jones, A., Holgate, S.T., 2018. Evaluating the long-term consequences of air pollution in early life: geographical correlations between coal consumption in 1951/1952 and current mortality in England and Wales. BMJ Open 8, e018231.
Piekarska, B., Sybilski, A.J., Krzych-Fałta, E., et al., 2018. Effect of indoor air quality on the development of rhinitis in an urban population in Poland. Allergy Asthma Proc. 39, e71–e77.
Pindus, M., Orru, H., Maasikmets, M., Kaasik, M., Jõgi, R., 2016. Association between health symptoms and particulate matter from traffic and residential heating - results from RHINE III in tartu. Open Respir. Med. J. 10, 58–69.
Po, J.Y., FitzGerald, J.M., Carlsten, C., 2011. Respiratory disease associated with solid biomass fuel exposure in rural women and children: systematic review and meta-analysis. Thorax 66, 232–239.
Ramanakumar, A.V., Parent, M.E., Siemiatycki, J., 2007. Risk of lung cancer from residential heating and cooking fuels in Montreal, Canada. Am. J. Epidemiol. 165, 634–642.
Sana, A., Somda, S.M.A., Meda, N., Bouland, C., 2018. Chronic obstructive pulmonary disease associated with biomass fuel use in women: a systematic review and meta-analysis. BMJ Open Respir. Res. 5, e000246.
Sloan, C.D., Andrew, A.S., Gruber, J.F., et al., 2012. Indoor and outdoor air pollution and lung cancer in New Hampshire and Vermont. Toxicol. Environ. Chem. 94.
Sood, A., Petersen, H., Blanchette, C.M., et al., 2010. Wood smoke exposure and gene promoter methylation are associated with increased risk for COPD in smokers. Am. J. Respir. Crit. Care Med. 182, 1098–1104.
Stanković, A., Nikolić, M., Arandjelović, M., 2011. Effects of indoor air pollution on respiratory symptoms of non-smoking women in Niš, Serbia. Multidiscip. Respir. Med. 6, 351–355.
Steinle, S., Reis, S., Sabel, C.E., et al., 2015. Personal exposure monitoring of PM2.5 in indoor and outdoor microenvironments. Sci. Total Environ. 508, 383–394.
Thorn, J., Brisman, J., Torén, K., 2001. Adult-onset asthma is associated with self-reported mold or environmental tobacco smoke exposures in the home. Allergy 56, 287–292.
Triche, E.W., Belanger, K., Bracken, M.B., et al., 2005. Indoor heating sources and respiratory symptoms in nonsmoking women. Epidemiology 16, 377–384.
Wu, A.H., Henderson, B.E., Pike, M.C., Yu, M.C., 1985. Smoking and other risk factors for lung cancer in women. J. Natl. Cancer Inst. 74, 747–751.
Yap, P.S., Garcia, C., 2015. Effectiveness of residential wood-burning regulation on decreasing particulate matter levels and hospitalizations in the San Joaquin Valley Air Basin. Am. J. Publ. Health 105, 772–778.

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Bisphenol A and declining semen quality: A systematic review to support the derivation of a reference dose for mixture risk assessments

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ABSTRACT

To support a mixture risk assessment with a focus on male reproductive health, we conducted a systematic review of associations between bisphenol A (BPA) exposures and declines in semen quality, based on animal and epidemiological studies. Contrary to a widely held view that there is "conflicting" evidence of such associations, our review and confidence rating approach reveals that animal studies provide convincing evidence of declines of semen quality after gestational BPA exposures. Many of the reported negative findings can be attributed to deficiencies in study sensitivity, insufficient control of background contamination and probable confounding through hormonal interference due to the use of soy-containing diets. We did not evaluate animal studies of adult BPA exposures. Divergent findings in "medium to high" and "medium" confidence epidemiological studies can be explained in terms of differences in exposure conditions. We attempted the estimation of a BPA reference dose based on animal studies. Due to variations in the no-observed adverse effect levels (NOAELs) in high confidence studies, possible reference doses ranged from 0.0001 to 0.0099 μg/kg/d. In choosing 0.003 μg/kg/d we struck a balance between caution suggested by studies at the lower end of the doses and the weight of evidence from studies with higher NOAELs. This weighting was motivated by the intended use of the value in a mixture risk assessment which meant arriving at a *reasonable estimate* of BPA exposures likely without effects on semen quality. We realise that our approach does not conform with the standards necessary for deriving tolerable daily intakes (TDIs) for single chemical exposures, which is not our interest here. BPA exposures currently experienced by European populations and beyond are in excess of 0.003 μg/kg/d and even fall in the range where some epidemiological studies observed effects on semen quality as a result of BPA exposures in adulthood.

1. Introduction

Bisphenol A (BPA) is a widely used industrial chemical that can disrupt several hormone systems and produce a variety of toxic effects. As a monomer in polycarbonate plastics and epoxy resins, it leaches into food and liquids. Polycarbonates are widely used as food contact materials, bottles and other containers. Epoxy resins make up the protective lining inside food cans and the coatings of water pipes and tanks. BPA is also present in thermal paper used as cash receipts. While BPA use in baby bottles is now prohibited in the European Union, it is still permitted in food contact material and containers, with a migration limit of 50 ng/g food. As a result, BPA exposure is widespread and food items stored in cans (e.g. fish, tomatoes) and plastic bottles (e.g. milk) contribute significantly to the daily intake of the general population (Karrer et al., 2020).

The European Chemicals Agency (ECHA) has included BPA in the list of substances of very high concern on the basis of endocrine disrupting properties (ECHA, 2018). Evidence has been mounting that BPA interferes with the signalling processes important for healthy male reproductive development. As is common with many endocrine disruptors, BPA affects multiple endpoints that constitute a syndrome of effects related to poor male reproductive health. It can antagonise the binding of testosterone to the androgen receptor (AR) (Ermler et al., 2011). After exposure of rats during gestation it produces changes in the anogenital distance (AGD) of male offspring (Christiansen et al., 2014) and declines in semen quality (Hass et al., 2016), both indicators of diminished androgen action in fetal life. Several epidemiological studies report associations between BPA exposures in adult life and declines in several parameters of semen quality (Adoamnei et al., 2018; Ji et al., 2018; Lassen et al., 2014).

The precise mechanisms by which BPA affects semen quality are not resolved, but several possibilities can be envisaged. Cell-autonomous activation of the AR in Sertoli cells is an absolute requirement for

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Abbreviations

AF	Assessment factor
AGD	Anogenital distance
AR	Androgen receptor
AUC	Area under the curve
BPA	Bisphenol A
COSTER	Conduct of Systematic Reviews in Toxicology and Environmental Health Research
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
GD	Gestational day
HED	Human equivalent dose
HEDF	Human equivalent dose factor
HI	Hazard Index
LOAEL	Lowest observed adverse effect level
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OHAT	Office of Health and Translation
PECO	Population, Exposure, Control, Outcome
PND	Postnatal day
TDI	Tolerable daily intake
t-TDI	temporary tolerable daily intake
WHO	World Health Organisation

Sertoli cells to be able to support spermatogenesis and to allow germ cells to complete meiosis (De Gendt et al., 2004). The ability of BPA to disrupt germ cell meiosis by producing abnormal proportions of stages VII – VIII of the spermatogenic cycle (Shi et al., 2018) could therefore be attributed to its AR-antagonist properties. However, there are other effects, including disruption of the epigenetic programming necessary for spermatogenesis, as evidenced by expression changes in DNA methyl transferases (Shi et al., 2019) and increased oxidative stress in testicular tissues (Ullah et al., 2019).

Experimental studies have shown that BPA can act in combination with other AR antagonists *in vitro* (Orton et al., 2014). In multi-component mixture studies of gestational exposures in the rat, BPA acted in concert with anti-androgens to produce retained nipples in male offspring (Axelstad et al., 2014) and declines in semen quality (Axelstad et al., 2018).

Numerous other chemicals can also affect male reproductive health, including phthalates, parabens, analgesics, polychlorinated dioxins, polychlorinated biphenyls, polybrominated biphenyl ethers and certain azole pesticides (Kortenkamp, 2020). Exposures to these substances are widespread (Apel et al., 2020; Moos et al., 2017; Bauer et al., 2021; Martin et al., 2017). This calls for systematic investigations of the impact of simultaneous exposure to multiple chemicals on male reproductive health.

One widely used mixture risk assessment method is the Hazard Index (HI) (Teuschler and Hertzberg, 1995). It employs risk quotients of exposure and health-based guidance values or reference doses that are familiar from single chemical risk assessment. By summing up the risk quotients of all chemicals included in the mixture risk assessment it examines fold-exceedances of "acceptable" mixture exposures relative to an index value of 1. To achieve consistency of the mixture risk assessment, these risk quotients must be built with reference doses for similar toxicity endpoints. Utilisation of reference doses for different toxicities, e.g. carcinogenicity for one mixture component and lung toxicity for another, must be avoided as such mixing of toxicities increases the uncertainty of the assessment. Thus, the search for the most sensitive toxicity endpoint, the so-called critical toxicity, which is required for deriving health-based guidance values in single chemical risk assessments, is not the sole criterion in mixture risk assessments. It must be complemented by estimating doses associated with a common adverse outcome.

Apart from disrupting male sexual differentiation, BPA affects a multitude of other processes, with adverse outcomes. In their recent Draft Scientific Opinion on BPA, the Panel on Food Contact Materials, Enzymes and Processing Aids of the European Food Safety Authority (EFSA) identified the immune system as the most sensitive target of BPA exposure. BPA also produces metabolic effects, developmental neurotoxicity and adverse effects on female reproductive organs. To protect the immune system from BPA exposures, the EFSA Panel derived a new tolerable daily intake (TDI) of 0.04 ng/kg body weight/day (EFSA, 2021), considerably lower than the previous temporary TDI of 4 μg/kg body weight/day (EFSA, 2015). However, a value derived for immunotoxicity cannot be relied on for a mixture risk assessment for male reproductive health. It is therefore necessary to derive a BPA reference dose specifically for reproductive effects.

In view of the widespread declines in semen quality in Western countries (Levine et al., 2017), and with the intention of interpreting these unfavourable trends in the framework of a mixture risk assessment, we selected semen quality as the basis for deriving a BPA reference dose.

We conducted a systematic review of the epidemiological literature and of the body of evidence from animal experimental studies to address two separate but related questions: what is the strength of evidence of associations between BPA exposure and declines in semen quality? What is a BPA reference dose for semen quality declines that can be used in a mixture risk assessment of male reproductive health, specifically with a focus on semen quality? We placed particular emphasis on gestational BPA exposures because germ stem cell populations are established in fetal and neonatal life, and only after this period spermatogenesis can begin. Disruption of these processes can have life-long, irreversible effects. In the mouse, this period is from gestational day (GD) 7 to postnatal day (PND) 8, in the rat from GD 9 to PND 10 (de Rooij and Vergouwen 1991; Olaso and Habert 2000). For obvious reasons, it is difficult to establish accurately such periods in humans, but the equivalent window is presumed to be in the first trimester of pregnancy (Sharpe, 2020).

2. Materials and methods

2.1. Literature search and screening

Literature search and screening, study evaluation, data extraction and evidence synthesis methods are set out in detail in the systematic review protocol developed following the COSTER recommendations (Whaley et al., 2020; Martin et al., 2021; and Supplementary Material S1). Briefly, epidemiological studies and experimental studies with BPA describing declines in semen quality were identified by conducting literature searches in PubMed, Web of Science, Scopus until July 2020, updated in August 2021. Citation searches of key papers were also conducted. PECO statements and literature search algorithms are available in Supplementary Material S1.

We incorporated all experimental studies with laboratory animals that analysed total sperm count, sperm concentration, motility, morphology or vitality as outcome measures, but did not consider DNA damage or aneuploidy. Studies with non-mammalian species were excluded, as were studies where BPA was administered to adult animals, outside the period when germ cell stem populations are established between GD 7 to PND 10. We also excluded studies where BPA was injected (sub-cutaneously or intra-peritoneally) as these routes bypass

liver metabolism and can lead to inflated BPA tissue concentrations.

We included epidemiological studies among adult men (between 18 and 40 years of age) that reported semen quality parameters (total sperm count, sperm concentration, motility, morphology or vitality). Case-control studies, cohort studies and cross-sectional studies were considered, but case reports and reviews were excluded. Only studies that had assessed BPA exposure as urinary concentrations were eligible. Measurements in plasma, serum or cord blood were excluded, due to the absence of kinetic models that allow estimation of daily intakes based on the concentrations measured in these fluids. Studies that measured BPA concentrations by using ELISA were also deemed unreliable and were not considered. Studies reporting associations between BPA and DNA damage in sperm, or aneuploidy were also excluded, as these effects are not related to disruptions of male reproductive health by hormonal factors.

The literature review process was coordinated and managed using the freely available CADIMA tool (https://www.cadima.info/index.php/area/evidenceSynthesisDatabase). Title/abstract, full text screening and data extraction was performed by at least two reviewers.

2.2. Study evaluation

The internal validity (risk of bias) of individual studies was assessed using separate criteria and considerations for human epidemiological and for animal studies. Our main concerns were risk of bias (understood as factors that affect the magnitude or direction of effect) and insensitivity (factors that limit the ability of a study to detect an effect that is in fact present).

To appraise the internal validity of experimental studies with mammalian laboratory animals, we used the internal validity appraisal protocol (risk of bias assessment) for BPA as detailed in (EFSA, 2017) and (EFSA, 2019). EFSA developed this protocol following the NTP OHAT Risk of Bias Tool (described in the NTP OHAT 2019 Handbook for conducting a literature-based health assessment, p 33 (NTP, 2015). We used key elements similar to those defined by EFSA (2019) for appraising BPA studies and analysed exposure characterisation (purity of test compound, consistent administration, and absence of contamination of the test compound), outcome assessment (blinding of assessors) and power of detecting effects (sufficient numbers of animals per dose group).

To assess specific quality issues related to studies of BPA and semen quality, we introduced three further key elements in our appraisal. One of these concerns the control of BPA contamination by using polycarbonate-free caging. BPA can leach from polycarbonate caging (Howdeshell et al., 2003) and may thus obscure the effects of experimentally administered BPA. Second, the use of phytoestrogen-free chow is important as phytoestrogen-containing chow may introduce hormonal disturbances which mask the effects of BPA on semen quality (Ruhlen et al., 2011). The third additional key element concerns the inclusion of a positive control with established detrimental effects on semen quality (often ethinylestradiol, estradiol or diethylstilbestrol). This is necessary to demonstrate the proficiency of the investigators to detect changes in semen quality and shows that the experimental system is sufficiently sensitive. The complete list of appraisal elements can be found in the published protocol (https://doi.org/10.5281/zenodo.5083147, Supplementary Material S1) and in Table 1.

Each element was scored using the NTP OHAT categories ++ *Definitely low risk*, + *Probably low risk*, ~ *Probably high risk* and ~~ *Definitely high risk*. Key elements were assessed first, and a study that failed a key element was not evaluated further. We adopted the system in EFSA (2019) and rated each study in terms of three Tiers, with *Tier 1* signifying high confidence where all three EFSA key elements and all our three additional key elements are scored + or ++ and no more than 1 question not addressing these key elements is scored ~ or ~~ (see EFSA, 2019; Table 2, p 8). *Tier 2* signifies medium confidence and denotes all combinations not covered in Tier 1 or 3. Studies were placed in *Tier 3* (low confidence) when any one of the three EFSA key elements and the additional key elements was scored ~ or ~~ or when more than 50% of the questions not addressing these key elements were scored ~ or ~~. The risk of bias assessment protocol is shown in the published protocol, together with instructions how to rate each element of the protocol in terms of the risk categories.

We examined epidemiological studies of associations between BPA and semen quality using the procedures detailed by Radke et al. (2018), with evaluations of exposure measurement, outcome measurement, participant selection, confounding and analysis. By applying the criteria detailed in Radke et al. (2018) and listed in the published protocol (https://doi.org/10.5281/zenodo.5083147) we judged the quality of each study regarding its utility for hazard identification by reaching a consensus in each evaluation domain with the categories *Good*, *Adequate*, *Poor*, or *Critically Deficient*. The ratings for each evaluation domain were combined to obtain an overall study confidence rating of *High*, *Medium*, *Low*, or *Uninformative* (Table 2).

2.3. Data synthesis

We provided a narrative synthesis to summarise the characteristics and findings of the eligible studies, in terms of BPA exposure ranges not associated with declines in human studies, or in terms of NOAELs or LOAELs in animal studies. In these summaries we only considered human epidemiological studies rated as high or medium confidence, and experimental animal studies rated as high confidence (*Tier 1*). To enable quantitative comparisons between bisphenol A exposures in human studies and experimental studies with animals, we converted urinary bisphenol A levels into daily intakes for humans by employing the toxicokinetic model detailed in Koch et al. (2012).

2.4. Evidence synthesis

We first assessed whether the evidence linking BPA with declines in semen quality, from both human and animal studies, is sufficiently robust to support hazard identification. To address this question, we employed methods for weighing evidence from two lines of evidence, human and animal studies, following the principles described in EFSA guidance (EFSA 2017). The evidence was synthesised by considering aspects of an association that may suggest causation, according to the Bradford Hill criteria, based on EFSA's adaptation: consistency, exposure–response relationship, strength of association, temporality, biological plausibility, and coherence.

We synthesised evidence from animal studies and human epidemiological studies separately to derive a reference dose for mixture risk assessment. For animal experiments we utilised the framework in Radke et al. (2018) modified by the approach detailed in EFSA (EFSA, 2019), as follows: The evidence is categorised as *Robust* when there are sets of studies with a *Tier 1* confidence rating with consistent findings of adverse effects on semen quality across multiple laboratories and species. Any evidence that cannot be reasonably explained by the respective study design or differences in animal model is from a set of experiments of lower confidence (*Tier 2* or *Tier 3*). The category *Moderate* is assigned when a set of evidence does not reach the degree of certainty required for *Robust*, but which includes at least one *Tier 1* confidence study and information strengthening the likelihood of a causal association. The results are largely consistent, but notable uncertainties remain. *Slight* describes a scenario in which there is a suggestion of a possible effect on semen quality, but the evidence is conflicting or weak, with only *low* confidence experiments available. *Indeterminate* is used when no animal studies are available or where the evidence is highly inconsistent and primarily of *low* confidence. *Compelling evidence of no effect* is used when *high* confidence experiments demonstrate a lack of biologically significant effects across multiple species, both sexes, and a broad range of exposure levels.

We synthesised evidence from human studies by adopting the

Table 1
Evaluation of experimental animal studies and semen quality after BPA administration.

	Study description		Key appraisal elements						Study outcomes		Study evaluation	
Reference	Species	Outcome measures	Purity of chemical	Diet soy free?	Randomisation, concealment, blinding	Number of animals per group	Sensitivity of model, positive control	Background contamination with BPA?	Outcomes	Comments	Tier	Overall confidence
Cagen et al. 1999	Rat, Wistar	daily sperm production	>99%	no	not reported	25 to 28	DES, ineffectve	not reported	No effect		3	Low
Chatsantiprapa et al. 2016	Mouse, CD1	number of motile sperm	99%	not reported	not reported	12	none	not reported	Decrease in number of motile sperm	AGDi shortening at 50 ug/kg d but, not at 500 ug/kg d	1	High
Chiocarelli et al. 2020	Mouse, CD1	% viable sperm, % motile	99%	yes	yes	5	none	no	Decrease in live and motile sperm		1	High
Delclos et al. 2014	Rat, SD	sperm counts, motility	>99%	yes	yes	18 to 23	EE2, active	no	No effect		1	High
Dere et al. 2018	Rat, SD	Spermatid heads	>99%	yes	yes	10 to 20	none	no	No effect		3	Low
Ema et al. 2001	Rat	Number of sperm, % motile, % progressive motile, % abnormal, % tailless	>99%	not reported	yes	25	none	no	No effect	No effects on sperm counts or other sperm parameters; **decrease** in abnormal and tailless sperm at 20 ug/kg d, AGD changes	3	Low
Hass et al. 2016	Rat, Wistar	Number of sperm	>99.5%	yes	yes	17 to 21	none	no	Decrease in sperm number	Effect not seen at higher doses.	1	High
Howdeshell et al. 2008	Rat, LE	Number of sperm	>99%	no	yes	16 to 18	EE2, active	possible, due to polycarbonate cages	No effect		3	Low
Kendig et al. 2012	Mouse, CD1	Number of sperm, % motile	USEPA / NIEHS standard	yes	yes	8	EE2, ineffective	no	No effect	Sperm counts and motility **increased** at higher doses	3	Low
Kobayashi et al. 2010	Mouse, C57BL/6J	Number of sperm, % motile sperm	>99.6%	no	yes	12	none	not reported	Decrease in motile sperm	Sperm motility in F2 males was only impacted at the highest dose. Doses are approximated from ppm to ug/kg/d and were higher at some timepoints	3	Low
Kobayashi et al. 2012	Rat, SD	Number of sperm, % motile, % progressive motile	>99.6%	no	not reported	10	none	not reported	No effect on motility	Sperm counts not assessed or not given	3	Low
Meng et al. 2018	Mouse, C57BL/6	Number of sperm, % abnormal sperm	>99%	no	not reported	7	none	not reported	Decrease in sperm number, increase in malformed sperm		3	Low
Nagao et al. 2002	Mouse, C57BL/6N	Number of sperm	>99%	yes	not reported	25	none	not reported	No effect	The only effect observed was a decrease in the weight of the seminal vesicle at the highest dose	3	Low
Rahman et al. 2017	Mouse, CD1	Sperm conc, % motile	>99%	yes	not reported	3, in 3 independent experiments	none	no	Decrease in sperm conc		1	High
Salian et al. 2009	Rat, Hol	Number of sperm, % motile	>99%	yes	yes	24	DES, active	not reported	Decrease in sperm number and motility		1	High
Shi et al. 2018	Mouse, CD1	Sperm conc; % motile	>99%	not reported	yes	5	none	not reported	Decrease in sperm number		1	High
Shi et al. 2019	Mouse, CD1	Sperm conc, motility	>99%	not reported	yes	6	none	not reported	Decrease in sperm number and motility		1	High
Sporndly-Nees et al. 2018	Rat, F344	Morphologically abnormal sperm	>99%	yes	yes	8 to 12	none	no	No effect	Long lag between end of exposure (PND 21) and measurement of sperm morphology (12 months), sperm numbers not measured	3	Low
Tinwell et al. 2002	Rat, SD, Alderley	Number of sperm	>99%	no	Females assigned to dose groups based on body weight ranking	7 litters	EE2, active	possible, due to polycarbonate caging	Decrease in sperm number	Effect only seen in Alderley Park rats, not in SD rats	3	Low
Tyl et al. 2008	Mouse, CD1	Number of sperm, % motile, % abnormal	99.70%	no	not reported	10 to 28	E2, inactive	not reported	No effect	High levels of phytoestrogens measured in diet	3	Low
Tyl et al. 2002	Rat, SD	Number of sperm, % motile, % abnormal	99.5% pure	no	yes	10 to 30	none	no	Decrease in sperm number	Decrease on sperm counts at highest dose	3	Low
Ullah et al. 2019	Rat, SD	Number of sperm, % motile, % viable	>99%	yes	yes	8	none	no	Decrease in sperm number		1	High
Vilela et al. 2014	Mouse, Vesper	Number of sperm	>99%	yes	not reported	15 or 11 for two lowest doses, 8 for highest	DES, active	not reported	Decrease in sperm number and motility		1	High
Vom Saal et al. 1998	Mouse CF1	Number of sperm	not reported	no	not reported	5 to 7	none	no	Decrease in sperm number		3	Low
Yang et al. 2015	Rat, SD	Number of sperm, % motile	>99%	not reported	not reported	3	none	not reported	Decrease in sperm number and motility		1	High
Yoshino et al. 2002	Rat, F344	Number of sperm, % motile, morphology	99.90%	not reported	yes	5 to 10	none	not reported	No effect		3	Low

Table 2
Evaluation of epidemiology studies of associations of BPA with semen quality.

Reference	Study description			Semen quality outcomes					Study evaluation					
	Population	Exposure sampling	Outcome	N	C	Mot	Mor	Vit	Exposure	Outcome	Participant selection	Confounding	Analysis	Overall confidence
Adoamnei et al. 2018	Cross-sectional; Students, 18 - 23 y	spot urine; adjusted for creatinine	Number, concentration, motility, morphology	v	v	~	~	nd	P	G	G	G	G	M/H
Benson et al. 2021	Cross-sectional; young men from Danish National Birth Cohort	single spot urine sample, corrected for creatinine	Number, concentration, motility, morphology	~	~	~	~	nd	P	G	G	P	G	L
Caporossi et al 2020	Cross-sectional; male partners of sub-fertile couples	spot urine; adjusted for creatinine	Number, concentration, motility, morphology	~	~	~	~	nd	P	G	A	G	G	M
Chen et al. 2013	Case-control; infertile men, idiopathic	morning urine, adjusted for creatinine	Number, concentration	~	~	nd	nd		P	P	A	G	G	L
Den Hond et al. 2015	case-control; male patients of fertility clinics	spot urine, adjusted for creatinine	Number, concentration, motility, morphology	~	~	~	~	nd	P	G	A	P	A	L
Goldstone et al. 2015	Cohort; Men > 18 y	spot urine; adjusted for creatinine	Number, concentration, motility, morphology	~	~	~	~	nd	P	A	G	G	G	M
Pollard et al. 2019	Cohort; Men 18 - 40 y	first morning urine; 8 to 9 samples per subject; adjustment for creatinine	Number, concentration, morphology	~	~	nd	v	nd	A	A	G	P	A	M
Ji et al. 2018	Cross-sectional; men 18 - 55 y	single spot urine, adjusted for creatinine	Number, concentration, motility	~	v	v	nd	nd	P	G	G	G	G	M/H
Kim, Ko et al. 2019	Cross-sectional; male patients of fertility clinic	single spot urine, corrected for specific gravity	Concentration, motility	nd	~	~	nd	nd	P	P/CD	A	P	P	U
Knez et al. 2014	Cross-sectional; men fertility clinic, 34 y average	single spot morning urine, adjusted for creatinine	Number, concentration, motility, morphology, vitality	v	v	v	~	v	P	A	P	G	A	L
Lassen et al. 2014	General population, "young men" undergoing physical examination for military service	single spot urine, osmolality adjusted	Number, concentration, motility, morphology	~	~	v	~	nd	P	G	G	G	G	M/H
Li et al. 2011	Cohort, occupationally exposed	two spot urine samples, pre- and post-shift; for controls one spot sample; volume-based- and creatinine-corrected	Number, concentration, motility, morphology, vitality	v	v	v	~	v	A	G	G	G	G	M/H
Meeker et al. 2010	Cohort, male partners of subfertile couples 18 - 55 y	spot urine samples, various sampling schemes; some 2-3 samples; corrected for specific gravity	Number, concentration, motility, morphology	~	v	v	v	nd	A	G	A	G	G	M/H
Mendiola et al. 2010	Cohort, partners of pregnant women, general population	no details on urine samples, correction for creatinine	Number, concentration, motility, morphology	~	~	~	~	nd	P	G	A	G	G	M
Omran et al. 2018	Case-control, infertile men	single spot urine sample, creatinie adjusted	Concentration, motility, morphology	nd	~	~	~	nd	P	A	P	P/CD	A	U
Radwan et al. 2018	Cross-sectional; men attending fertility clinic, but with normal sperm parameters	single spot urine sample, creatinine adjusted	Number, concentration, motility, morphology	~	~	v	~	nd	P	G	A	G	G	M

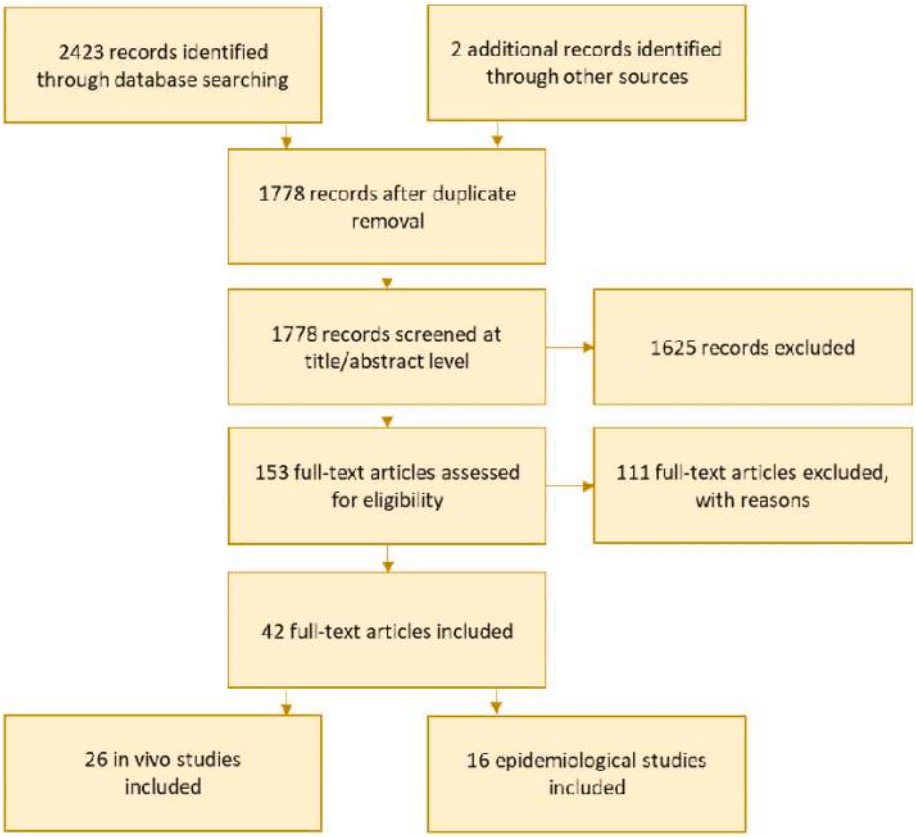

Fig. 1. Literature flow diagramme for animal studies and epidemiological studies of BPA exposures and semen quality.

framework developed by Radke et al. (2018) which assigns strength of evidence conclusions of *Robust*, *Moderate*, *Slight*, *Indeterminate*, and *Compelling evidence of no effect*. *Robust* describes evidence from *high* or *medium* confidence independent studies that report an association between BPA exposure and declines in semen quality, with reasonable confidence that alternative explanations, including chance, bias, and confounding, can be ruled out across studies. *Moderate* is used to describe a situation where there is a smaller number of studies (at least one *high* or *medium confidence* study with supporting evidence), with some heterogeneous results, that do not reach the degree of confidence required for *robust*. *Slight* is assigned when there are one or more studies reporting an association between bisphenol A and declining semen quality, but where considerable uncertainty exists. The evidence is limited to a set of consistent *low* confidence studies, or higher confidence studies with unexplained heterogeneity. *Indeterminate* is used when either there are no studies available in humans or when the evidence is highly inconsistent and primarily of *low* confidence. *Compelling evidence of no effect* requires several *high* confidence epidemiological studies returning null results.

2.5. Derivation of a BPA reference dose for declines in semen quality

To derive a bisphenol A reference dose with respect to declines in semen quality, we followed the procedure sketched out in EFSA (2017). Briefly, we made quantitative comparisons for each line of evidence (per animal species, and human) where it was possible to derive a point of departure (NOAEL or benchmark dose).

Where necessary, NOAELs were extrapolated from LOAELs by using a standard assessment factor (AF = 3). We based these comparisons on high quality studies (*high* or *medium* confidence human studies, *Tier 1* animal studies).

In humans, smaller doses than in animals are required to achieve the same effective tissue concentrations. To address species-specific differences in the toxicokinetics of BPA, we applied the points of departure identified (or extrapolated) from animal data to derive a human equivalent dose (HED) by application of human equivalent dose factors (HEDF), as detailed in EFSA (2021). The HEDs were then compared with BPA exposure ranges from epidemiological studies.

For comparisons of dosages used in animal studies with exposures experienced by humans, we converted urinary BPA levels to estimated daily intakes using the model developed by Koch et al. (2012).

3. Results

The literature selection process for human epidemiological studies and animal studies is shown in Fig. 1.

We first assessed the strength of evidence for an association between BPA exposure and declines in semen quality and then attempted to estimate a reference dose for this health endpoint for use in mixture risk assessments.

3.1. Strength of evidence: experimental studies in animals

Study selection and evaluation: Twenty-six experimental studies of BPA exposure and deteriorations of semen quality in rats and mice met our eligibility criteria (Table 1). Except for the study by Vom Saal et al. (1998), there were no concerns regarding the purity of the test compound, its consistent administration or possible contaminations, as BPA of a purity >99% was used in all studies.

Some studies, however, raised concerns about hormonal disturbances introduced through soy-containing diets. This is of relevance, as dosing of rats with genistein from GD 7 to the end of pregnancy led to declines in semen quality (Delclos et al., 2001) and feeding soy-containing diets obscured the effects of diethylstilboestrol on semen quality (Ruhlen et al., 2011). In several studies there was direct evidence that the diets used contained phytoestrogens. This was the case with

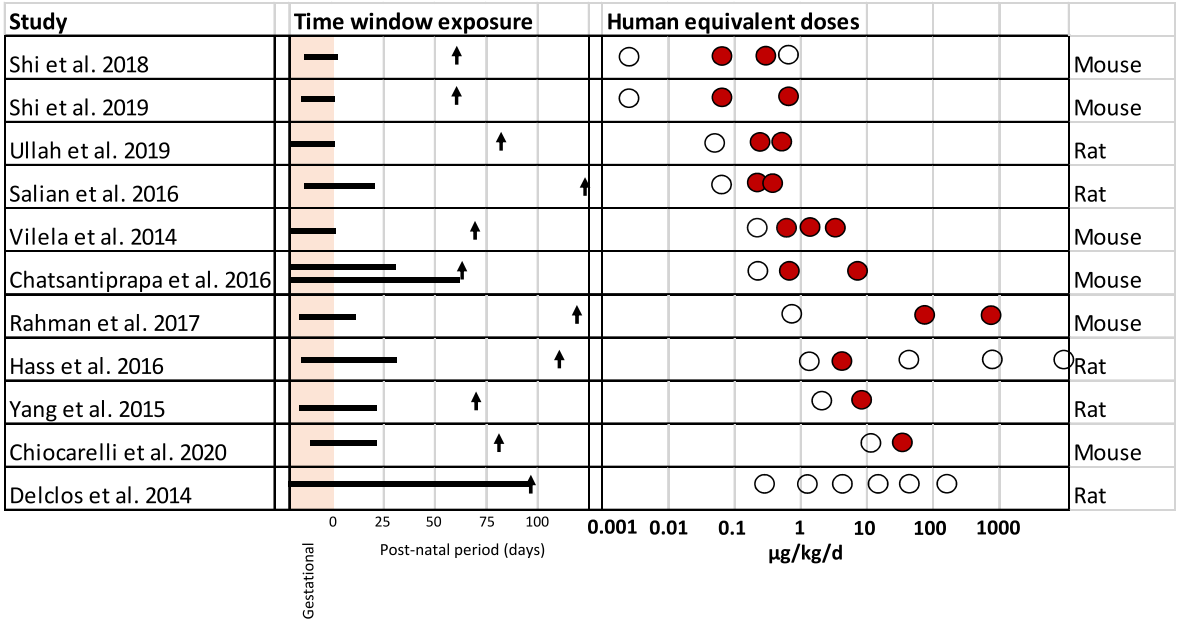

Fig. 2. Summary of high confidence (*Tier 1*) animal studies of BPA and semen quality. Black horizontal bars in "Time window exposure" show the periods of BPA administration, arrows depict time points when semen was sampled. The gestational period is shaded pink. Open circles in "Human equivalent doses" are doses equivalent to no-observed adverse effect levels (NOAELs) which were extrapolated from lowest-observed adverse effect levels (LOAELs) by application of an assessment factor of 3. Red circles are doses associated with declines in sperm numbers, grey circles show doses without effects on sperm numbers (if these are also the lowest tested doses, they are NOAELs). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Estimation of reference doses for semen quality declines from animal studies.

	LOAEL (µg/kg d)	NOAEL (µg/kg d)	Species	HEDF	HED (µg/kg d)	RfD (µg/kg d)
Shi et al., 2018	0.5	**0.17**	Mouse	0.0155	0.0026	0.0001
Shi et al., 2019	0.5	**0.17**	Mouse	0.0155	0.0026	0.0001
Salian et al., 2009	1.2	**0.4**	Rat	0.165	0.0660	0.0026
Ullah et al., 2019	1.5	0.5	Rat	0.165	0.0825	0.0033
Vilela et al., 2014	40	**13.3**	Mouse	0.0155	0.2062	0.0082
Chatsantiprapa et al., 2016	50	**16**	Mouse	0.0155	0.2480	0.0099

LOAEL: Lowest observed adverse effect level; NOAEL: No-observed adverse effect level; HEDF: Human equivalent dose factor; HED: Human equivalent dose; RfD: Reference dose derived by dividing HED values by 25.
NOAEL values shown in bold are extrapolations from studies where only LOAELs, but not NOAELs were observed. In these cases, LOAELs were divided by 3 and the resulting values taken as "extrapolated" NOAELs.

Cagen et al. (1999) who used a rodent diet containing dehulled soybean meal. Rat chow 5001, 5002 and 5008 used by Howdeshell et al. (2008), Tyl et al. (2002, 2008) and Vom Saal et al. (1998) also contains phytoestrogens. The same applies to standard CE2 diet fed by Kobayashi et al. (2010, 2012) or standard chow by Meng et al. (2018). Accordingly, we rated these studies as low confidence (*Tier 3*) and did not conduct further detailed evaluations. Ema et al. (2001) did not report on the phytoestrogen content of the diet used, but we considered this as probably low risk because they observed endocrine-related BPA effects (changes in anogenital distance, but not semen parameters). The same applies to Chatsantiprapa et al. (2016), Shi et al. (2018, 2019) and Yang et al. (2015). Yoshino et al. (2002) also provided insufficient information on the phytoestrogen content and did not observe endocrine BPA effects. There were doubts about the proficiency of this study to demonstrate BPA effects on semen quality, as positive controls were not included. Accordingly, we evaluated Yoshino et al. (2002) as low confidence and assigned this study to *Tier 3*.

All remaining studies were evaluated as "definitely" or "probably low risk" in terms of randomisation, concealment and blinding and in relation to statistical power of detecting an effect (sufficient number of animals). Only Rahman et al. (2017) and Yang et al. (2015) employed fewer than 5 animals per dose group but possible concerns about insufficient power were made baseless by their observations of BPA-related effects on parameters of semen quality.

In addition to Yoshino et al. (2002), several other studies gave reason to doubt the sensitivity of the model used and its proficiency in detecting effects on semen quality. There was direct evidence for a lack of sensitivity in Kendig et al. (2017), Cagen et al. (1999) and Tyl et al. (2008) who employed ethinylestradiol, DES or estradiol, respectively, as a positive control but were unsuccessful in observing effects ("definitely high risk"). In all the other remaining cases (Dere et al., 2018; Ema et al., 2001; Nagao et al., 2002; Spörndly-Nees et al., 2018), there was indirect evidence for lack of sensitivity as positive controls were not used and BPA effects on semen parameters were not observed. This resulted in a rating of probably high risk and assignment to *Tier 3*.

With the remaining studies there were no concerns regarding BPA background contamination or any of the other evaluation elements. Chioccarelli et al. (2020) and Yang et al. (2015) did not provide adequate information about the statistical methods they used for estimating BPA doses associated with small effects, which we deemed to have an impact on study validity ("probably high risk"). However, this did not influence the overall confidence rating of "high" (*Tier 1*) for these studies.

Overall study confidence ratings: In summary, 11 of the 26 eligible

Author	Study design	Number of subjects	BPA urinary levels without effect (µg/g creatinine)	Estimated daily intakes (µg/kg d)
Adoamnei 2018	cross-sectional	215	1.3 - 2.8	**0.04 - 0.08**
Pollard 2019	cohort	161	2.39	**0.07**
Ji 2018	cross-sectional	500	0.32 - 1.12	**0.01 - 0.03**
Lassen 2014	cohort	297	3.71 - 6.44	**0.10 - 0.18**

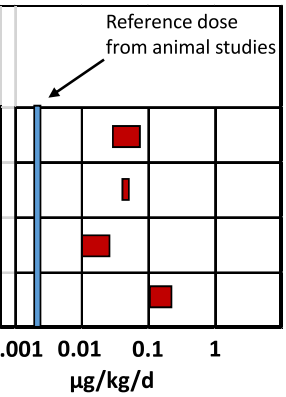

Fig. 3. Comparison of BPA reference dose with BPA exposure ranges not associated with semen quality declines in selected epidemiological studies. The red horizontal bars represent estimated daily BPA intakes for which effects on semen quality were not noted. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

studies obtained a confidence rating of "high" (*Tier 1*). In these studies, all six key evaluation elements were evaluated as "definitely" or "probably low risk", with no more than one other element rated as "definitely" or "probably high risk". The other 15 studies only achieved a confidence rating of "low" and accordingly had to be placed in *Tier 3*. Several of the *Tier 3* studies failed multiple key elements of the evaluation (Table 1). There were no *Tier 2* studies.

Evidence synthesis: As shown in Table 1, 10 of the 11 studies with a high confidence rating reported effects of BPA on semen quality parameters. The only high confidence study that did not observe effects was by Delclos et al. (2014).

Of the 15 studies we evaluated as being of low confidence, 5 reported BPA effects, while 10 did not observe declining semen quality after BPA exposure.

In summary, there are 10 independent studies with a "high" (*Tier 1*) confidence rating which reported declining semen quality after BPA exposure in multiple strains of two species, rat and mouse. Accordingly, the overall strength of evidence that associates BPA with poor semen quality in experimental studies can be evaluated as "robust".

3.2. Strength of evidence: human epidemiological studies

Study selection and evaluation: We identified 16 studies that matched our eligibility criteria (Table 2). These studies are case-control, cohort or cross-sectional with participants drawn from the general population, occupational cohorts, or couples from infertility clinics. The studies varied in size from 105 (Caporossi et al., 2020) to 1590 participants (Chen et al., 2013).

BPA measurements corresponding to prenatal exposures would be ideal for investigating associations with semen quality, as the *in utero* environment is critical for semen quality in adulthood (Skakkebaek et al., 2015). However, none of the eligible studies related semen quality to maternal exposures. The few studies that investigated exposures during development had to be excluded due to measurement of bisphenol A in serum, cord blood or seminal fluids for which toxicokinetic models for conversion to daily intakes are missing (Hart et al., 2018; Vitku et al., 2016).

In adult men, the best timing of exposure measurements would be around 90 days before taking a semen sample, because spermatogenesis takes approximately 75 days, with an additional 12 days of maturation as the sperm travels through the epididymis. However, none of the eligible studies adopted such a timing. Instead, most studies collected urine samples for BPA measurements at the same time, or near the time of semen analysis (Caporossi et al., 2020; Chen et al., 2013; Ji et al., 2018; Knez et al., 2014; Lassen et al., 2014). The exception is Pollard et al. (2019) who measured BPA exposures 3 days before sampling semen. Many studies did not give explicit details as to the timing of exposure measurements (Adoamnei et al., 2018; Benson et al., 2021; Den Hond et al., 2015; Goldstone et al., 2015; Kim et al., 2019; Li et al., 2011; Meeker et al., 2010; Mendiola et al., 2010; Omran et al., 2018; Radwan et al., 2018).

BPA has a relatively short half-life of excretion of 4–5 h. This may result in considerable variations of urinary BPA concentrations. To take account of these variations, sampling at multiple time points is recommended (Agier et al., 2020). However, most of the eligible studies based their BPA exposure estimates on single spot urine samples.

Due to the shortcomings regarding timing and frequency of urine sampling, we rated the exposure assessments in most of the studies as "poor". The only exceptions are Pollard et al. (2019), Li et al. (2011) and Meeker et al. (2010) who employed multiple BPA measurements which we regarded as somewhat mitigating the shortcomings regarding the timing of exposure measurements. Accordingly, the exposure assessments in these three studies were rated as "adequate".

We evaluated the outcome measurements as "good" when semen analyses were conducted according to the WHO (2010) guidelines. This applied to almost all studies, except when several of the core semen quality parameters (sample volume, sperm concentration, motility and morphology) were not examined, as in Chen et al. (2013) and Kim et al. (2019). We rated the outcome measurements in these two studies as "poor". Studies with missing descriptions of semen quality measurements were classed as critically deficient (Kim et al., 2019). In some cases, motility assessments could not be performed, as semen samples were collected at home and then shipped for analysis. We rated these studies as "adequate" (Pollard et al., 2019; Goldstone et al., 2015). Knez et al. (2014) and Li et al. (2011) also examined sperm vitality.

Studies that chose subjects from the general population, with no apparent selection effects and high participation rates were evaluated as "good" in relation to participant selection (Adoamnei et al., 2018; Benson et al., 2021; Goldstone et al., 2015; Ji et al., 2018; Lassen et al., 2014; Pollard et al., 2019). We classed occupational studies and those in infertility clinic settings as "adequate" (Caporossi et al., 2020; Chen et al., 2013; Den Hond et al., 2015; Meeker et al., 2010; Mendiola et al., 2010; Radwan et al., 2018). Where details on participant selection were missing, we applied the rating of "poor" (Kim et al., 2019; Omran et al., 2018).

Key confounders that must be considered in semen quality studies include age, abstinence time, smoking, body mass index, and chronic diseases (Sánchez-Pozo et al., 2013). Although not as well established as risk factors, alcohol use and stress may also warrant consideration. Most eligible studies adjusted for the key confounders, and accordingly we evaluated them as "good" in terms of confounder analysis. Where abstinence time was not included as a confounder or where information about abstinence time was missing or where subjects with abstinence times of fewer than 2 days were included in the analysis, we applied a rating of "poor" (Benson et al., 2021; Den Hond et al., 2015; Pollard

et al., 2019; Kim et al., 2019; Omran et al., 2018).

Ideally, evaluations of associations of chemical exposures with semen quality should analyse semen parameters as continuous variables, to minimise misclassification and to obtain sufficient statistical power. Furthermore, results should be presented with standard errors and confidence intervals and not just shown as "significant". Most of the studies met these requirements and were rated as "good" in terms of data analysis. Chen et al. (2013), Pollard et al. (2019) and Meeker et al. (2010) dichotomised semen quality parameters, and accordingly, we downgraded this evaluation aspect in these studies to "adequate". Kim et al. (2019) provided insufficient detail of their statistical analysis and had to be rated as "poor".

Overall study confidence ratings: Due to the importance of the exposure assessment component, we judged that no study with an exposure assessment rating of "poor" should obtain an overall confidence rating of "high". Studies where all other aspects were evaluated as "good" could achieve a maximum overall confidence rating of "medium to high" (M/H). If three or more components were evaluated as "poor", we applied an overall rating of "uninformative" (U). With two aspects classed as "poor", the overall rating was pegged at "low" (L). Table 2 shows the study confidence ratings we established according to these decision rules.

Evidence synthesis: As shown in Table 2, 8 studies returned null findings and 8 reported associations of declining semen parameters with BPA exposures. Of the 8 null studies, 3 achieved an overall confidence rating of "medium" (Caporossi et al., 2020; Goldstone et al., 2015; Mendiola et al., 2010) while the others were evaluated either as "low" or "uninformative". Among the 8 studies that found associations with BPA, 5 were "medium to high", and 2 "medium" and one study was rated "low" (Knez et al., 2014).

The disparity between the 3 "medium" confidence null studies and those that reported associations can be attributed to differences in exposure conditions: Caporossi et al. (2020), Goldstone et al. (2015) and Mendiola et al. (2010) all examined populations with rather low BPA urinary levels. This may well have precluded the detection of associations with BPA. Thus, rather than yielding conflicting evidence (unexplained positive and negative results in similarly exposed human populations) the eligible studies produced mixed results explained by differing exposure levels.

In summary, there are 7 independent studies of "medium" or "medium to high" confidence with positive findings. Accordingly, the overall strength of evidence of associations between BPA exposures and declines in semen quality can be evaluated as "robust".

3.3. Weight of evidence

There is robust evidence from animal studies that BPA exposures during gestation lead to declines in semen quality. In humans, evidence of the consequences of BPA exposures in fetal life is currently not available. However, the associations of BPA exposure in adult life with declines in semen quality are robust and support the conclusion that the patterns seen in animal experiments are relevant to humans. They are sufficiently robust to support hazard identification and characterisation. Accordingly, we proceeded to attempt a derivation of a BPA reference dose for declines in semen quality (hazard characterisation).

3.4. Derivation of a reference dose for declines in semen quality

Experimental studies in animals: Fig. 2 summarises all *Tier 1* studies with respect to the time windows of exposures used and the BPA doses associated with statistically significant declines in semen quality (sperm numbers and motility). All studies covered the critical period when germ cell stem populations are established (mouse: GD 7 to PND 8, rat: GD 9 to PND 10).

Due to higher rates of metabolism and excretion in rodents, the doses required to attain comparable tissue levels in mice, rats or humans differ. Normally, higher doses than in humans are required to achieve similar tissue levels in rodents. The availability of serum-concentration time course data allows making such comparisons on a quantitative basis, in terms of Areas under the Curve (AUC). To adjust for kinetic differences, and to make the exposures comparable, AUCs resulting from comparable doses in animal species are divided by AUCs in humans to obtain Human Equivalent Dose Factors (HEDF). Human Equivalent Doses (HED) are then obtained by multiplying the doses used in rodent studies with the appropriate HEDF (0.0155 for mouse and 0.165 for rat studies, respectively) (EFSA, 2021). We focused on studies with at least two different dose groups, in addition to untreated controls. As shown in Fig. 2, the lowest doses associated with declines in sperm numbers varied from a HED of 0.0077 μg/kg/d (Shi et al., 2018, 2019) to 77.5 μg/kg/d (Rahman et al., 2017), with most studies reporting activity at HEDs between 0.24 and 8.25 μg/kg/d.

In estimating a BPA reference dose, we first calculated HEDs based on no-observed adverse effect levels (NOAELs). In almost all studies, the lowest used treatment doses produced effects which precluded the determination of a NOAEL. In these cases, we extrapolated NOAELs from the reported lowest treatment doses (lowest observed adverse effect levels, LOAELs) by application of an AF of 3. HEDs from studies with observed or extrapolated NOAELs above 1 μg/kg/d were not considered further. To account for species differences and vulnerable individuals, we adopted the procedure described in EFSA (2015, 2021) and divided the HEDs by an assessment factor of 25, widely used by EFSA for chemical risk assessment. This produced the reference doses listed in Table 3. The only study that reported a NOAEL was by Ullah et al. (2019). Their NOAEL was 0.5 μg/kg/d in the rat, which by combination with an HEDF of 0.165 and an AF of 25 produces a reference dose of 0.0033 μg/kg/d. A very similar reference dose of 0.0026 μg/kg/d was estimated based on the data in Salian et al. (2009). Based on the findings by Shi et al. (2018, 2019) in mice, we estimated 0.0001 μg/kg/d, and the mouse studies by Vilela et al. (2014) and Chatsantiprapa et al. (2016) produced 0.0082 and 0.0099 μg/kg/d, respectively. It appears that the estimates derived from Ullah et al. (2019) and Salian et al. (2009) occupy a mid-point, and accordingly, we adopted 0.003 μg/kg/d as a BPA reference dose in mixture risk assessments for declines in semen quality.

Comparison of BPA reference dose with data from human epidemiological studies: We compared the reference dose estimate derived from animal studies with BPA exposures in epidemiological studies below the ranges associated with declines in semen quality ("no-observed effect ranges"). We based our comparison on studies among the general population and excluded occupationally exposed cohorts and populations from fertility clinics. This left four studies eligible for this comparison: Adoamnei et al. (2018), Pollard et al. (2019), Ji et al. (2018) and Lassen et al. (2014) (Fig. 3).

The authors of these studies categorised BPA exposures into ranges of urinary concentrations which they analysed in terms of statistically significant associations with declines in semen quality. We converted the urinary BPA concentrations reported in these studies into estimated daily intakes, by using the model developed by Koch et al. (2012). This allowed us to identify exposure ranges apparently no longer associated with semen quality. As shown in Fig. 3, the reference dose of 0.003 μg/kg/d estimated from animal studies is below the "no-observed effect range" of between 0.01 and 0.18 μg/kg/d reported in the four epidemiological studies (Fig. 3).

3.5. Comparison with BPA exposure estimates

Using the model by Koch et al. (2012), the urinary BPA concentrations from samples collected in 2009 in human biomonitoring exercises by Koch et al. (2012) and Frederiksen et al. (2020) were converted into estimated daily intakes of 0.14 and 0.16 μg/kg/d, respectively (95th percentiles). The median daily intakes were reported as 0.035 and 0.048 μg/kg/d, respectively. These estimates from German and Danish

subjects agree well with those for Norwegian populations published by Karrer et al. (2020) (median: 0.035 μg/kg/d, most probable range: 0.02–0.1 μg/kg/d).

Thus, European populations experience BPA exposures 12 to 16-fold (median) and up to 48-fold (95th percentile) above the reference dose we estimated from animal studies. These exposures fall in the ranges where declines in semen quality were observed in epidemiological studies (Adoamnei et al., 2018: 0.045–0.08 μg/kg/d; Pollard et al., 2019: 0.07 μg/kg/d; Ji et al., 2018: 0.01–0.03 μg/kg/d; Lassen et al., 2014: 0.1–0.18 μg/kg/d).

4. Discussion

The evidence linking BPA exposures to declines in semen quality is often characterised as "conflicting" or "varied". In contrast with such views, the application of the systematic review method, together with a rigorous confidence rating approach, reveals that there is convincing evidence of declines of semen quality after gestational BPA exposures in animal studies. In addition, human epidemiological studies provide supporting evidence for poor semen quality after BPA exposure in adult life. This is despite the noted weaknesses in exposure assessments which will have increased the likelihood of null findings through exposure misclassification (Agier et al., 2020).

We propose that a great deal of the negative findings in animal studies can be attributed to deficiencies in study sensitivity and to insufficient control of background contamination with BPA due to the use of polycarbonate caging. Howdeshell et al. (2003) demonstrated that BPA leaches from polycarbonate cages. In prepubertal mice housed in such cages, Howdeshell et al. saw increases in uterine weights (albeit not statistically significant). Some studies also raised concerns about confounding through hormonal interference by phytoestrogens from soy-containing diets. Ruhlen et al. (2011) observed that soy-containing diets obscure the effects of diethylstilboestrol on semen quality. Furthermore, gestational exposures of rats to phytoestrogens such as genistein led to declines in semen quality (Delclos et al., 2001).

Apart from general concerns about the quality of exposure assessments, the confidence in some epidemiological studies was compromised by deficiencies in outcome measurements and adjustments for confounding. Nevertheless, the inconsistent findings from "medium to high" and "medium" confidence studies can be explained in terms of differences in exposure conditions. This is to be distinguished from "conflicting evidence" in the sense of conflicting findings due to unexplained factors.

On this robust basis, we attempted the estimation of BPA exposures very likely not associated with declines in semen quality. This estimate is intended for use in future mixture risk assessments of male reproductive health.

Our value is derived from the data in animal studies. For most "high" confidence studies (*Tier 1*), the HEDs calculated from the corresponding LOAELs or NOAELs fall in the range between around 0.0026 and 0.25 μg/kg/d (Fig. 2, Table 3). By application of an AF of 25, these HEDs translate into 0.0001–0.01 μg/kg/d as possible BPA reference doses. Our choice of 0.003 μg/kg/d approximates the data from Salian et al. (2009) and Ullah et al. (2019). We judged that the higher estimates of 0.008 and 0.01 μg/kg/d which could have been chosen based on Vilela et al. (2014) and Chatsantiprapa et al. (2016), respectively, would have been insufficiently conservative, considering that Shi et al. (2018, 2019) reported effects at approximately 100-fold lower doses.

The reason why we did not opt for 0.0001 μg/kg/d, as supported by Shi et al. (2018, 2019), lies in the purpose of this exercise. Rather than providing a high degree of protection, as is essential when deriving health-based guidance values or TDIs, the intended use of our value in a mixture risk assessment dictated our interest in a *reasonable estimate* of BPA exposures likely without effects on semen quality. This led us to weigh the low doses in Shi et al. (2018, 2019) against the higher levels observed in the other animal studies. We realise that this procedure does not conform with the standards necessary for deriving tolerable daily intakes for single chemical exposures. We would like to emphasise that our business here is *not* in deriving a health-based guidance value or TDI for BPA which indeed would require a higher degree of conservatism and perhaps a correspondingly lower reference dose.

Our quantitative comparison of dose ranges in animal studies with exposure levels in epidemiological studies was for orientation only and did not influence our choice of a BPA reference dose. We recognise that such comparisons are problematic as the human studies related semen quality to contemporaneous BPA exposures, not gestational exposures. To our knowledge, epidemiological studies of gestational BPA exposures are not available. However, there is evidence from animal studies that BPA exposure in adulthood also leads to poor semen quality (not reviewed here, but for examples see Wang et al., 2016; Ullah et al., 2018).

While our evaluations were in progress, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) completed their re-evaluation of the 2015 temporary TDI for BPA. They proposed a new TDI of 0.04 ng/kg body weight/day (EFSA 2021), 100,000-times lower than the previous value of 4 μg/kg body weight/day (EFSA 2015). This new estimate considers immunotoxic effects as critical. Of relevance to our assessment, the EFSA Panel considered BPA effects on semen parameters that result from gestational or post-natal exposures until weaning as unlikely, based on animal studies that appeared up to 2018. However, in their assessment, EFSA did not consider several studies we rated as high confidence (Tier 1), such as Chatsantiprapa et al. (2016), Vom Saal et al. (1998), Vilela et al. (2014), and Yang et al. (2015). Shi et al. (2019) and Ullah et al. (2019) were published outside EFSA's evaluation period. Thus, the two studies that most heavily influenced our estimate, Salian et al. (2009) and Ullah et al. (2019), did not find entry into EFSA's evaluation. Our assessment agrees with the appraisal of studies in a bisphenol S evaluation by Beausoleil et al. (2022) who regarded Shi et al. (2019) and Ullah et al. (2019) as key studies.

In contrast to their view of the strength of evidence from gestational and post-natal animal studies, EFSA judged effects of BPA exposures on semen quality (motility and viability) in adulthood as likely. This appraisal is based on the study by Wang et al. (2016) in adult mice who reported a LOAEL of 10 μg/kg/d for BPA effects on sperm motility. With an AF = 3, this gives an extrapolated NOAEL of 3.3 μg/kg/d, in good agreement with the lower limit benchmark dose of 3.41 μg/kg/d calculated by the EFSA Panel. Combined with a mouse HEDF of 0.0155 and a further AF of 25, this produces a reference dose of 0.0015 μg/kg/d, two-fold lower than our estimate of 0.003 μg/kg/d.

We intend to utilise the reference dose derived here in a mixture risk assessment for male reproductive health, with a focus on declines in semen quality. This will be of importance in view of the reported declines in semen quality, mainly in Western countries (Levine et al., 2017). The assessment will include multiple chemicals, such as polychlorinated dibenzo-dioxins, polychlorinated biphenyls, phthalates, bisphenol F and S, parabens and many more. It is hoped that this will bring the contours of chemical exposures that impact on fertility into view.

Declaration of competing interest

The authors declare there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.113942.

References

Adoamnei, E., Mendiola, J., Vela-Soria, F., Fernández, M.F., Olea, N., Jørgensen, N., Swan, S.H., Torres-Cantero, A.M., 2018. Urinary bisphenol A concentrations are associated with reproductive parameters in young men. Environ. Res. 161, 122–128. https://doi.org/10.1016/j.envres.2017.11.002.

Agier, L., Slama, R., Basagaña, X., 2020. Relying on repeated biospecimens to reduce the effects of classical-type exposure measurement error in studies linking the exposome to health. Environ. Res. 186, 109492. https://doi.org/10.1016/j.envres.2020.109492.

Apel, P., Kortenkamp, A., Koch, H.M., Vogel, N., Rüther, M., Kasper-sonnenberg, M., Conrad, A., Brüning, T., Kolossa-gehring, M., 2020. Time course of phthalate cumulative risks to male developmental health over a 27-year period : biomonitoring samples of the German Environmental Specimen Bank. Environ. Int. 137, 105467. https://doi.org/10.1016/j.envint.2020.105467.

Axelstad, M., Christiansen, S., Boberg, J., Scholze, M., Jacobsen, P.R., Isling, L.K., Kortenkamp, A., Hass, U., 2014. Mixtures of endocrine-disrupting contaminants induce adverse developmental effects in preweaning rats. Reproduction 147, 489–501. https://doi.org/10.1530/REP-13-0447.

Axelstad, M., Hass, U., Scholze, M., Christiansen, S., Kortenkamp, A., Boberg, J., 2018. EDC impact: reduced sperm counts in rats exposed to human relevant mixtures of endocrine disrupters. Endocr. Connect. 7, 139–148. https://doi.org/10.1530/EC-17-0307.

Bauer, A.Z., Swan, S.H., Kriebel, D., Liew, Z., Taylor, H.S., Bornehag, C.G., Andrade, A. M., Olsen, J., Jensen, R.H., Mitchell, R.T., Skakkebaek, N.E., Jégou, B., Kristensen, D. M., 2021. Paracetamol use during pregnancy — a call for precautionary action. Nat. Rev. Endocrinol., 0123456789 https://doi.org/10.1038/s41574-021-00553-7.

Beausoleil, C., Le Magueresse-Battistoni, B., Viguié, C., Babajko, S., Canivenc-Lavier, M. C., Chevalier, N., Emond, C., Habert, R., Picard-Hagen, N., Mhaouty-Kodja, S., 2022. Regulatory and academic studies to derive reference values for human health: the case of bisphenol S. Environ. Res. 204 https://doi.org/10.1016/j.envres.2021.112233.

Benson, T.E., Gaml-Sørensen, A., Ernst, A., Brix, N., Hougaard, K.S., Hærvig, K.K., Bonde, J.P.E., Tøttenborg, S.S., Lindh, C.H., Ramlau-Hansen, C.H., Toft, G., 2021. Urinary bisphenol a, f and s levels and semen quality in young adult Danish men. Int. J. Environ. Res. Publ. Health 18, 1–12. https://doi.org/10.3390/ijerph18041742.

Cagen, S.Z., Waechter, J.M., Dimond, S.S., Breslin, W.J., Butala, J.H., Jekat, F.W., Joiner, R.L., Shiotsuka, R.N., Veenstra, G.E., Harris, L.R., 1999. Normal reproductive organ development in wistar rats exposed to bisphenol A in the drinking water. Regul. Toxicol. Pharmacol. 30, 130–139. https://doi.org/10.1006/rtph.1999.1340.

Caporossi, L., Alteri, A., Campo, G., Paci, E., Tranfo, G., Capanna, S., Papaleo, E., Pigini, D., Viganò, P., Papaleo, B., 2020. Cross sectional study on exposure to BPA and phthalates and semen parameters in men attending a fertility center. Int. J. Environ. Res. Publ. Health 17. https://doi.org/10.3390/ijerph17020489.

Chatsantiprapa, K., Sophon, T., Sattayasai, J., 2016. Effects of continuous exposure to bisphenol A on male and female mice from prenatally to adulthood. Thai Journal of Pharmceutical Sciences 40 (2), 61–69.

Chen, M., Tang, R., Fu, G., Xu, Bin, Zhu, P., Qiao, S., Chen, X., Xu, Bo, Qin, Y., Lu, C., Hang, B., Xia, Y., Wang, X., 2013. Association of exposure to phenols and idiopathic male infertility. J. Hazard Mater. 250–251, 115–121. https://doi.org/10.1016/j.jhazmat.2013.01.061.

Chioccarelli, T., Manfrevola, F., Migliaccio, M., Altucci, L., Porreca, V., Fasano, S., Cobellis, G., Cescon, M., 2020. Fetal-perinatal exposure to bisphenol-A affects quality of spermatozoa in adulthood mouse. Internet J. Endocrinol. https://doi.org/10.1155/2020/2750501, 2020.

Christiansen, S., Axelstad, M., Boberg, J., Vinggaard, A.M., Pedersen, G.A., Hass, U., 2014. Low-dose effects of Bisphenol a on early sexual development in male and female rats. Reproduction 147, 477–487. https://doi.org/10.1530/REP-13-0377.

De Gendt, K., Swinnen, J.V., Saunders, P.T.K., Schoonjans, L., Dewerchin, M., Devos, A., Tan, K., Atanassova, N., Claessens, F., Lécureuil, C., Heyns, W., Carmeliet, P., Guillou, F., Sharpe, R.M., Verhoeven, G., 2004. A Sertoli cell-selective knockout of the androgen receptor causes spermatogenic arrest in meiosis. Proc. Natl. Acad. Sci. U. S. A. 101, 1327–1332. https://doi.org/10.1073/pnas.0308114100.

de Rooij, D.G., Vergouwen, R.P., 1991. The estimation of damage to testicular cell lineages. Prog. Clin. Biol. Res. 372, 467–480.

Delclos, K.B., Bucci, T.J., Lomax, L.G., Latendresse, J.R., Warbritton, A., Weis, C.C., Newbold, R.R., 2001. Effects of dietary genistein exposure during development on male and female CD (Sprague-Dawley) rats. Reprod. Toxicol. 15, 647–663. https://doi.org/10.1016/S0890-6238(01)00177-0.

Delclos, K.B., Camacho, L., Lewis, S.M., Vanlandingham, M.M., Latendresse, J.R., Olson, G.R., Davis, K.J., Patton, R.E., Da costa, G.G., Woodling, K.A., Bryant, M.S., Chidambaram, M., Trbojevich, R., Juliar, B.E., Felton, R.P., Thorn, B.T., 2014. Toxicity evaluation of bisphenol a administered by gavage to sprague dawley rats from gestation day 6 through postnatal day 90. Toxicol. Sci. 139, 174–197. https://doi.org/10.1093/toxsci/kfu022.

Den Hond, E., Tournaye, H., De Sutter, P., Ombelet, W., Baeyens, W., Covaci, A., Cox, B., Nawrot, T.S., Van Larebeke, N., D'Hooghe, T., 2015. Human exposure to endocrine disrupting chemicals and fertility: a case-control study in male subfertility patients. Environ. Int. 84, 154–160. https://doi.org/10.1016/j.envint.2015.07.017.

Dere, E., Anderson, L.M., Huse, S.M., Spade, D.J., McDonnell-Clark, E., Madnick, S.J., Hall, S.J., Camacho, L., Lewis, S.M., Vanlandingham, M.M., Boekelheide, K., 2018. Effects of continuous bisphenol A exposure from early gestation on 90 day old rat testes function and sperm molecular profiles: a CLARITY-BPA consortium study. Toxicol. Appl. Pharmacol. 347, 1–9. https://doi.org/10.1016/j.taap.2018.03.021.

ECHA, (European Chemicals Agency), 2018. Seven new substances added to the Candidate List, entry for bisphenol-A updated [WWW Document], 11.11.21.

EFSA, 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. PART II - Toxicological assessment and' 13 (1) https://doi.org/10.2903/j.efsa.2015.3978.

EFSA, Hardy, A., Benford, D., Halldorsson, T., Jeger, M.J., Knutsen, H.K., More, S., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Schlatter, J.R., Silano, V., Solecki, R., Turck, D., Benfenati, E., Chaudhry, Q.M., Craig, P., Frampton, G., Greiner, M., Hart, A., Hogstrand, C., Lambre, C., Luttik, R., Makowski, D., Siani, A., Wahlstroem, H., Aguilera, J., Dorne, J., Fernandez Dumont, A., Hempen, M., Valtueña Martínez, S., Martino, L., Smeraldi, C., Terron, A., Georgiadis, N., Younes, M., 2017. Guidance on the use of the weight of evidence approach in scientific assessments. EFSA J. 15 (8) https://doi.org/10.2903/j.efsa.2017.4971.

EFSA, 2019. Testing the study appraisal methodology from the 2017 Bisphenol A (BPA) hazard assessment protocol. EFSA Support. Publ. 16. https://doi.org/10.2903/sp.efsa.2019.en-1732. (Accessed 8 February 2022).

EFSA, 2021. Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. Panel on Food Contact Material, Enzymes and Processing Aids (CEP). Scientific Opinion endorsed for public consultation. https://connect.efsa.europa.eu/RM/s/publicconsultation2/a0l1v00000E8BRD/pc0109.

Ema, M., Fujii, S., Furukawa, M., Kiguchi, M., Ikka, T., Harazono, A., 2001. Rat two-generation reproductive toxicity study of bisphenol A. Reprod. Toxicol. 15, 505–523. https://doi.org/10.1016/S0890-6238(01)00160-5.

Ermler, S., Scholze, M., Kortenkamp, A., 2011. The suitability of concentration addition for predicting the effects of multi-component mixtures of up to 17 anti-androgens with varied structural features in an in vitro AR antagonist assay. Toxicol. Appl. Pharmacol. 257, 189–197.

Frederiksen, H., Nielsen, O., Koch, H.M., Skakkebaek, N.E., Juul, A., Jørgensen, N., Andersson, A.M., 2020. Changes in urinary excretion of phthalates, phthalate substitutes, bisphenols and other polychlorinated and phenolic substances in young Danish men; 2009–2017. Int. J. Hyg Environ. Health 223, 93–105. https://doi.org/10.1016/j.ijheh.2019.10.002.

Goldstone, A.E., Chen, Z., Perry, M.J., Kannan, K., Louis, G.M.B., 2015. Urinary bisphenol A and semen quality, the LIFE study. Reprod. Toxicol. 51, 7–13. https://doi.org/10.1016/j.reprotox.2014.11.003.

Hart, R.J., Doherty, D.A., Keelan, J.A., Minaee, N.S., Thorstensen, E.B., Dickinson, J.E., Pennell, C.E., Newnham, J.P., McLachlan, R., Norman, R.J., Handelsman, D.J., 2018. The impact of antenatal Bisphenol A exposure on male reproductive function at 20–22 years of age. Reprod. Biomed. Online 36, 340–347. https://doi.org/10.1016/j.rbmo.2017.11.009.

Hass, U., Christiansen, S., Boberg, J., Rasmussen, M.G., Mandrup, K., Axelstad, M., 2016. Low-dose effect of developmental bisphenol A exposure on sperm count and behaviour in rats. Andrology 4, 594–607. https://doi.org/10.1111/andr.12176.

Howdeshell, K.L., Peterman, P.H., Judy, B.M., Taylor, J.A., Orazio, C.E., Ruhlen, R.L., vom Saal, F.S., Welshons, W.V., 2003. Bisphenol A is released from used polycarbonate animal cages into water at room temperature. Environ. Health Perspect. 111, 1180–1187. https://doi.org/10.1289/ehp.5993.

Howdeshell, K.L., Furr, J., Lambright, C.R., Wilson, V.S., Ryan, B.C., Gray, L.E., 2008. Gestational and lactational exposure to ethinyl estradiol, but not bisphenol a, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male long evans hooded rat. Toxicol. Sci. 102, 371–382. https://doi.org/10.1093/toxsci/kfm306.

Ji, H., Miao, M., Liang, H., Shi, H., Ruan, D., Li, Y., Wang, J., Yuan, W., 2018. Exposure of environmental Bisphenol A in relation to routine sperm parameters and sperm movement characteristics among fertile men. Sci. Rep. 8, 1–9. https://doi.org/10.1038/s41598-018-35787-5.

Karrer, C., Andreassen, M., von Goetz, N., Sonnet, F., Sakhi, A.K., Hungerbühler, K., Dirven, H., Husøy, T., 2020. The EuroMix human biomonitoring study: source-to-dose modeling of cumulative and aggregate exposure for the bisphenols BPA, BPS, and BPF and comparison with measured urinary levels. Environ. Int. 136, 105397. https://doi.org/10.1016/j.envint.2019.105397.

Kendig, E.L., Buesing, D.R., Christie, S.M., Cookman, C.J., Robin, B., Hugo, E.R., Kasper, S.N., Kendziorski, J.A., Ungi, K.R., 2017. Estrogen-like disruptive effects of dietary exposure to bisphenol A or 17α-ethinyl estradiol in CD1 mice. https://doi.org/10.1177/1091581812463254.Estrogen-Like, 31, 537-530.

Kim, H.K., Ko, D.H., Lee, W., Kim, K.R., Chun, S., Song, J., Min, W.K., 2019. Body fluid concentrations of bisphenol A and their association with in vitro fertilization outcomes. Hum. Fertil. 1–9. https://doi.org/10.1080/14647273.2019.1612104.

Knez, J., Kranvogl, R., Breznik, B.P., Vončina, E., Vlaisavljević, V., 2014. Are urinary bisphenol A levels in men related to semen quality and embryo development after medically assisted reproduction? Fertil. Steril. 101 https://doi.org/10.1016/j.fertnstert.2013.09.030.

Kobayashi, K., Kubota, H., Ohtani, K., Hojo, R., Miyagawa, M., 2012. Lack of effects for dietary exposure of bisphenol A during in utero and lactational periods on reproductive development in rat offspring. J. Toxicol. Sci. 37, 565–573. https://doi.org/10.2131/jts.37.565.

Kobayashi, K., Ohtani, K., Kubota, H., Miyagawa, M., 2010. Dietary exposure to low doses of bisphenol A: effects on reproduction and development in two generations of C57BL/6J mice. Congenital. Anom. 50, 159–170. https://doi.org/10.1111/j.1741-4520.2010.00279.x.

Koch, H.M., Kolossa-Gehring, M., Schröter-Kermani, C., Angerer, J., Brüning, T., 2012. Bisphenol A in 24 h urine and plasma samples of the German Environmental Specimen Bank from 1995 to 2009: a retrospective exposure evaluation. J. Expo. Sci. Environ. Epidemiol. 22, 610–616. https://doi.org/10.1038/jes.2012.39.

Kortenkamp, A., 2020. Molecular and Cellular Endocrinology Which chemicals should be grouped together for mixture risk assessments of male reproductive disorders. Mol. Cell. Endocrinol. 499, 110581. https://doi.org/10.1016/j.mce.2019.110581.

Lassen, T.H., Frederiksen, H., Jensen, T.K., Petersen, J.H., Joensen, U.N., Main, K.M., Skakkebaek, N.E., Juul, A., Jørgensen, N., Andersson, A.M., 2014. Urinary bisphenol a levels in young men: association with reproductive hormones and semen quality. Environ. Health Perspect. 122, 478–484. https://doi.org/10.1289/ehp.1307309.

Levine, H., Jørgensen, N., Martino-Andrade, A., Mendiola, J., Weksler-Derri, D., Mindlis, I., Pinotti, R., Swan, S.H., 2017. Temporal trends in sperm count: a systematic review and meta-regression analysis. Hum. Reprod. Update 23, 646–659. https://doi.org/10.1093/humupd/dmx022.

Li, D.K., Zhou, Z., Miao, M., He, Y., Wang, J., Ferber, J., Herrinton, L.J., Gao, E., Yuan, W., 2011. Urine bisphenol-A (BPA) level in relation to semen quality. Fertil. Steril. 95, 625–630. https://doi.org/10.1016/j.fertnstert.2010.09.026 e4.

Martin, O.V., Evans, R.M., Faust, M., Kortenkamp, A., 2017. A human mixture risk assessment for neurodevelopmental toxicity associated with polybrominated diphenyl ethers used as flame retardants. Environ. Health Perspect. 125 https://doi.org/10.1289/EHP826.

Martin, O.V., Baig, A., Ermler, S., McPhie, J., Scholze, M., Kortenkamp, A., 2021. Protocol for a systematic review of associations of bisphenol A exposure with declining semen quality in males to support derivation of a reference dose for mixture risk assessments for male reproductive health. Zenodo. https://doi.org/10.5281/zenodo.5083147.

Meeker, J.D., Ehrlich, S., Toth, T.L., Wright, D.L., Calafat, A.M., Trisini, A.T., Ye, X., Hauser, R., 2010. Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. Reprod. Toxicol. 30, 532–539. https://doi.org/10.1016/j.reprotox.2010.07.005.

Mendiola, J., Jørgensen, N., Andersson, A.M., Calafat, A.M., Ye, X., Redmon, J.B., Drobnis, E.Z., Wang, C., Sparks, A., Thurston, S.W., Liu, F., Swan, S.H., 2010. Are environmental levels of bisphenol A associated with reproductive function in fertile men? Environ. Health Perspect. 118, 1286–1291. https://doi.org/10.1289/ehp.1002037.

Meng, Y., Lin, R., Wu, F., Sun, Q., Jia, L., 2018. Decreased capacity for sperm production induced by perinatal bisphenol A exposure is associated with an increased inflammatory response in the offspring of C57BL/6 male mice 1–11. https://doi.org/10.3390/ijerph15102158.

Moos, R.K., Apel, P., Schröter-Kermani, C., Kolossa-Gehring, M., Brüning, T., Koch, H.M., 2017. Daily intake and hazard index of parabens based upon 24h urine samples of the German environmental specimen bank from 1995 to 2012. J. Expo. Sci. Environ. Epidemiol. 27, 591–600. https://doi.org/10.1038/jes.2016.65.

Nagao, T., Saito, Y., Usumi, K., Yoshimura, S., Ono, H., 2002. Low-dose bisphenol A does not affect reproductive organs in estrogen-sensitive C57BL/6N mice exposed at the sexually mature, juvenile, or embryonic stage. Reprod. Toxicol. 16, 123–130. https://doi.org/10.1016/S0890-6238(02)00003-5.

NTP, 2015. Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systemic Review and Evidence Integration 1–98.

NTP, 2019 Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration, https://ntp.niehs.nih.gov/ntp/ohat/pubs/handbookmarch2019_508.pdf, accessed 8February 2022.

Olaso, R., Habert, R., 2000. Genetic and cellular analysis of male germ cell development. J. Androl. 21, 497–511.

Omran, G.A., Gaber, H.D., Mostafa, N.A.M., Abdel-Gaber, R.M., Salah, E.A., 2018. Potential hazards of bisphenol A exposure to semen quality and sperm DNA integrity among infertile men. Reprod. Toxicol. 81, 188–195. https://doi.org/10.1016/j.reprotox.2018.08.010.

Orton, F., Ermler, S., Kugathas, S., Rosivatz, E., Scholze, M., Kortenkamp, A., 2014. Mixture effects at very low doses with combinations of anti-androgenic pesticides, antioxidants, industrial pollutant and chemicals used in personal care products. Toxicol. Appl. Pharmacol. 278, 201–208. https://doi.org/10.1016/j.taap.2013.09.008.

Pollard, S.H., Cox, K.J., Blackburn, B.E., Wilkins, D.G., Carrell, D.T., Stanford, J.B., Porucznik, C.A., 2019. Male exposure to bisphenol A (BPA) and semen quality in the home observation of periconceptional exposures (HOPE) cohort. Reprod. Toxicol. 90, 82–87. https://doi.org/10.1016/j.reprotox.2019.08.014.

Radke, E.G., Braun, J.M., Meeker, J.D., Cooper, G.S., 2018. Phthalate exposure and male reproductive outcomes: a systematic review of the human epidemiological evidence. Environ. Int. 121, 764–793. https://doi.org/10.1016/j.envint.2018.07.029.

Radwan, M., Wielgomas, B., Dziewirska, E., Radwan, P., Kałużny, P., Klimowska, A., Hanke, W., Jurewicz, J., 2018. Urinary bisphenol A levels and male fertility. Am. J. Men's Health 12, 2144–2151. https://doi.org/10.1177/1557988318799163.

Rahman, M.S., Kwon, W.S., Karmakar, P.C., Yoon, S.J., Ryu, B.Y., Pang, M.G., 2017. Gestational exposure to bisphenol A affects the function and proteome profile of F1 spermatozoa in adult mice. Environ. Health Perspect. 125, 238–245. https://doi.org/10.1289/EHP378.

Ruhlen, R.L., Taylor, J.A., Mao, J., Kirkpatrick, J., Welshons, W.V., Vom Saal, F.S., 2011. Choice of animal feed can alter fetal steroid levels and mask developmental effects of endocrine disrupting chemicals. J. Dev. Orig. Health Dis. 2, 36–48. https://doi.org/10.1017/S2040174410000711.

Salian, Smita, Doshi, Tanvi, Vanage, Geeta, 2009. Perinatal exposure of rats to bisphenol A affects the fertility of male offspring. Life Sci. 85, 742–752.

Sánchez-Pozo, M.C., Mendiola, J., Serrano, M., Mozas, J., Björndahl, L., Menkveld, R., Lewis, S.E.M., Mortimer, D., Jorgensen, N., Barratt, C.L.R., Fernández, M.F., Castilla, J.A., 2013. Proposal of guidelines for the appraisal of SEMen QUAlity studies (SEMQUA). Hum. Reprod. 28, 10–21. https://doi.org/10.1093/humrep/des355.

Sharpe, RM, 2020. Androgens and the masculinization programming wondow: human-rodent differences. Biochem. Soc. Trans. 48, 1725.

Shi, M., Sekulovski, N., MacLean, J.A., Hayashi, K., 2018. Prenatal exposure to bisphenol A analogues on male reproductive functions in mice. Toxicol. Sci. 163, 620–631. https://doi.org/10.1093/toxsci/kfy061.

Shi, M., Whorton, A.E., Sekulovski, N., Maclean, J.A., Hayashi, K., 2019. Prenatal exposure to bisphenol A, E, and S induces transgenerational effects on male reproductive functions in mice. Toxicol. Sci. 172, 303–315. https://doi.org/10.1093/toxsci/kfz207.

Skakkebaek, N.E., Rajpert-De Meyts, E., Buck Louis, G.M., Toppari, J., Andersson, A.M., Eisenberg, M.L., Jensen, T.K., Jørgensen, N., Swan, S.H., Sapra, K.J., Ziebe, S., Priskorn, L., Juul, A., 2015. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. Physiol. Rev. 96, 55–97. https://doi.org/10.1152/physrev.00017.2015.

Spörndly-Nees, E., Boberg, J., Ekstedt, E., Holm, L., Fakhrzadeh, A., Dunder, L., Kushnir, M.M., Lejonklou, M.H., Lind, P.M., 2018. Low-dose exposure to Bisphenol A during development has limited effects on male reproduction in midpubertal and aging Fischer 344 rats. Reprod. Toxicol. 81, 196–206. https://doi.org/10.1016/j.reprotox.2018.08.007.

Teuschler, L.K., Hertzberg, R.C., 1995. Current and future risk assessment guidelines, policy, and methods development for chemical mixtures. Toxicology 105, 137–144. https://doi.org/10.1016/0300-483X(95)03207-V.

Tyl, R.W., Myers, C.B., Marr, M.C., Sloan, C.S., Castillo, N.P., Veselica, M.M., Seely, J.C., Dimond, S.S., Miller, J.P. Van, Shiotsuka, R.N., Beyer, D., Hentges, S.G., Waechter, J. M., 2008. Two-Generation reproductive toxicity study of dietary bisphenol A in CD-1. Swiss) Mice 104, 362–384. https://doi.org/10.1093/toxsci/kfn084.

Tyl, R.W., Myers, C.B., Marr, M.C., Thomas, B.F., Keimowitz, A.R., Brine, D.R., Veselica, M.M., Fail, P.A., Chang, T.Y., Seely, J.C., Shiotsuka, R.N., 2002. Three-Generation reproductive toxicity study of dietary bisphenol A in CD. Sprague-Dawley Rats 146, 121–146.

Ullah, A., Pirzada, M., Jahan, S., Ullah, H., Razak, S., Rauf, N., 2019. Prenatal BPA and its analogs BPB , BPF , and BPS exposure and reproductive axis function in the male offspring of Sprague Dawley rats. https://doi.org/10.1177/0960327119862335.

Ullah, A., Pirzada, M., Jahan, S., Ullah, H., Turi, N., Ullah, W., Siddiqui, M.F., Zakria, M., Lodhi, K.Z., Khan, M.M., 2018. Impact of low-dose chronic exposure to bisphenol A and its analogue bisphenol B, bisphenol F and bisphenol S on hypothalamo-pituitary-testicular activities in adult rats: a focus on the possible hormonal mode of action. Food Chem. Toxicol. 121, 24–36. https://doi.org/10.1016/j.fct.2018.08.024.

Vilela, J, Hartmann, A, Silva, EF, Cardoso, T, Corcini, CD, Varela-Junior, AS, Martinez, PE, Colares, EP, 2014. Sperm impairments in adult vesper mice (*Calomys cauda*) caused by *in utero* exposure to bisphenol A. Andrologia 46, 971–978. https://doi.org/10.1111/and.12182.

Vitku, J., Heracek, J., Sosvorova, L., Hampl, R., Chlupacova, T., Hill, M., Sobotka, V., Bicikova, M., Starka, L., 2016. Associations of bisphenol A and polychlorinated biphenyls with spermatogenesis and steroidogenesis in two biological fluids from men attending an infertility clinic. Environ. Int. 89–90, 166–173. https://doi.org/10.1016/j.envint.2016.01.021.

Vom Saal, F.S., Cooke, P.S., Buchanan, D.L., Palanza, P., Thayer, K.A., Nagel, S.C., Parmigiani, S., Welshons, W.V., 1998. A physiologically based approach to the study of bisphenol a and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. Toxicol. Ind. Health 14, 239–260. https://doi.org/10.1177/074823379801400115.

Whaley, P., Aiassa, E., Beausoleil, C., Beronius, A., Bilotta, G., Boobis, A., de Vries, R., Hanberg, A., Hoffmann, S., Hunt, N., Kwiatkowski, C.F., Lam, J., Lipworth, S., Martin, O.V., Randall, N., Rhomberg, L., Rooney, A.A., Schünemann, H.G., Wikoff, D., Wolffe, T., Halsall, C., 2020. Recommendations for the conduct of systematic reviews in toxicology and environmental health research (COSTER). Environ. Int. 143, 105926. https://doi.org/10.1016/j.envint.2020.105926.

Wang, H.F., Liu, M., Li, N., Luo, T., Zheng, L.P., Zeng, X.H., 2016. Bisphenol a impairs mature sperm functions by a CatSper-relevant mechanism. Toxicol. Sci. 152, 145–154. https://doi.org/10.1093/toxsci/kfw070.

WHO, 2010. https://apps.who.int/iris/handle/10665/44261. (Accessed 8 February 2021).

Yang, Y., Hong, Y., Chae, S.A., 2015. Reduction in semen quality after mixed exposure to bisphenol A and isobutylparaben in utero and during lactation periods 1–10. https://doi.org/10.1177/0960327115608927.

Yoshino, Hiroko, Ichihara, Toshio, Kawabe, Mayumi, Imai, Norio, Hagiwara, Akihiro, Asamoto, Makoto, Shirai, Tomoyuki, 2002. Lack of significant alteration in the prostate or testis of F344 rat offspring after transplacental or lactational exposure to bisphenol A. J. Toxicol. Sci. 27 (5), 433–439.

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Household air pollution from wood-burning cookstoves and C-reactive protein among women in rural Honduras

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ABSTRACT

Household air pollution from solid fuel combustion was estimated to cause 2.31 million deaths worldwide in 2019; cardiovascular disease is a substantial contributor to the global burden. We evaluated the cross-sectional association between household air pollution (24-h gravimetric kitchen and personal particulate matter ($PM_{2.5}$) and black carbon (BC)) and C-reactive protein (CRP) measured in dried blood spots among 107 women in rural Honduras using wood-burning traditional or *Justa* (an engineered combustion chamber) stoves. A suite of 6 additional markers of systemic injury and inflammation were considered in secondary analyses. We adjusted for potential confounders and assessed effect modification of several cardiovascular-disease risk factors.

The median (25th, 75th percentiles) 24-h-average personal $PM_{2.5}$ concentration was 115 μg/m^3 (65,154 μg/m^3) for traditional stove users and 52 μg/m^3 (39, 81 μg/m^3) for *Justa* stove users; kitchen $PM_{2.5}$ and BC had similar patterns. Higher concentrations of $PM_{2.5}$ and BC were associated with higher levels of CRP (e.g., a 25% increase in personal $PM_{2.5}$ was associated with a 10.5% increase in CRP [95% CI: 1.2–20.6]). In secondary analyses, results were generally consistent with a null association. Evidence for effect modification between pollutant measures and four different cardiovascular risk factors (e.g., high blood pressure) was inconsistent. These results support the growing evidence linking household air pollution and cardiovascular disease.

1. Introduction

Short-term and long-term exposure to particulate matter is associated with increased risk for cardiovascular morbidity and mortality (Atkinson et al., 2014; Brook et al., 2010; Franklin et al., 2015; Gold and Mittleman, 2013; Hoek et al., 2013; Newby et al., 2015; Polichetti et al., 2009; C. A. Pope, 2000). The use of solid biomass fuels, such as wood, dung or crop residue, for cooking and heating results in high levels of chronic exposure to particulate matter and black carbon (BC). The majority of the household air pollution burden of disease is among those living in low and middle income countries (LMIC), where approximately 80% of worldwide cardiovascular deaths occur (Bowry et al., 2015).

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Exposure to household air pollution was estimated to be responsible for 91.5 million disability-adjusted life years and 2.31 million deaths in 2019 (Bennitt et al., 2021; Health Effects Institute, 2020). There is evidence that exposure to household air pollution is associated with cardiovascular disease (CVD) (Lee et al., 2020; Shupler et al., 2020).

Numerous plausible mechanistic pathways may explain the association between particulate matter and cardiovascular disease including systemic inflammation, endothelial dysfunction, and oxidative stress (Brook et al., 2010; Caravedo et al., 2016; Pearson et al., 2003; C. Arden Pope et al., 2016). The expression of circulating C-reactive protein (CRP) is an indication of systemic general inflammatory activity and a predictor of future cardiovascular disease as well as all-cause mortality (Johnson et al., 2004; Ridker, 2003). Supporting evidence exists for exposure to ambient air pollution and increased levels of CRP (W. Li et al., 2017; Y. Li, Rittenhouse-Olson, Scheider and Mu, 2012). Additional biomarkers of inflammation such as (Serum Amyloid A [SAA], Interleukin 1-β [IL-1β], IL-8, Tumor Necrosis Factor-α [TNF-α], Intercellular Adhesion Molecule 1 [ICAM-1], and Vascular Cell Adhesion Molecule [VCAM-1]), are also indicators of increased endothelial inflammation and cardiovascular disease (Teixeira et al., 2014). There is suggestive evidence from the field of household air pollution that demonstrates associations between cleaner-burning stoves and measured household air pollution and these biomarkers (Caravedo et al., 2016; Dutta et al., 2012; Olopade et al., 2017).

Quantifying associations between household air pollution and cardiovascular disease in rural, low-resource settings is challenging, especially when clinical disease outcomes are measured (Mcdade et al., 2007). Biomarkers of subclinical cardiovascular disease are feasible to measure in the field and have been utilized in household air pollution studies (Caravedo et al., 2016; Dutta et al., 2012; Dutta et al., 2013; Olopade et al., 2017; Young et al., 2019). Although standard practice is to measure biomarkers in serum drawn from venous blood, logistical challenges in the field often prevent direct blood draws due to a lack of equipment and facilities for sample processing and preservation (Mcdade et al., 2007). The use of dried blood spots to quantify inflammatory markers has grown in utility over the past decade in both clinical and research applications due to the increased convenience, low-cost, and reliability of the methods (Miller and McDade, 2012). The use of dried blood spots to measure CRP is particularly promising. Through our feasibility study in Nicaragua among 54 women, we demonstrated low within-person variability in CRP concentrations as measured daily in dried blood over a 4-day period and positive associations between exposure to household air pollution and increased levels of CRP among wood-burning cookstove users (Young et al., 2019). Specifically, we reported that a 25% increase in kitchen $PM_{2.5}$ was associated with a 7.4% increase in CRP measured in dried blood spots (Young et al., 2019).

In this cross-sectional study among 107 rural Honduran women, we used a minimally invasive method of finger-stick dried blood spots to assess the association between systemic inflammation and the previous 24-h average exposure to household air pollution. In this study, we built upon our previous work in Nicaragua and added several important indicators of exposure including measured concentrations of personal $PM_{2.5}$ and kitchen and personal BC. Our primary analysis focused on CRP, a decision based largely on the role of CRP as an important indicator of future disease risk and the results of our feasibility study (Young et al., 2019). Although CRP itself is not likely causally involved in cardiovascular events, it is consistently reflective of underlying pro-inflammatory pathways that mediate disease (Eiriksdottir et al., 2011). For our secondary analyses, we measured additional markers of systemic injury and inflammation (SAA, IL-1β, IL-8, TNF-α, ICAM-1, and VCAM-1). Further, we hypothesized that the association between household air pollution and CRP may be stronger among a subset of the population with risk factors for cardiovascular disease such as increased age, higher body mass index (BMI), higher levels of glycated hemoglobin (HbA1c) and higher blood pressure (Dubowsky et al., 2006).

2. Materials and methods

Study protocols were approved by the Colorado State University Institutional Review Board.

2.1. Study population

This cross-sectional study was conducted in 11 rural communities surrounding La Esperanza which is home to approximately 15,000 people and located in the mountainous region of Western Honduras. Participants were a convenience sample recruited into the study from a sample of more than 500 households screened in a household survey. We obtained exposure and health measurements from 150 female primary cooks near La Esperanza between February and April 2015 (dry season). Enrollment criteria required that the primary cook own a traditional stove or a cleaner-burning *Justa* cookstove (built at least 4 months prior to the interview) and be 25–56 years old, a non-smoker, and not pregnant. Traditional cookstoves in the communities are typically self-built wood-burning stoves, with a metal griddle and a chimney. *Justa* cookstoves are a common wood-burning stove model in Central America. The *Justa* design includes a rocket-elbow combustion chamber, metal griddle, and chimney. Study participants provided informed consent and, following data collection, received an incentive of USD$5 worth of food items for their participation.

2.2. Exposure to household air pollution

We measured kitchen and personal 24-h concentrations of $PM_{2.5}$ and BC. Fine particle concentrations were measured gravimetrically using Triplex cyclones with a particle cut size of 2.5 μm (BGI by Mesa Labs, Butler NJ, USA). Air was pulled through the cyclone by an external pump (SKC AirCheck XR5000, SKC Inc, Eighty Four, PA, USA) that was pre-calibrated daily using a flow meter (DryCal Dc-Lite, Bios International, Mesa Labs, NJ, USA) and set to a flow rate of 1.5 L/min. $PM_{2.5}$ was collected on 37-mm polytetrafluoroethylene (PTFE)-coated glass fiber filters (Fiberfilm™ T60A20, Pall Corporation, Port Washington KY, USA). Prior to sampling, the filters were equilibrated for at least 24-h and then pre-weighed at Colorado State University (CSU) using a microbalance (Mettler Toledo Microbalance, model MX5, resolution and repeatability of 1-μg, Columbus, OH, USA).

Kitchen exposure monitors were placed between 76 and 127 cm above the stove and away from open windows and doors. For personal exposure, the sampling pump was placed in a small bag that each participant wore over their shoulder for the 24-h period (except when bathing or sleeping). Women were instructed to place the bag with the monitor next to their bathing area or bed when not wearing it. The inlet to the cyclone was clipped to a strap on the woman's chest near her breathing zone. After collection of the sample, filters were stored at −22 °C and then transported to CSU, equilibrated for at least 24-h, and post-weighed for particulate matter mass. One filter blank was collected every two weeks (n = 7).

We calculated the 24-h average gravimetric $PM_{2.5}$ concentration as the change in sample filter mass (adjusted for the average mass change in blank filters) divided by the total volume of air sampled. The $PM_{2.5}$ mass limit of detection (LOD) was calculated as follows: average mass of blank filters plus 3 times the standard deviation (SD) of the sample blank filter masses. All samples with a mass less than the LOD of 54 μg (7 kitchen samples and 7 personal samples) were replaced with a value of $LOD/\sqrt{2}$ (MacDougall et al., 1980). Due to a broken DryCal volumetric flow meter needed to calibrate the $PM_{2.5}$ sampling pump, we were unable to collect PM exposure measures from the first 41 houses recruited into the study. Additional samples were excluded from analysis due to: AirCheck pumps running for less than 75% of the intended time (<18 h) (three personal and two kitchen samples), negative filter weight (one personal $PM_{2.5}$ sample), and missing flow post-calibration data in the field (one kitchen $PM_{2.5}$ sample).

BC concentrations were estimated based on the optical transmission of light through the filters (Hansen et al., 1984) using a transmissometer (model OT-21, Magee Scientific, USA). Transmission data were converted to mass concentrations based on published mass-absorption values for combustion aerosols (Chylek et al., 1981) and corrected for a filter loading artifact wherein an underestimation occurs at high sample loading (Kirchstetter and Novakov, 2007). We calculated a BC concentration using the sample air flow and duration. The LOD was estimated to be equivalent to a minimum concentration 0.86 μg/m^3 which corresponds to three times the standard deviation of BC concentrations from 54 blank samples (additional blank filters were used from field sampling campaigns in Honduras conducted within the same year to estimate the reference values for the transmissometer). Values below the LOD (3 kitchen samples and 10 personal samples) were substituted by LOD/$\sqrt{2}$. For more detailed information on BC methodologies, please see Chylek et al. (1981), Kirchstetter and Novakov, 2007, and supporting information in our previous publication (Young et al., 2018).

2.3. Markers of systemic injury and inflammation

Markers of systemic injury and inflammation were assessed via dried blood (Mei et al., 2001). To obtain the dried blood spots, each woman had her middle or ring finger cleaned with a 70% alcohol swab. Once the finger was dry, it was pricked with a sterile disposable 1.75 mm point BD Genie™ lancet (BD, Franklin Lakes, USA). The first drop of blood was wiped away using sterile gauze to prevent contamination from possible tissue fluid or skin. Blood was then spotted onto a standardized filter paper (See Fig. 1) (903 Protein Saver Card, Schleicher & Schuell, NH). Participants provided up to 5 spots on the card. The samples were obtained in the morning between 7:30am and 12:00pm, immediately following the 24-h exposure assessment. After collection, samples were allowed to dry for 24-h at room temperature, placed in baggies with desiccant and humidity indicator cards, and stored frozen at −22 °C in Honduras. The samples were transported (off ice for less than 24 h) and stored at CSU at −80 °C. Samples were shipped overnight (on dry ice) from CSU to the National Health and Environmental Effects Laboratory of the U.S. Environmental Protection Agency for analysis. Analyses were performed on the Meso Scale Multiplex instrument (Meso Scale Discovery, Gaithersburg, MD). The V-PLEX Plus Vascular Injury Panel 2 (human) kit was used to measure ICAM-1, VCAM-1, CRP, SAA. The Human Pro-Inflammatory-4 II Base Kit was used for proinflammatory mediators (IL-Iβ, TNF-α, IL-6, and IL-8). One 6 mm circle was punched from one blood spot on each card. The punches were transferred to 96-well plates (one punch per well) and a 200 μL extraction buffer (PBS with 0.5% tween-20) was added to each well. Each plate was sealed and placed on a shaker table overnight in a 4 °C refrigerator. Plates were stored in a −80 °C freezer prior to analysis. For analysis, the extraction liquids were measured in a 1:200 dilution factor for the V-PLEX plus vascular injury kit and without any dilution for the pro-inflammatory mediator kit. The QuickPlex 120 instrument directly outputs data. The reliability (intra-plate variability) and reproducibility (inter-plate variability) were tested for all assays and were below a 10% coefficient of variation. We evaluated the lower LOD for each inflammatory marker according to the company specifications (Meso Scale Discovery, Gaithersburg, MD). Samples with a value below the LOD for the inflammatory marker were substituted with the LOD/$\sqrt{2}$ (MacDougall et al., 1980). Five of the inflammatory markers had no values less than the LOD (CRP, SAA, IL-8, ICAM-1, and VCAM-1). For IL-1β, we substituted one value less than the LOD (LOD = 0.04 ng/mL), while we substituted 37 values for TNF-α (LOD = 0.04 ng/mL). For IL-6, 89% (127 samples) were below the LOD of 0.06 ng/mL, therefore we did not evaluate this marker in further analyses.

Fig. 1. Protein saver card and dried blood spot.
Photo Credit: Joanna B Pinneo Photography, https://www.joannabpinneophoto.com/index.

2.4. Additional information

2.4.1. Population demographics and covariates

The study team administered in-person demographic surveys in the homes of participants. Responses were recorded on a tablet into an electronic data collection system, Open Data Kit (Brunette et al., 2013). We ascertained data on the number of beds per person in the household, years of formal education, access to electricity, the number of assets owned (cars, bikes, motorbikes, televisions, radios, refrigerators, sewing machines), and diet to calculate a dietary diversity score. For the dietary diversity score, women reported all food eaten in the previous 24-h period and the number of portions (Arps, 2011). The final dietary diversity score was a sum of the number of food groups a woman had eaten at least one portion of in the past 24 h. Scores ranged from 1 to 10 and served as an indicator of socioeconomic status (SES). Surveys were also used to collect information on cooking and exposure to secondhand smoke. Women self-reported any medication use at the time of the study. Anthropometric data were gathered at the homes of women. Participant's waist circumference, weight (kilograms), and height (meters) were measured. BMI was calculated by dividing the weight by the square of the height.

2.4.2. Effect modification covariates

To estimate diabetes-related information for our effect modification analyses, we measured HbA1c with a 5 μl finger stick sample of blood. The sample was analyzed in the field with the A1CNow+® system (PTS Diagnostics, Indianapolis, USA). We categorized participants into having normal levels of HbA1c (HbA1c <5.7%) or elevated levels (HbA1c ≥ 5.7%) (American Diabetes Association, 2018). Blood pressure was measured using the SphygmoCor XCEL Central Blood Pressure Measurement System (AtCor Medical Pty Ltd, Australia), recorded at the brachial artery on the woman's right arm with a 23–33 cm cuff. Three consecutive measurements were taken for each participant after a 10-min rest period. The average of the last two measurements was recorded. We categorized blood pressure into normal blood pressure (systolic <120 mmHg and diastolic <80 mmHg) and elevated blood pressure (systolic ≥120 mmHg and/or diastolic ≥80 mmHg) (Whelton et al., 2018). BMI was estimated as described above.

2.5. Statistical analysis

Data were analyzed using SAS® software version 9.4 (SAS Institute, Inc., Cary, NC, USA) and R, version 3.4.1 (R Core Team, Vienna, Austria).

Descriptive statistics were determined for population characteristics and pollutant concentrations for the entire population and stratified by stove type used within the home. Spearman rank correlation coefficients were calculated between pollutants. Descriptive statistics and Spearman

correlation coefficients were also calculated for the 7 inflammatory markers.

In primary analyses we evaluated the associations between $PM_{2.5}$ (personal and kitchen) and BC (personal and kitchen) with each of the inflammatory markers using a separate multiple linear regression model for each exposure-outcome combination. We logarithmically transformed both $PM_{2.5}$ and BC exposure concentrations as well as all inflammatory markers to meet the assumptions of linear regression. For all analyses, we removed participants who self-reported use of hypertension medications (n = 3), use of vitamins and/or folic acid (n = 22), or anti-inflammatory medications (n = 11). Potential confounding variables were chosen a priori based on previous literature and included age, education, a marker of anthropometry, and a marker of socioeconomic status. We evaluated options for markers of anthropometry (BMI, height, weight, and waist circumference) and measures of socio-economic status (including dietary diversity score, number of assets owned, electricity, beds per person, and education level) based on crude associations with the biomarkers. In our final models, we controlled for age, body-mass index, assets owned (<2 assets or ≥2 assets), electricity (yes/no) and years of school (<6 or ≥6 years). We also conducted sensitivity analyses and re-ran final models: 1.) including a term for community in order to account for non-independence of participants within communities, and 2). the removal of five participants who reported occasional secondhand smoke exposure.

We explored effect modification by risk factors for cardiovascular disease including age (<40 or ≥40 years), BMI (<25.1 or ≥25.1 kg/m^2, the median value in our dataset), HbA1c levels (normal [<5.7] vs. elevated [≥5.7%]) and a one-time measure of blood pressure (normal [systolic <120 mmHg and diastolic <80 mmHg] vs. elevated [systolic ≥120 mmHg and/or diastolic ≥80 mmHg]) by adding an interaction term for each of the candidate modifying factors and each of the exposure variables in the models. We considered effect modification results to be significant based on a p-value of 0.05 or less for the interaction term.

3. Results

We enrolled 150 women in our cross-sectional study; a total of 146 women were included in our sample. Two participants declined to provide dried blood samples, and two women had incomplete samples. Population characteristics are presented in Table 1. The average age of women in the study was 37.3 years (SD: 8.9), average BMI was 25.9 kg/m^2 (SD: 4.1), and about half the population (n = 67) had less than 6 years of education. Most variables were similar between the two stove groups; there was a suggestive difference in education status (51.4% of traditional stove users had less than 6 years of education compared to 41.7% of *Justa* stove users).

Table 1
Population characteristics among nonsmoking primary female cooks using traditional or cleaner-burning *Justa* stoves, rural Honduras (N = 146).

	Total (N = 146)	Traditional (N = 73)	*Justa* (N = 73)
	N (%) or Mean (SD)	N (%) or Mean (SD)	N (%) or Mean (SD)
Age (years)	37.3 (8.9)	38.4 (9.4)	36.1 (7.9)
Body mass index (kg/m^2)	25.9 (4.1)	25.8 (4.5)	26.0 (3.8)
Categorized BMI			
<25.1	67 (46%)	36 (49.3%)	31 (42%)
≥25.1	79 (54%)	37 (50.7%)	42 (58%)
Elevation (meters)[a]	1913 (103)	1990 (91)	1936 (109)
Years of education[b]			
Less than six years	67 (46.5%)	37 (51.4%)	30 (41.7%)
Six or more years	77 (53.5%)	35 (48.6%)	42 (58.3%)
Electricity[a]			
No	119 (82.1%)	61 (83.6%)	58 (80.6%)
Yes	26 (17.9%)	12 (16.4%)	14 (19.4%)
Number of assets			
Less than 2	69 (47.3%)	33 (45.2%)	36 (49.3%)
Two or more	77 (52.7%)	40 (54.8%)	37 (50.7%)
Years spent cooking with biomass	25.8 (9.7)	26.8 (10.5)	24.8 (8.8)
Self-reported exposure to secondhand smoke	5 (3.4%)	5 (3.6%)	0 (0%)
Systolic blood pressure	118.6 (12.7)	120.4 (12.2)	116.8 (13.1)
Diastolic blood pressure	73.1 (8.6)	73.8 (9.4)	72.4 (8.9)
Blood Pressure			
Normal (systolic <120 mmHg and diastolic <80 mmHg)	118 (80.8%)	54 (74%)	64 (88)%
Elevated (systolic ≥120 mmHg and/or diastolic ≥80 mmHg)	28 (19%)	19 (26%)	9 (12%)
HbA1c[c]	5.5 (0.75)	5.5 (0.4)	5.6 (1.0)
Glycated Hemoglobin			
Normal (HbA1c <5.7%)	104 (74%)	54 (76%)	50 (73%)
Elevated (HbA1c ≥ 5.7%)	36 (25%)	17 (24%)	19 (27%)

[a] N = 145; Traditional = 73, Justa = 73.
[b] N = 144; Traditional = 73, Justa = 72.
[c] N = 140; Traditional = 71, Justa = 69.

3.1. Exposure concentrations

For the exposures, the final sample sizes for the pollutant measurements was 105 for personal $PM_{2.5}$, 106 for personal BC and kitchen $PM_{2.5}$, and 107 for kitchen BC.Tthe final sample sizes for $PM_{2.5}$ and BC differed by one measurement each due to the a negative filter weight (one personal $PM_{2.5}$ sample) and missing flow post-calibration data in the field (one kitchen $PM_{2.5}$ sample); these measurements were retained in the BC analyses. Twenty-four hour minimum, maximum, median, 25th and 75th percentile concentrations of each pollutant are shown in Table 2. As expected, kitchen $PM_{2.5}$ (n = 106) was higher than personal $PM_{2.5}$ (n = 105) with median concentration of 132 μg/m^3 (25th and 75th percentile: 62 μg/m^3, 374 μg/m^3) compared to 80 μg/m^3 (25th and 75th percentile: 51 μg/m^3, 137 μg/m^3). The same pattern holds for kitchen and personal BC. In addition, women who owned traditional stoves were exposed to higher concentrations of each of the two pollutants than women who owned *Justa* stoves (Table 2). For example, the median 24-h-average personal $PM_{2.5}$ concentration was 115 μg/m^3 (25th and 75th percentile: 65 μg/m^3, 154 μg/m^3) for traditional stove users (n = 62) and 52 μg/m^3 (25th and 75th percentile: 39 μg/m^3, 81 μg/m^3) for *Justa* stove users (n = 43). The 24-h averages of the four pollutant measures (kitchen and personal $PM_{2.5}$ and kitchen and personal BC) were strongly correlated. Within pollutants, there was a positive correlation between kitchen concentrations and personal concentrations to $PM_{2.5}$ (Spearman rho = 0.80) and kitchen and personal BC (Spearman rho = 0.77). $PM_{2.5}$ and BC exposures were correlated among kitchen measurements (Spearman rho = 0.89) and personal measurements (Spearman rho = 0.78).

3.1.1. Markers of systemic injury and inflammation

Overall summary statistics for the seven inflammatory markers measured in dried blood spots are presented in Table 3. We had 110 participants with valid blood spot data. Participants had a median CRP level of 13.6 per ng/mL (25th-75th: 5.8–27.5 per ng/ml). Several markers were moderately correlated, CRP and SAA (Spearman rho = 0.49) and ICAM-1 and VCAM-1 (Spearman rho = 0.71), while other markers exhibited low or no correlation (Table 3).

3.1.2. Primary analysis of CRP

In our linear models, our sample size was limited to participants who had valid exposure measurements, valid dried blood spot samples, and who did not take medications (Kitchen $PM_{2.5}$: n = 74; personal $PM_{2.5}$: n = 73; Kitchen BC: n = 75; personal BC: n = 75; stove-type: n = 73). The analytical sample did not differ by demographic characteristics from the full study sample (data not presented). Higher levels of $PM_{2.5}$ were associated with higher levels of CRP (Table 4). For example, a 25%

Table 2
24-h average kitchen and personal fine particulate matter and black carbon concentrations, traditional and *Justa* stove users, rural Honduras.

	All Participants						Traditional Stove Users						*Justa* Stove Users					
	n	Min	25th	Median	75th	Max	n	Min	25th	Median	75th	Max	n	Min	25th	Median	75th	Max
24-h average kitchen $PM_{2.5}$ *(μg/m³)*	*106*	18	62	132	374	1654	*62*	18	91	181	511	1654	*44*	18	38	71	159	1134
24-h average personal $PM_{2.5}$ *(μg/m3)*	*105*	18	51	80	137	346	*62*	18	65	115	154	346	*43*	18	39	52	81	174
24-h average kitchen Black Carbon *(μg/m3)*	*107*	1	9	21	82	1172	*63*	1	15	44	114	1172	*44*	1	4	11	19	469
24-h average personal Black Carbon *(μg/m3)*	*106*	1	4	7	18	123	*62*	1	7	14	32	123	*44*	1	1	4	9	47

$PM_{2.5}$: fine particulate matter.

Table 3
Median (25th and 75th percentiles) and Spearman rank correlation between markers of systemic injury and inflammatory (N = 110).

	Median (25th, 75th percentile)	TNFa	CRP	SAA	ICAM-1	VCAM-1	IL-1B
IL-8	5.8 (4.9, 8.2)	0.26	−0.09	−0.02	0.13	0.09	0.22
TNF-a	0.06 (0.04, 0.07)	1	0.39	0.04	0.48	0.42	0.14
CRP[a]	13.6 (5.8, 27.5)	–	1	0.49	0.39	0.20	0.00
SAA[a]	28.9 (17.9, 52.8)	–	–	1	0.18	0.11	0.11
ICAM-1[a]	6.9 (5.9, 8.5)	–	–	–	1	0.71	0.1
VCAM-1[a]	11.2 (9.8, 13.3)	–	–	–	–	1	0.18
IL-1 β	0.20 (0.1, 0.20)	–	–	–	–	–	1

Interleukin 8 (IL-8), Tumor Necrosis Factor-α (TNF-α), C-Reactive Protein (CRP), Serum Amyloid A (SAA), Intercellular Adhesion Molecule 1 (ICAM-1), Vascular Cell Adhesion Molecule (VCAM-1) Interleukin 1-β (IL-1β).
[a] Concentrations presented per ng/mL.

Table 4
Estimated adjusted[a] percentage difference in C-reactive protein per 25% increase in 24-h average measured pollutant concentration, or by stove type among traditional and *Justa* stove users, rural Honduras.

Pollutant		*N*	*Percentage Difference in CRP*	*95% CI*
Kitchen $PM_{2.5}$ (μg/m³)[b]		74	4.2	−1.1, 9.7
Personal $PM_{2.5}$ (μg/m³)[b]		73	10.5	1.2, 20.6[d]
Kitchen BC (μg/m³)[b]		75	3.9	0.1, 7.8[d]
Personal BC (μg/m³)[b]		73	4.2	−0.5, 9.1
Stove type[c]	*Traditional*	54	24.6	−33.4, 133.1
	Justa (ref)	56		

Cl: Confidence interval; $PM_{2.5}$: fine particulate matter; CRP (C-reactive protein).
[a] Sample size included only participants with both exposure and CRP. Models were adjusted for age, body mass index (BMI), number of assets (<2 or ≥2), electricity (yes/no), years of education (<6 or ≥6).
[b] In continuous exposure models, CRP and measured pollution were both log transformed. Beta coefficients were entered into the formula ((1.25^β)-1) and multiplied by 100. We can interpret the estimate of the continuous pollution exposures as a percent increase in inflammatory marker for each 25% increase in exposure. Example: There is a 10.5% higher CRP level with a 25% higher personal $PM_{2.5}$ concentration.
[c] Inflammatory marker was log-transformed. Categorical variable beta coefficients were entered into the formula (e^β-1)*100). The estimates for the categorical measures of exposure can be interpreted as the percent difference in inflammatory marker when comparing traditional stove to the reference (Justa stove).
[d] Significant at the 0.05 level.

increase in personal $PM_{2.5}$ concentrations resulted in a 10.5% (95% CI: 1.2–20.6) increase in CRP concentrations after controlling for potential confounders (age, body mass index (BMI), number of assets (<2 or ≥2), electricity (yes/no), years of education (<6 or ≥6). Because of the natural-log transformation of our modeled $PM_{2.5}$ exposure variable, the 25% increase in personal $PM_{2.5}$ associated with the 10.5% increase in CRP represents a different absolute difference in $PM_{2.5}$ at varying points along the observed $PM_{2.5}$ continuum; for example, at the 25th percentile the corresponding absolute difference is 13 μg/m³ (i.e., from 51 μg/m³ to 64 μg/m³) and at the 75th percentile the corresponding absolute difference is 34 μg/m³ (i.e., from 137 μg/m³ to 171 μg/m³). Similar positive results were observed between higher kitchen concentrations of BC and higher CRP concentrations, while there was a suggestive positive association between kitchen $PM_{2.5}$ and CRP as well as personal BC and CRP.

We observed that women who used traditional stoves had CRP levels that were 24.6% higher than women who used *Justas* stoves (95% CI: 33.4–133.1) (Table 4).

3.1.4. Secondary analysis of markers of systemic injury and inflammation

Among our secondary biomarkers, we also observed associations between all four continuous pollutants and SAA (Supplement Table 1). A 25% increase in personal $PM_{2.5}$ exposure was associated with an 8.3% increase in SAA concentrations (95% CI: 2.3, 14.7). Additionally, a 25% higher kitchen black-carbon concentration was associated with 1.9% higher IL-8 concentration (95% CI: 0.4–3.5) in finger prick blood samples. We did not observe any associations between household air pollution concentrations and IL-1β, ICAM-1, VCAM-1, or TNF-α.

In general, the cookstove type (traditional or *Justa*) was not associated with secondary markers of inflammation with the exception of SAA. We observed that women who owned a traditional stove had a 59.8% higher SAA level compared to women who used a *Justa* cookstove (95% CI: 10.2–131.8). Separate sensitivity analyses including a term for community and removing participants exposed to second-hand smoke did not influence results (results not shown).

3.2. Effect modification

For effect modification analyses, 18 of 110 women reported no cardiovascular risk factors. Of those who reported only one risk factor, 13 had only age >40 years as a risk factor, 15 had only high BMI (≥25.1 kg/m²), five women had only high blood pressure, and 11 were classified as only having high HbA1c (Fig. 2), the others had multiple risk factors. Although we hypothesized effects of household air pollution on CRP to be stronger among those in our population with cardiovascular disease

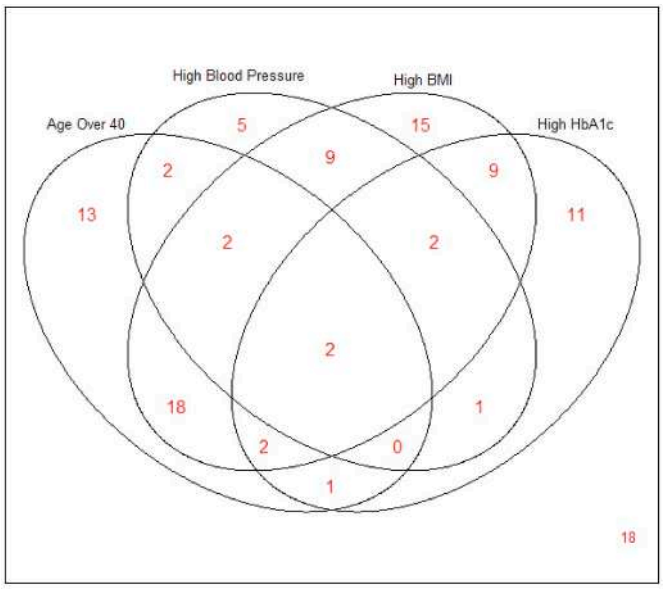

Fig. 2. Venn diagram of risk factors for cardiovascular disease (N = 110)
†18 participants did not have any risk factor
BMI = body mass index, HbA1c = glycated hemoglobin.

risk factors; patterns were not consistent with this hypothesis (Fig. 3). For example, we observed a stronger positive association between household air pollution and CRP among women who were classified as "normal" compared to the elevated HbA1c group. A 25% higher kitchen $PM_{2.5}$ concentration was associated with an 8.6% (95% CI: 2.6, 15.0) higher CRP concentration among women with elevated HbA1c, while a 25% higher personal $PM_{2.5}$ concentration was associated with a 20.7% higher CRP among women with normal HbA1c (95% CI: 10.0, 32.4); (p-interaction = 0.01) (Fig. 3). We did not observe evidence of effect modification on the association of personal $PM_{2.5}$ and CRP for other factors including categories of BMI (p-interaction = 0.22) or blood pressure (p-interaction = 0.46). Similarly inconsistent effect modification results were observed for the secondary markers of systemic injury and inflammation (Supplement Figures 1-4). Further, we did not observe consistent patterns of pollutant concentration distributions by risk factor groupings that may have helped with interpreting effect modification results (Supplemental Table 2). For example, if those with normal HbA1c were, on average, in a lower distribution range of $PM_{2.5}$ as compared to those with elevated HbA1c, these results could have suggested that the stronger effect of $PM_{2.5}$ on CRP among those with normal HbA1c was due to the exposure-response being stronger at lower PM concentrations.

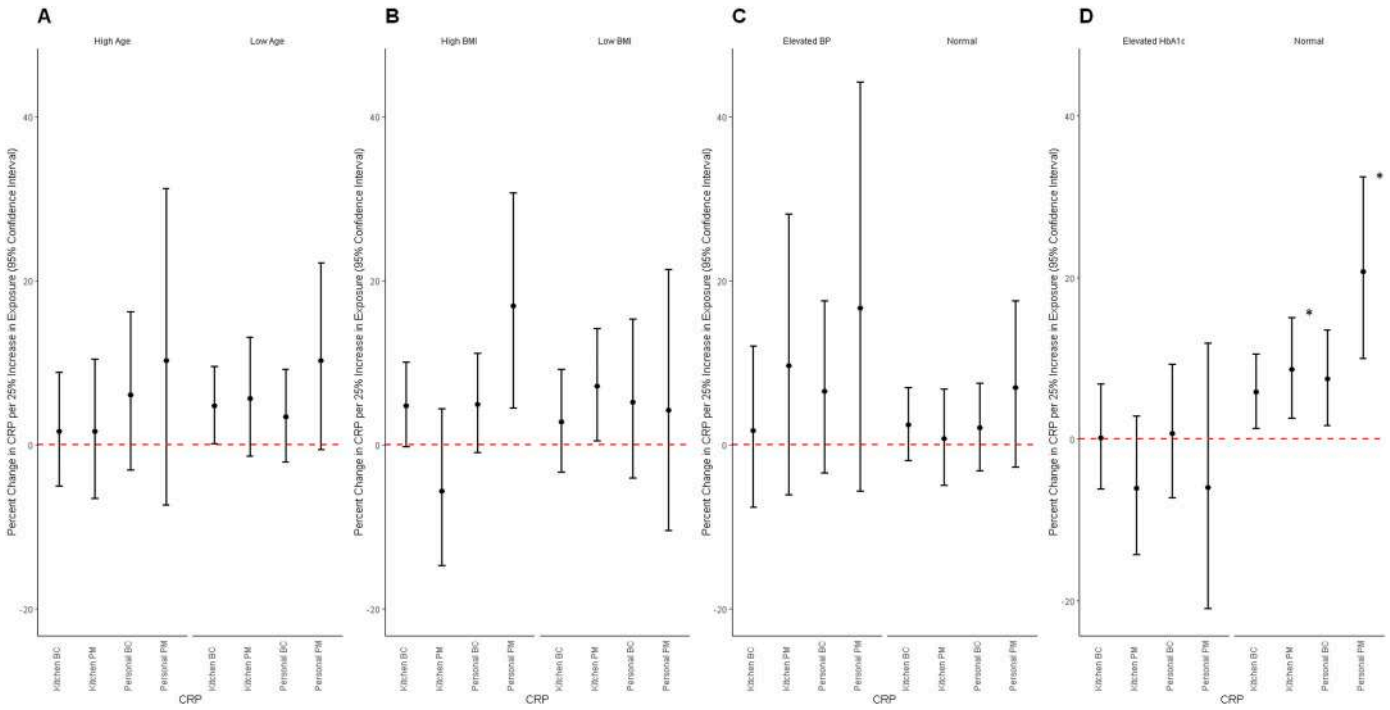

Fig. 3. Associations between 24-h average pollutant concentrations and levels of C-reactive protein (CRP)
Models were adjusted for age, body mass index (BMI), number of assets (<2 or ≥2), electricity (yes/no), years of education (<6 or ≥6)
A: Model for age; Low age = <40 years old (N = 47 kitchen PM, N = 48 for personal PM, kitchen BC and personal BC); high age = ≥40 years old (N = 27 for kitchen PM and kitchen BC, N = 25 for personal PM and personal BC)
B: Model for BMI; Low BMI = BMI <25.1 kg/m^3 (N = 31 for kitchen PM and kitchen BC, N = 30 for personal PM and personal BC); high BMI = ≥ 25.1 kg/m (N = 44 kitchen BC, N = 43 for personal PM, kitchen BC and personal BC)
C: Model for blood pressure; Normal blood pressure = normal blood pressure (systolic <120 mmHg and diastolic <80 mmHg) (N = 53 for kitchen PM, personal PM, and personal BC, N = 54 for kitchen BC); Elevated BP = elevated blood pressure (systolic ≥120 mmHg and/or diastolic ≥80 mmHg) (N = 19 for kitchen PM and kitchen BC, N = 18 for personal PM and personal BC)
D: Model for HbA1c: Normal HbA1c = HbA1c <5.7% (N = 61 for kitchen PM and kitchen BC, N = 60 for personal PM and personal BC); Elevated HbA1c = HbA1c ≥ 5.7
*Statistically significant at the 0.05 level.

4. Discussion

4.1. CRP

We observed evidence of associations between higher household air pollution, i.e., $PM_{2.5}$ and BC, and higher levels of CRP among female primary cooks. CRP is an important clinical biomarker and indicator of systemic inflammation with seemingly little diurnal or seasonable variability (Langrish et al., 2012; Pearson et al., 2003). CRP has demonstrated low within-person variability in previous studies; a measure of CRP on four consecutive days in Nicaragua had an interclass correlation coefficient (ICC) of 0.88 (Young et al., 2019). Although limited by a cross-sectional design, these results suggest that inhalation of smoke from cooking with biomass may result in systemic inflammation with potential implications for future cardiovascular disease risk.

Household air pollution studies with less sensitive measures of exposure (i.e. indicators for stove type) show mixed results for associations with CRP. A study in India demonstrated that women using biomass stoves had 3.3 times as much serum CRP than age-matched liquefied petroleum gas (LPG) users (Dutta et al., 2012). On the contrary, two studies in Peru among exposed and non-exposed biomass users have shown negative and null associations between biomass users and CRP levels (Caravedo et al., 2016; Kephart et al., 2020). A cross-sectional analysis of measured 48-h concentrations of kitchen and personal $PM_{2.5}$ and BC in Peru also showed null associations with CRP in dried blood spots (Fandiño-Del-Rio et al., 2021).

Our results are largely consistent with literature showing associations between measured ambient air pollution, especially particulate matter, and CRP (Liu et al., 2019). Although a direct comparison between the current results and past work should be interpreted carefully (due to differences in exposure instrumentation, exposure ranges, and sample size), our results are consistent with our previous feasibility work with measured household air pollution, demonstrating that among 54 women in Nicaragua, a 25% increase in 48-h household $PM_{2.5}$ was associated with a 7.4% (95% CI: 0.7–14.5%) increase in CRP measured in dried blood spots (Young et al., 2019). Our measured kitchen exposures demonstrated that a 25% increase in 24-h average kitchen $PM_{2.5}$ was associated with a 4.2% increase in CRP (95% CI: 1.1 – 9.7%). Our current study adds additional information on personal exposure to $PM_{2.5}$ and personal and kitchen BC and CRP concentrations.

4.2. Secondary analysis of markers of systemic injury and inflammation

Our results on the secondary markers of injury and inflammation were mixed and add to the inconsistent results from the field of household air pollution. We observed associations between all measures of household air pollution and SAA. The concentrations of SAA usually correlate with those of CRP (as observed in our study), however some studies indicate SAA to be a more sensitive marker to inflammatory disease than CRP (Johnson et al., 2004; Willerson and Ridker, 2004). We observed limited evidence of associations between household air pollution and other markers of inflammation; however, other studies demonstrate some associations between exposure to household air pollution and increased levels of systemic inflammation. Studies from India have demonstrated that users of biomass stoves compared to liquid-petroleum gas stoves had higher levels of serum IL-8 and TNF-α (Dutta et al., 2012, 2013). A study in Nigeria demonstrated that pregnant women who switched from firewood stoves to ethanol-burning stoves demonstrated lower TNF-α, and higher measured concentrations of $PM_{2.5}$ were associated with higher levels of IL-8 and TNF-α, but not IL-6 (Olopade et al., 2017). In Peru, measured 48-h concentrations of kitchen BC were associated with TNF-α (Fandiño-Del-Rio et al., 2021). There may be several reasons for observed associations with CRP and not with other makers of inflammation. It is possible that the complex biological pathways for inflammatory mediators may vary by time from exposure or source of combustion, and thus may trigger a response from some inflammatory markers and not others (Wu et al., 2018). Additionally, it is possible that CRP may be more stable in dried blood (Young et al., 2019) or that other biomarkers may have larger variability for which our study sample may have been underpowered to detect associations with household air pollution.

4.3. Effect modification

Our findings suggest inconsistencies in observed associations between air pollution concentrations and markers of systemic injury and inflammation by cardiovascular disease risk factors (Eckel, 1997; Leon, 2015; Ortega et al., 2016; Sanidas et al., 2017). These types of risk factors have been shown to modify the associations between air pollution and indicators of cardiovascular health (Brook et al., 2010; Dubowsky et al., 2006); however, a systematic review of the effects of air pollution on CRP did not find consistent results for effect modification by categories such as obesity or diabetic status (Y. Li et al., 2012). Contrary to our results of an effect of household air pollution on CRP only among those with normal HbA1c (but in line with our initial hypothesis), Dubowsky et al. observed that associations between ambient $PM_{2.5}$ and CRP were elevated among people classified as diabetic, overweight, and hypertensive seniors ($\geq$60 years old) in the U.S. (Dubowsky et al., 2006). There is some precedence for our finding, however; in a study of long-term exposure to ambient air pollution among residents in Germany, annual levels of $PM_{2.5}$ were associated with higher measured CRP among participants who did not have diabetes (Pilz et al., 2018). In a study of household air pollution defined as biomass users vs. non-users, Caravedo et al. explored effect modification by age categories (35–44 years, 45–54, 55–64, and 65 and over) and sex, however they found no interaction between these factors and stove type on markers of inflammation (CRP, SAA, ICAM-1 or VCAM-1) (Caravedo et al., 2016). It is challenging to compare studies of measured air pollutants with those using binary measures of stove use and also for those being conducted in different areas with often unmeasured contextual differences; however, further research with more robust study designs should evaluate factors that may modify these associations (Clark and Peel, 2014).

4.4. Strengths and limitations

Our study builds on our work in Nicaragua and demonstrates the ability to successfully collect dried blood spots in the field setting, and several validation studies have shown high correlations between serum and dried blood spot samples (Miller and McDade, 2012; Qian, 2015; Schmid et al., 2004; Skogstrand et al., 2008). Although this study is cross-sectional, the use of this methodologic approach in future prospective studies will help elucidate the potential impacts of household air pollution on cardiovascular disease risk without relying on the need to follow participants for long periods of time to clinical disease ascertainment or the need to rely on retrospective exposure assessment in case-control studies. The relatively few studies evaluating the association between measured $PM_{2.5}$ or black carbon and biomarkers of systemic inflammation (Dutta et al., 2013; Fandiño-Del-Rio et al., 2021; Olopade et al., 2017; Young et al., 2019) demonstrate inconsistent results; our study contributes additional data to help elucidate the association. A strength of our study is the relatively large sample size and the measured concentrations of exposures and biomarker outcomes. We measured both kitchen and personal $PM_{2.5}$ and BC, rather than relying on proxy-measures for exposure (i.e. stove type or biomass use).

Selection bias in our cross-sectional study is a concern due to the recruitment of a convenience sample. However, we believe it is unlikely that selection or participation would be associated with both the exposure and the outcome. Additionally, our one-time measurement of household air pollution and markers of systemic injury and inflammation may not be representative of long-term conditions. Measurement error for biomarkers on the Multiplex instrument could have occurred,

but we do not believe any error would be differential with respect to exposure. A limitation of CRP is that is can be impacted by acute events (e.g., respiratory infections) which could reduce the precision of our results. Residual confounding is a potential limitation; however, the population was fairly homogenous with respect to other measured characteristics. Establishing temporality between exposure and outcome is always a concern for cross-sectional studies; we at least partially addressed this limitation by requiring that the *Justa* stove was installed at least 4 months prior to study enrollment. The cross-sectional nature also reduces our ability to draw conclusions regarding the duration of stove use and potential time needed to observe changes in the outcome. Finally, our stove type results (traditional vs. cleaner-burning *Justa*) may not be generalizable to other populations or stove and fuel types.

5. Conclusions

Our results indicated that higher exposure to household air pollution was associated with higher levels of CRP, a commonly accepted indicator of future cardiovascular disease risk, among women in rural Honduras. Additional analyses also demonstrated associations with other markers of systemic inflammation, although these were less consistent. Positive associations between exposure to $PM_{2.5}$ and BC with SAA demonstrate a need to further investigate the importance of SAA in the mechanistic pathway from air pollution exposure to disease. The inclusion of measured BC adds to the limited and inconsistent research on the role of BC from household air pollution and inflammatory markers. Our work further demonstrates the feasibility of dried blood spots as a tool to evaluate markers of systemic inflammation in study areas with limited resources. Our results support the body of research demonstrating an association between air pollution and indicators of cardiovascular disease risk and adds evidence from the field of household air pollution.

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Disclaimer

This paper does not necessarily reflect EPA policy.

Declaration of competing interest

Two of the authors, Sebastian Africano and Anibal Pinel and are members of the implementing non-governmental organizations that deploy the cookstove technology studied in this paper. Results of research like this are often shown as evidence of the effectiveness of this particular cookstove technology in publications, including blogs, articles, and grant proposals, which may lead to future funding of these initiatives by individual and/or institutional supporters of the respective organizations. As such, we disclose this information for your review.

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Appendix A. Supplementary data

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References

American Diabetes Association, 2018. Classification and diagnosis of diabetes: standards of medical care in diabetes–2018. Diabetes Care 41 (Suppl. 1), S13–S27. https://doi.org/10.2337/dc18-S002.

Arps, Shahna, 2011. Socioeconomic status and body size among women in Honduran Miskito communities. Ann. Hum. Biol. 38 (4), 508–519. https://doi.org/10.3109/03014460.2011.564206.

Atkinson, R.W., Kang, S., Anderson, H.R., Mills, I.C., Walton, H.A., 2014. Epidemiological time series studies of PM2.5 and daily mortality and hospital admissions: a systematic review and meta-analysis. Thorax 69 (7), 660–665. https://doi.org/10.1136/thoraxjnl-2013-204492.

Bennitt, F.B., Wozniak, S.S., Causey, K., Burkart, K., Brauer, M., 2021. Estimating disease burden attributable to household air pollution: new methods within the Global Burden of Disease Study. Lancet Global Health 9, S18. https://doi.org/10.1016/s2214-109x(21)00126-1.

Bowry, Ashna D.K., Lewey, Jennifer, Dugani, Sagar B., Choudhry, Niteesh K., 2015. The burden of cardiovascular disease in low- and middle-income countries: epidemiology and management. Can. J. Cardiol. 31 (9), 1151–1159. https://doi.org/10.1016/j.cjca.2015.06.028.

Brook, R.D., Rajagopalan, S., Pope, C.A., Brook, J.R., Bhatnagar, A., Diez-Roux, A.V., et al., 2010. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American heart association. Circulation 121 (21), 2331–2378. https://doi.org/10.1161/CIR.0b013e3181dbece1.

Brunette, Waylon, Sundt, Mitchell, Dell, Nicola, Chaudhri, Rohit, Breit, Nathan, Borriello, Gaetano, 2013. Open Data Kit 2.0: Expanding and Refining Information Services for Developing Regions.

Caravedo, M.A., Herrera, P.M., Mongilardi, N., de Ferrari, A., Davila-Roman, V.G., Gilman, R.H., et al., 2016. Chronic exposure to biomass fuel smoke and markers of endothelial inflammation. Indoor Air 26 (5), 768–775. https://doi.org/10.1111/ina.12259.

Chylek, P., Ramaswamy, V., Cheng, R., Pinnick, R.G., 1981. Optical properties and mass concentration of carbonaceous smokes. Appl. Opt. 20 (17), 2980–2985. https://doi.org/10.1364/AO.20.002980.

Clark, Maggie L., Peel, Jennifer L., 2014. Perspectives in household air pollution research : who will benefit from interventions ? Curr Envir Health Rpt 250–257. https://doi.org/10.1007/s40572-014-0021-0.

Dubowsky, Sarah D., Suh, Helen, Schwartz, Joel, Coull, Brent A., Gold, Diane R., 2006. Diabetes, obesity, and hypertension may enhance associations between air pollution and markers of systemic inflammation. Environ. Health Perspect. 114 (7), 992–998. https://doi.org/10.1289/ehp.8469.

Dutta, Anindita, Ray, Manas Ranjan, Banerjee, Anirban, 2012. Systemic inflammatory changes and increased oxidative stress in rural Indian women cooking with biomass fuels. Toxicol. Appl. Pharmacol. 261 (3), 255–262. https://doi.org/10.1016/j.taap.2012.04.004.

Dutta, Anindita, Roychoudhury, Sanghita, Chowdhury, Saswati, Ray, Manas Ranjan, 2013. Changes in sputum cytology, airway inflammation and oxidative stress due to chronic inhalation of biomass smoke during cooking in premenopausal rural Indian women. Int. J. Hyg Environ. Health 216 (3), 301–308. https://doi.org/10.1016/j.ijheh.2012.05.005.

Eckel, Robert H., 1997. Obesity and heart disease. LP – 3250 Circulation 96 (9), 3248. Retrieved from. http://circ.ahajournals.org/content/96/9/3248.abstract.

Eiriksdottir, G., Harris, T.B., Launer, L.J., Gudnason, V., Folsom, A.R., Andrews, G., et al., 2011. Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data. BMJ 342 (7794), 425. https://doi.org/10.1136/bmj.d548.

Fandiño-Del-Rio, Magdalena, Kephart, Josiah, L., Williams, Kendra N., Malpartida, Gary, Boyd Barr, Dana, Steenland, Kyle, et al., 2021. Household air pollution and blood markers of inflammation: a cross-sectional analysis. Indoor Air 31 (5), 1509–1521. https://doi.org/10.1111/ina.12814.

Franklin, Barry A., Brook, Robert, Arden Pope, C., 2015. Air pollution and cardiovascular disease. Curr. Probl. Cardiol. 40 (5), 207–238. https://doi.org/10.1016/j.cpcardiol.2015.01.003.

Gold, Diane R., Mittleman, Murray A., 2013. New insights into pollution and the cardiovascular system: 2010 to 2012. Circulation 127 (18), 1903–1913. https://doi.org/10.1161/CIRCULATIONAHA.111.064337.

Hansen, A.D.A., Rosen, H., Novakov, T., 1984. The aethalometer — An instrument for the real-time measurement of optical absorption by aerosol particles. The Science of the Total Environment 36, 191–196. https://doi.org/10.1016/0048-9697(84)90265-1.

Health Effects Institute, 2020. State of Global Air 2020. Special Report. Boston, MA.

Hoek, Gerard, Krishnan, Ranjini M., Beelen, Rob, Peters, Annette, Ostro, Bart, Brunekreef, Bert, Kaufman, Joel D., 2013. Long-term air pollution exposure and cardio- respiratory mortality: a review. Environ. Health 12 (1), 43. https://doi.org/10.1186/1476-069X-12-43.

Johnson, B., Delia, Kip, Kevin E., Marroquin, Oscar C., Ridker, Paul M., Kelsey, Sheryl F., Shaw, Leslee J., et al., 2004. Serum amyloid A as a predictor of coronary artery disease and cardiovascular outcome in women: the national heart, lung, and blood institute-sponsored women's ischemia syndrome evaluation (wise). Circulation 109 (6), 726–732. https://doi.org/10.1161/01.CIR.0000115516.54550.B1.

Kephart, Josiah L., Fandiño-Del-Rio, Magdalena, Koehler, Kirsten, Bernabe-Ortiz, Antonio, Bernabe-Ortiz, Antonio, Miranda, J. Jaime, Gilman, Robert H., Checkley, William, 2020. Indoor air pollution concentrations and cardiometabolic

health across four diverse settings in Peru: a cross-sectional study. Environ. Health: A Global Access Science Source 19 (1), 1–13. https://doi.org/10.1186/s12940-020-00612-y.

Kirchstetter, Thomas W., Novakov, T., 2007. Controlled generation of black carbon particles from a diffusion flame and applications in evaluating black carbon measurement methods. Atmos. Environ. 41 (9), 1874–1888. https://doi.org/10.1016/j.atmosenv.2006.10.067.

Langrish, J.P., Bosson, J., Unosson, J., Muala, A., Newby, D.E., Mills, N.L., et al., 2012. Cardiovascular effects of particulate air pollution exposure: time course and underlying mechanisms. J. Intern. Med. 272 (3), 224–239. https://doi.org/10.1111/j.1365-2796.2012.02566.x.

Lee, Kuan Ken, Bing, Rong, Kiang, Joanne, Bashir, Sophia, Spath, Nicholas, Stelzle, Dominik, et al., 2020. Adverse health effects associated with household air pollution: a systematic review, meta-analysis, and burden estimation study. Lancet Global Health 8 (11), e1427–e1434. https://doi.org/10.1016/S2214-109X(20)30343-0.

Leon, Benjamin M., 2015. Diabetes and cardiovascular disease: epidemiology, biological mechanisms, treatment recommendations and future research. World J. Diabetes 6 (13), 1246. https://doi.org/10.4239/wjd.v6.i13.1246.

Li, Y., Rittenhouse-Olson, Kate, Scheider, William L., Mu, Lina, 2012. Effect of particulate matter air pollution on C-reactive protein: a review of epidemiologic studies. Rev. Environ. Health 27 (4), 133–149. https://doi.org/10.1515/reveh-2012-0012.Effect.

Li, W., Dorans, Kirsten S., Wilker, Elissa H., Rice, Mary B., Ljungman, Petter L., Schwartz, Joel D., et al., 2017. Short-term exposure to ambient air pollution and biomarkers of systemic inflammation. Arterioscler. Thromb. Vasc. Biol. 37 (9), 1793–1800. https://doi.org/10.1161/ATVBAHA.117.309799.

Liu, Qisijing, Gu, Xuelin, Deng, Furong, Mu, Lina, Baccarelli, Andrea A., Guo, Xinbiao, Wu, Shaowei, 2019. Ambient particulate air pollution and circulating C-reactive protein level: a systematic review and meta-analysis. Int. J. Hyg Environ. Health 222 (5), 756–764. https://doi.org/10.1016/j.ijheh.2019.05.005.

MacDougall, D., Crummet, W.B., Amore, F.J., Crosby, D.G., Estes, F.L., Freeman, D.H., et al., 1980. Guidelines for data acquisition and data quality evaluation in environmental chemistry. Anal. Chem. 52 (14), 2242–2249. https://doi.org/10.1021/ac50064a004.

Mcdade, Thomas W., Williams, Sharon, Snodgrass, J. Josh, 2007. What a drop can do : dried blood spots as a. Demography 44 (4), 899–925.

Mei, Joanne V., Alexander, J. Richard, Adam, Barbara W., Hannon, W. Harry, 2001. Innovative non- or minimally-invasive technologies for monitoring health and nutritional status in mothers and Young children use of filter paper for the collection and analysis of human whole. Blood Specimens 1, 1631–1636.

Miller, Elizabeth M., McDade, THomas W., 2012. A highly sensitive immunoassay for interleukin-6 in dried blood spots. Am. J. Hum. Biol. 24 (6), 863–865. https://doi.org/10.1002/ajhb.22324.A.

Newby, David E., Mannucci, Pier M., Tell, Grethe S., Baccarelli, Andrea A., Brook, Robert D., Donaldson, Ken, et al., 2015. Expert position paper on air pollution and cardiovascular disease. Eur. Heart J. 36 (2), 83–93. https://doi.org/10.1093/eurheartj/ehu458.

Olopade, Christopher O., Frank, Elizabeth, Bartlett, Emily, Alexander, Donee, Dutta, Anindita, Ibigbami, Tope, et al., 2017. Effect of a clean stove intervention on inflammatory biomarkers in pregnant women in Ibadan, Nigeria: a randomized controlled study. Environ. Int. 98, 181–190. https://doi.org/10.1016/j.envint.2016.11.004.

Ortega, Francisco B., Lavie, Carl J., Blair, Steven N., 2016. Obesity and cardiovascular disease. Circ. Res. 118 (11), 1752–1770. https://doi.org/10.1161/CIRCRESAHA.115.306883.

Pearson, Thomas A., Mensah, George A., Alexander, R. Wayne, Anderson, Jeffrey L., Cannon, Richard O., Criqui, Michael, et al., 2003. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the centers for disease control and prevention and the American Heart Association. Circulation 107 (3), 499–511. https://doi.org/10.1161/01.CIR.0000052939.59093.45.

Pilz, Veronika, Wolf, Kathrin, Breitner, Susanne, Rückerl, Regina, Koenig, Wolfgang, Rathmann, Wolfgang, et al., 2018. International Journal of Hygiene and C-reactive protein (CRP) and long-term air pollution with a focus on ultra fi ne particles. Int. J. Hyg Environ. Health 221 (3), 510–518. https://doi.org/10.1016/j.ijheh.2018.01.016.

Polichetti, Giuliano, Cocco, Stefania, Spinali, Alessandra, Trimarco, Valentina, Nunziata, Alfredo, 2009. Effects of particulate matter (PM10, PM2.5 and PM1) on the cardiovascular system. Toxicology 261 (1–2), 1–8. https://doi.org/10.1016/j.tox.2009.04.035.

Pope, C.A., 2000. Epidemiology of fine particulate air pollution and human health: biologic mechanisms and who's at risk? Environ. Health Perspect. 108 (Suppl. 4), 713–723. https://doi.org/10.1289/ehp.00108s4713.

Pope, C. Arden, Bhatnagar, Aruni, McCracken, James P., Abplanalp, Wesley, Conklin, Daniel J., Timothy, O'Toole, 2016. Exposure to fine particulate air pollution is associated with endothelial injury and systemic inflammation. Circ. Res. 119 (11), 1204–1214. https://doi.org/10.1161/CIRCRESAHA.116.309279.

Qian, Yuchen, 2015. Comparing and Validating Dried Blood Spots with Plasma for Cytokine Assessments in Environmental Exposure Settings.

Teixeira, Bruno Costa, Lopes, André Luiz, Macedo, Rodrigo Cauduro Oliveira, Correa, Cleiton Silva, Ramis, Thiago Rozales, Ribeiro, Jerri Luiz, et al., 2014. Inflammatory markers, endothelial function and cardiovascular risk. Jornal Vascular Brasileiro 13 (2), 108–115. https://doi.org/10.1590/jvb.2014.054.

Ridker, Paul M., 2003. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 107 (3), 363–369. https://doi.org/10.1161/01.CIR.0000053730.47739.3C.

Sanidas, Elias, Papadopoulos, Dimitris P., Grassos, Harris, Velliou, Maria, Tsioufis, Kostas, Barbetseas, John, Papademetriou, Vasilios, 2017. Air pollution and arterial hypertension. A new risk factor is in the air. Journal of the American Society of Hypertension 11 (11), 709–715. https://doi.org/10.1016/j.jash.2017.09.008.

Schmid, Christoph, Wiesli, Peter, Bernays, René, Bloch, Konrad, Zapf, Jürgen, Zwimpfer, Cornelia, et al., 2004. Decrease in sE-selectin after pituitary surgery in patients with acromegaly. Clin. Chem. 50 (3), 650–652. https://doi.org/10.1373/clinchem.2003.028779.

Shupler, Matthew, Hystad, Perry, Birch, Aaron, Miller-Lionberg, Daniel, Jeronimo, Matthew, Arku, RaphaelE., et al., 2020. Household and personal air pollution exposure measurements from 120 communities in eight countries: results from the PURE-AIR study. The Lancet Planetary Health 4 (10), e451–e462. https://doi.org/10.1016/S2542-5196(20)30197-2.

Skogstrand, Kristin, Ekelund, Charlotte K., Thorsen, Poul, Vogel, Ida, Jacobsson, Bo, Nørgaard-Pedersen, Bent, Hougaard, David M., 2008. Effects of blood sample handling procedures on measurable inflammatory markers in plasma, serum and dried blood spot samples. J. Immunol. Methods 336 (1), 78–84. https://doi.org/10.1016/j.jim.2008.04.006.

Whelton, Paul K., Carey, Robert M., Aronow, Wilbert S., Casey, Donald E., Collins, Karen J., Dennison Himmelfarb, Cheryl, et al., 2018. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American college of cardiology/American heart association task force on clinical Pr. J. Am. Coll. Cardiol. 71 (19), e127–e248. https://doi.org/10.1016/j.jacc.2017.11.006.

Willerson, J.T., Ridker, Paul M., 2004. Inflammation as a cardiovascular risk factor. Circulation 109, II-2–II–10. https://doi.org/10.1161/01.CIR.0000129535.04194.38.

Wu, Weidong, Jin, Yuefei, Carlsten, Chris, 2018. Inflammatory health effects of indoor and outdoor particulate matter. J. Allergy Clin. Immunol. 141 (3), 833–844. https://doi.org/10.1016/j.jaci.2017.12.981.

Young, Bonnie N., Clark, Maggie L., Rajkumar, Sarah, Benka-Coker, Megan L., Bachand, Annette, Brook, Robert D., et al., 2018. Exposure to household air pollution from biomass cookstoves and blood pressure among women in rural Honduras: a cross-sectional study. Indoor Air (July), 1–13. https://doi.org/10.1111/ina.12507.

Young, Bonnie N., Peel, Jennifer L., Nelson, Tracy L., Bachand, Annette M., Heiderscheidt, Judy M., Luna, Bevin, et al., 2019. C-reactive protein from dried blood spots: application to household air pollution field studies. Indoor Air (July), 1–7. https://doi.org/10.1111/ina.12603.

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Inflammatory markers and lung function in relation to indoor and ambient air pollution

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ABSTRACT

Ambient air pollution causes a range of adverse health effects, whereas effects of indoor sources of air pollution are not well described in high-income countries. We compared hazards of ambient air pollution and indoor sources with respect to important biomarkers of cardiorespiratory effects in terms of lung function and systemic inflammation in a middle-aged Danish cohort.

Our cohort comprised 5199 men and women aged 49–63 years at the recruitment during April 2009 to March 2011, with information on exposure to second-hand smoke (SHS) and use of candles, wood stove, kerosene heater and gas cooker as well as relevant covariates. Ambient air pollution exposure was assessed as 2-year mean nitrogen dioxide (NO_2) at the address (mean ± SD: 17.1 ± 9.9 μg/m^3) and 4-day average levels of particulate matter with diameter <2.5 μm ($PM_{2.5}$; mean ± SD: 12.5 ± 6.0 μg/m^3) in urban background. Lung function was assessed as % predicted forced expiratory volume in the first second (FEV1) and inflammatory markers comprised interleukin-6 (IL-6), IL-10, IL-18, interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and high sensitivity C-reactive protein (hs-CRP). We used random-effect regression models controlling for potential confounders as well as models with further adjustment for self-reported health or for all other exposures.

In models adjusted for confounders FEV1 was inversely associated with exposure to NO_2, (−0,83% per 10 μg/m^3; 95% CI: −1.26; −0.41%), SHS (−0.56% per 1 of 5 categories increment; 95% CI: −0.89; −0.23%), and gas cooker without hood (−0.89%; 95% CI: −1.62; −0.17%), whereas use of wood stove and candles showed positive associations, although these attenuated by mutual adjustment for all exposures or self-reported health. IL-6 showed positive associations with NO_2 (6.30% increase in log-transformed values per 10 μg/m^3; 95% CI: 3.54; 9.05%), $PM_{2.5}$ (7.82% per 10 μg/m^3; 95% CI: 3.35; 12.4%), SHS (4.38% per increase of 1 of 5 categories; 95% CI: 2.22; 6.54%) and use of kerosene (13.8%; 95% CI: 2.51; 25.1%), whereas the associations with use of wood stove and candles were inverse. $PM_{2.5}$ and NO_2 showed positive associations with IFN-γ and TNF-α, while $PM_{2.5}$ further associated with IL-10 and IL-18. Hs-CRP was inversely associated with use of candles.

These results suggest that the levels of exposure to ambient air pollution and SHS are more harmful than are the levels of exposure to indoor combustion sources from candles and wood stoves in a high-income setting.

Abbreviations: $PM_{2.5}$, Particulate matter with diameter < 2.5μm; PM_{10}, Particulate matter with diameter <10 μm; SHS, Second hand smoke; IL-6, Interleukin-6; IL-10, Interleukin-10; IL-18, Interleukin-18; TNF-α, Tumor nekrosis factor alpha; INF-γ, Interferon gamma; FEV1, Forced expiratory volumen in the first second; hs-CRP, High-sensitivity C-reactive protein; PM, Particulate matter; NO_2, Nitrogen dioxide; BMI, Body mass index.

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1. Introduction

Ambient air pollution in terms of particulate matter (PM) and gases is known to cause a wide range of health effects, including cardiopulmonary disease with global loss of life most recently estimated to more than 10 million annually due to the combustion of fossil fuel (Vohra et al., 2021). Household air pollution due to the combustion of polluting fuels for cooking and heating is globally attributed to 1.8 million premature deaths and 61 million disability-adjusted life years (DALYs) mainly in low- and middle-income countries, according to a recent extensive review (Lee et al., 2020). . However, except for the well-documented effects of second hand smoke (SHS) exposure on risk of airway disease, especially with prenatal and childhood exposure and the risk of cardiovascular disease (Khoramdad et al., 2020; Rushton 2021), little is known on risks of indoor combustion emissions in high-income countries (Vardoulakis et al., 2020). In high-income countries, particularly in Northern areas, candles, wood stoves, gas cookers, and kerosene heaters are important indoor combustion sources (Siponen et al., 2019; Sorensen et al., 2005; Groot et al., 2021), which should be better addressed with respect to health impact.

Potential mechanisms of short and long-term cardiopulmonary health impact of ambient air pollution have been thoroughly investigated by biomarkers of inflammation and measures of lung function in population-based studies (Wu et al., 2019). Thus, both short and long-term exposure to ambient PM in terms of $PM_{2.5}$ (diameter$<$2.5 μm) and/or PM_{10} (diameter$<$10 μm) and long-term exposure to traffic-related air pollution estimated as NO_2 were associated with decreased forced expiratory volume in the first second (FEV1) in healthy adults in large meta-analysis-based settings (Adam et al., 2015; Edginton et al., 2019). Whereas exposure to SHS rather consistently has been associated with reduced FEV1, studies of associations with other exposure to indoor combustion-based pollutants from gas cookers, candles, and wood stoves are few and with conflicting results (Carey et al., 1999; Flexeder et al., 2019; Hersoug et al., 2010a; Karottki et al., 2014; Soppa et al., 2014).

A number of markers of inflammation have been studied in relation to both long- and short-term exposure to air pollution in population and panel-based settings. An increase in PM_{10}, $PM_{2.5}$ or traffic-related NO_2 assessed by ambient air monitoring or modelled at the address in large populations have been associated with increased levels of proinflammatory cytokines, especially interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), as well as acute phase reactants C-reactive protein (hs-CRP) and fibrinogen, with both short-term and long-term approaches (Hajat et al., 2015; Panasevich et al., 2009; Tang et al., 2020; Tsai et al., 2019). Only a high level of exposure in the kitchen with the use of biomass stoves in Peru has been linked to inflammatory markers with elevated TNF-α and decreased interleukin-10 (IL-10) but no association with hs-CRP or IL-6 (Fandino-Del-Rio et al., 2021). For SHS exposure, there is robust evidence for elevated levels of hs-CRP, whereas IL-6 shows weaker associations (Jones et al., 2016).

In order to address and compare hazards related to indoor combustion sources with ambient air pollution in a high-income setting, where multiple health impacts have been related to the relatively modest outdoor levels (Andersen et al., 2011; Bronnum-Hansen et al., 2018; Raaschou-Nielsen et al., 2012), we linked these exposures with lung function and inflammatory markers in a middle-aged Danish cohort of 5199 participants.

2. Material and methodes

2.1. Study population

From 2009 to 2011 a total of 17,937 persons from three established cohorts: the Metropolit 1953 Danish Male Birth Cohort (MP), the Copenhagen Perinatal Cohort (CPC), and the Danish Longitudinal Study on Work, Unemployment, and Health (DALWUH), were invited to participate in the Copenhagen Aging and Midlife Biobank (CAMB) data collection (Avlund et al., 2014). The study was approved by the Ethical Review Committee of the Capital Region of Copenhagen (H-A-2008-126). The MP cohort included 11,532 boys born in 1953 in the Copenhagen Metropolitan area, including the counties of Copenhagen, Frederiksberg, Gentofte, and Roskilde (Osler et al., 2006). The CPC cohort included all children born at the National University Hospital in Copenhagen between 1959 and 1961 (9125 children) (Mortensen 1997). The DALWUH cohort consisted of a random sample of 10% of the Danish men and women born in either 1949 or 1959, resulting in 7588 individuals included in the cohort (Christensen et al., 2004). For the CAMB data collection, members of the cohorts living in the eastern parts of Denmark were invited to participate (7750 from the MP cohort, 5282 from the CPC cohort, and 4906 from the DALWUH cohort). The recruitment to the study included both a self-administrated questionnaire and an invitation to clinical tests and blood samples. The questionnaire consisted of 96 questions on health, major life event, indoor environment, and working and family life. Between April 20, 2009 and March 2, 2011 7191 of the invited, answered the postal questionnaire, and 5576 participated in clinical tests shortly after (Lund et al., 2016). The final study population of 5199 (29.0%) was defined by the availability of a validated address for the assessment of exposure to traffic-related air pollution. Attrition analyses showed that responders to the questionnaire and participants attending the physical examination were more likely to be employed and had significantly higher education compared to non-responders/non-participants (based on data from the Danish registers). Use of the health care system (i.e., visits to the general practitioner during 2009) showed no statistically significant difference among the responders/participants and non-responders/non-participants, suggesting that participants and non-participants did not differ with regard to general health (Lund et al., 2016).

2.2. Exposure assessment

Exposure to traffic-related ambient air pollution in two years leading up to the clinical test was assessed in terms of a two-year average of NO_2 at the address for each participant by the Danish Air-GIS air pollution modeling system (see https://www.au.dk/AirGIS). The Air-GIS system has been validated in several studies, and the NO_2 modelling has been linked to a wide range of health outcomes and impact (Bronnum-Hansen et al., 2018; Ketzel et al., 2011; Raaschou-Nielsen et al., 2012). Furthermore, daily $PM_{2.5}$ levels and mean temperature were obtained from the urban background monitoring station in Copenhagen at H.C. Ørsted Institute, part of the Danish Air Quality Monitoring Programme (Ellermann 2020). Averages of $PM_{2.5}$ and mean temperature were derived for the four days leading up to all clinical sampling days. In earlier time-series studies, 4-day means of ambient levels of PM and temperature from urban background monitoring have proven the most predictive of health outcomes in the Copenhagen area (Andersen et al., 2008; Wichmann et al., 2013).

Indoor exposure variables were derived from the questionnaires. Exposure to SHS was assigned a value of 1–5 according to answers to the question, "How often are you exposed to passive smoking in your home?", with response options: never, less than once a week, 1–2 times a week, 3–4 times a week, or more than 4 times a week. Exposure to fumes from a gas cooker was assigned a value of 1 for no gas cooker, 2 for the presence of a gas cooker and a fume hood, and 3 for the presence of a gas cooker without a fume hood. Exposure to kerosene burning fumes was assigned a value of 1 for none and 2 for the use of kerosene for heating the home. Use of wood stove was assigned a value of 1 with a negative answer to the question, "Does your home have a wood-burning stove or fireplace?". With a positive answer, the variable was assigned a value of 2–5 according to answer to the question of frequency of use during winter: less than once a week, 1–2 times a week, 3–4 times a week, or more than 4 times a week during winter. The use of wood stove was collapsed into three levels: none or use less than once a week, 1–4 times a week, or more than 4 times a week for categorical analyses. The use of

candles was assigned a value of 1–5 according to answers to the following question: "In winter, how often do you have candles lit in the evenings?" with response options never, less than once a week, 1–2 times a week, 3–4 times a week, or more than 4 times a week. The use of candles was collapsed into three levels: never or use less than once a week, 1–4 times a week, or more than 4 times a week for categorical analyses.

2.3. Clinical test measurements

Clinical tests included measurement of body weight and height for determination of body mass index. Spirometry was performed as the best of two maneuvers by a Pneumotrac Spirometer with Spirotrac IV Software (Vitalograph, Ireland for determination of FEV1 (Miller et al., 2005). FEV1 in percent of predicted from gender, age, and height was used as the outcome variable. The circulating levels of the following inflammatory markers were further included as outcome variables: hs-CRP, IL-6, IL-10, IL-18, interferon gamma (IFN-γ), and TNF-α. Hs-CRP was analysed in plasma samples by Roche/Hitachi MODULAR, whereas cytokines were analysed in EDTA plasma by electro-chemiluminescence multiplex system on a Sector 2400 Imager with commercial kits from Meso Scale Discovery (Gaithersburg, USA) according to the manufactures instructions as describe elsewhere (Foverskov et a. 2019). Cytokines were log-transformed for normality of right-skewed distributed measurement values.

2.4. Covariates

Data on smoking status, alcohol consumption, education, and employment were obtained from the questionnaire. Smoking status was assigned a value of 1–4 for never, past, occasional, and current smokers. Alcohol consumption in terms of intake of alcohol units of 12 g per week was assigned a value of 1–4 as never (0 unit/week), low risk (<14/21 units/week for women/men), elevated risk (more than 14/21 to 35 units/week for women/men), and high risk (more than 35 units/week) use according to the Danish Health Authority's recommendation at the time of data collection (2009–2011). A continuous variable with information on 'duration of education' was derived from two categorical variables: school education recoded to 8–12 years and vocational training recoded to 0–5 years with a combined scale of 8–17. For a detailed description, see (Mortensen et al., 2014). Employment was assigned a value of 1–3 corresponding to full-time, part-time and no employment. Self-reported health was defined as poor, fair, good, very good or excellent and assigned a value of 1–5 accordingly.

2.5. Statistical analysis

Spearman correlation coefficients between the exposure variables and covariates were calculated. The associations between outcome and exposure variables were assessed by random-effects models with gender and subcohort as random intercepts. The exposure variables were entered as continuous variables in crude models and models adjusted for mean ambient temperature as 4-day average ahead of sampling (to account for seasonal effects), age, smoking status, exposure to SHS (except when considering that as exposure), alcohol consumption, BMI, duration of education and employment. Associations were expressed as % change derived from the regression coefficient with 95% confidence intervals for an increase of 1 (SHS, gas cooker with or without hood, kerosene heater, wood stove use, and candle use) or 10 μg/m^3 for NO_2 and $PM_{2.5}$ exposure levels. Further, joint models with adjustment for self-reported health or mutual adjustment for all exposure variables were also build for explanatory sensitivity analyses. For categorical analysis for graphical illustration SHS, wood stove use, and candle use were collapsed into three categories, whereas NO_2 and $PM_{2.5}$ exposure levels were categorized as less than 10 μg/m^3, from 10 to less than 20 μg/m^3 and above or equal to 20 μg/m^3. Probability values less than 0.05 were considered statistically significant. STATA version 14.2 StataCorp LP was used for all analyses.

3. Results

3.1. Characteristics of the study population and exposure variables

Population characteristics and summarized exposure and outcome variables are presented in Table 1. The population was aged between 49 and 63 years and the majority were male (58%) due to one of the three included cohorts being male. Self-reported health was generally good with less than 13% reporting fair or poor health. Among the participants 880 (17%) had FEV1% predicted below 80% indicating the usual cut of for normal function, whereas 259 (5%) had hs-CRP levels above normal (<10 mg/L). High risk alcohol consumption, presence of gas cooker without a fume hood, and use of wood stove in the home were more frequent in men than in women, whereas the use of candles and full-time employment were more frequent among women. The other exposure variables and covariates were more evenly distributed between men and women. The level of inflammatory markers was generally higher among men than among women. Overall, use of candles was widespread with 41.4% burning candles more than 4 times per week. Wood stoves were present in 39.8% of the partipants homes with 20.9% reporting use more than 4 times per week, whereas gas cookers (16.3%) kerosene heaters (6.7%) were relatively rare.

Spearman correlation coefficients (r_s) between exposure variables and covariates are shown in Table 2. Of note was that the 2-year level of exposure to NO_2 at the address was moderately correlated with the presence of a gas cooker without a hood (r_s 0.26) and inversely correlated (r_s −0.24) with wood stove use. Of more note was the inverse correlation (r_s −0.21) between exposure to SHS and duration of education. The use of candles was weakly correlated (r_s 0.10) with alcohol consumption and inversely with poor self-reported health (r_s −0.090). The correlations between age and 4-day means of ambient temperature (r_s 0.11) and $PM_{2.5}$ (r_s 0.13) levels are a bit intriguing. Nevertheless, all correlations between exposure variables were relatively weak and unlikely to affect regression models including them together.

3.2. Associations of lung function and inflammatory markers with exposure variables

Two-year NO_2 exposure at the address was significantly associated with decreased FEV1 (−0,83% per 10 μg/m^3; 95% CI: −1.26; −0.41%) and increased levels of IL-6 (6.30% increase in log-transformed values per 10 μg/m^3; 95% CI: 3.54; 9.05%), TNF-α (0.95% increase in log-transformed values per 10 μg/m^3; 95% CI: 0.17; 1.74%), and IFN-γ (5.00% increase in log-transformed values per 10 μg/m^3; 95% CI: 1.35; 8.64%) in the main covariate-adjusted models, but not with other inflammatory markers in crude and covariate-adjusted models (Table 3, Fig. 1, Supplementary Material, Fig. S1). Further adjustment for self-reported health or other exposures had very little effect on these associations (Table 3). In analyses based on NO_2 exposure categories, an exposure-response pattern for these associations was only visible for IL-6 (Supplementary Material, Fig. S2).

Four-day average $PM_{2.5}$ exposure in the area was significantly associated with increased levels of IL-6 (7.82% increase in log-transformed values per 10 μg/m^3; 95% CI: 3.35; 12.4%), TNF-α (2.54% increase in log-transformed values per 10 μg/m^3; 95% CI: 1.23; 3.85%), IFNγ (9.22% increase in log-transformed values per 10 μg/m^3; 95% CI: 3.30; 15.2%), IL-18 (0.51% increase in log-transformed values per 10 μg/m^3; 95% CI: 0.15; 0.88%), and IL-10 (13.5% increase in log-transformed values per 10 μg/m^3; 95% CI: 1.98; 24.2%), but not hs-CRP (Table 3, Fig. 1, Supplementary Material, Fig. S1). Further adjustment for self-reported health or other exposures had very little effect on these associations (Table 3). For FEV1 an inverse association was only significant for participants exposed to 4-day average $PM_{2.5} \geq 20$ μg/m3 in a

Table 1
Characteristics of the 5199 participants in the Copenhagen Aging and Midlife Cohort.

	Categories or units (n)	Total n (%)	Men n (%)	Women n (%)
Age (years)	≥ 49 - 56	2978 (57.3)	1745 (47.7)	1233 (80.0)
	≥57 - 63	2220 (42.7)	1912 (52.3)	308 (20.0)
Smoking status	Never	1848 (35.6)	1276 (34.9)	572 (37.1)
	Previously	2136 (41,1)	1520 (41.6)	616 (40.0)
	Occasionally	135 (2.6)	93 (2.6)	42 (2.7)
	Current	1073 (20.7)	763 (20.9)	310 (20.1)
Alcohol consumption women/men	None	580 (11.3)	308 (8.5)	272 (17.9)
	<14/21 units/ week	2626 (51.1)	1953 (53.9)	673 (44.4)
	≥14/21, <35 units/week	1013 (19,7)	620 (17.1)	393 (25.9)
	≥35 units/week	924 (18.0)	746 (20.6)	178 (11.7)
Exposure to second hand smoke	Never	3558 (68.9)	2480 (68.2)	1078 (70.3)
	Less than once a week	749 (14.5)	540 (14.9)	209 (13.6)
	1-2 times a week	121 (2.3)	88 (2.4)	33 (2.2)
	3-4 times a week	55 (1.1)	42 (1.2)	13 (0.9)
	More than 4 times a week	687 (13.3)	487 (13.4)	200 (13.1)
Exposure to gas cooker Fumes	No gas cooker	4353 (83.7)	3052 (83.5)	1301 (84.4)
	Gas cooker and fume hood	485 (9.3)	317 (8.7)	168 (10.9)
	Gas cooker, no fume hood	360 (6.9)	288 (7.9)	72 (4.7)
Kerosene heater	No	4851 (93.3)	1434 (93.1)	3417 (93.4)
	Yes	348 (6.7)	108 (7.0)	240 (6.6)
Use of woodstove	None	3076 (60.2)	2095 (58.2)	981 (64.8)
	Less than once a week	403 (7.9)	307 (8.5)	96 (6.3)
	1-2 times a week	273 (5.3)	205 (5.7)	68 (4.5)
	3-4 times a week	295 (5.89)	212 (5.9)	83 (5.5)
	More than 4 times a week	1067 (20.9)	780 (21.7)	287 (18.9)
Use of candles	Never	217 (4.2)	175 (4.8)	42 (2.7)
	Less than once a week	833 (18.4)	616 (16.9)	217 (14.1)
	1-2 times a week	953 (18.4)	705 (19.3)	248 (16.1)
	3-4 times a week	1033 (19.9)	713 (19.6)	320 (20.8)
	More than 4 times a week	2147 (41.4)	1436 (39.4)	711 (46.3)
Employment	Full time	4298 (82.7)	1178 (76.4)	3120 (85.3)
	Part time	394 (7.6)	165 (10.7)	229 (6.3)
	None	507 (9.8)	199 (12.9)	308 (8.4)
Self-reported health	Poor	138 (1.9)	97 (2.0)	41 (1.8)
	Fair	750 (10.5)	507 (10.3)	243 (10.9)
	Good	2893 (40.4)	2004 (40.6)	889 (39.9)
	Very good	2763 (38.5)	1888 (38.2)	875 (39.2)
	Excellent	625 (8.7)	442 (9.0)	183 (8.2)
Education duration	years (n = 5199)	13.6 ± 2.4	13.6 ± 2.3	13.7 ± 2.3
Body mass index	kg/m^2 (n = 5197)	26.1 ± 4.2	26.5 ± 3.9	25.8 ± 4.8
Two-year NO_2 average at address	μg/m^3 (n = 5199)	17.1 ± 9.9	17.1 ± 9.8	17.3 ± 10.3
4-day mean ambient $PM_{2.5}$	μg/m^3 (n = 5162)	12.5 ± 6.0	12.5 ± 6.0	12.4 ± 6.3
4-day mean ambient temperature	°C (n = 5199)	8.8 ± 7.4	8.8 ± 7.4	9.1 ± 7.6
FEV1	% predicted (n = 5181)	92.9 ± 14.7	92.9 ± 15.1	93.1 ± 13.8
hs-CRP	mg/l (n = 5113)	2.37 ± 4.44	4.35 ± 4.52	2.37 ± 4.40
IL-6	pg/ml (n = 5092)	3.55 ± 13.9	3.67 ± 13.4	3.28 ± 15.3
IL-10	pg/ml (n = 4897)	12.0 ± 134.3	12.3 ± 51.5	11.3 ± 51.5
IL-18	pg/ml (n = 5116)	320 ± 426	329 ± 462	298 ± 326
INFγ	pg/ml (n = 4628)	1.11 ± 10.5	1.18 ± 012.2	0.93 ± 3.99
TNFα	pg/ml (n = 5115)	5.91 ± 13.0	6.04 ± 11.8	5.58 ± 15.6

NO_2: nitrogen dioxide; $PM_{2.5}$: particulate matter with diameter <2.5 μm; FEV1: % predicted forced expiratory volume in the first second; hs-CRP: high sensitivity C-reactive protein; IL-6: interleukin-6; IL-10: interleukin-10; IL-18: interleukin 18; IFN-γ: interferon gamma; TNF-α: tumor necrosis factor alpha. Values are mean ± SD in the lower part of the table.

categorized analysis, where exposure-response relationships appeared for most of the inflammatory markers (Supplementary Material, Fig. S2). As the study participants lived with different distances to the $PM_{2.5}$ monitor, we also included models for associations with 4-day average $PM_{2.5}$ restricted to participants living within 25 km of this (n = 3036), finding very similar results for the inflammatory markers (Table 3).

SHS exposure was significantly associated with decreased FEV1 and increased levels of IL-6, TNF-α, IL-18, IL-10 and hs-CRP, but not IFN-γ in a crude model. With covariate adjustment in the model, these associations attenuated considerably, although those for FEV1 (0.56% decrease per increase of 1 of 5 categories; 95% CI: −0.87; −0.23%), IL-6 (4.38% increase in log-transformed values per increase of 1 of 5 categories; 95% CI: 2.22; 6.54%), and IL-18 (0.19% increase in log-transformed values per increase of 1 of 5 categories; 95% CI: 0.02; 0.36%) remained significant (Table 3; Supplementary Material, Fig. 1), and the former two showed exposure-response relationships in categorized analyses (Supplementary Material, Fig. S2).

For exposure to gas cooker fumes, there was significantly lower FEV1 (0.89% decrease; 95% CI: −1.62; −0.17%), and higher IL-6 (5.30% increase in log-transformed values; 95% CI: 0.55; 10.0%), among those without a fume hood as compared to participants with electrical stoves (Supplementary Material, Fig. S2). However, in linear models including partipants with both gas stove and fume hood adjusting for co-variates the associations were not significant and with further mutual adjustment for other exposure variables, they almost disappeared (Table 3). In crude and adjusted models, the presence of a kerosene heater in the home was associated with increased levels of IL-6 (13.8% increase in log-transformed values; 95% CI: 2.51; 25.1%), whereas none of the associations with other outcome variables were significant (Table 3, Supplementary Material, Fig. S1).

Use of wood stove was significantly associated with increased FEV1 as well as decreased levels of IL-6, and hs-CRP, but not with IL-18, IL-10 or IFN-γ in a crude model (Table 3, Supplementary Material, Fig. S1).

Table 2
Spearman correlation coefficients (r_s) between ambient and indoor exposure variables and covariates among 4986 participants with data on all variables the Copenhagen Aging and Midlife Cohort.

	2-year NO_2 at address	Ambient $PM_{2.5}$	Ambient tempera ture	SHS	Gas cooker	Kerosene heater	Use of wood stove	Use of candles	Age	Smoking status	Alcohol consumption	Employ-ment	Education duration	BMI
Ambient $PM_{2.5}$ 4 days mean	0.144[a]													
Ambient temperature 4 days mean	−0.122[a]	−0.089[a]												
Second Hand Smoke (SHS)	0.048	0.025	0.040											
Gas cooker w/w'out hood	0.256[a]	−0.004	−0.042	0.008										
Kerosene heater	−0.025	−0.018	0.004	0.015	−0.044									
Use of wood stove	−0.239[a]	0.000	−0.015	−0.041	−0.066	0.042								
Use of candles	−0.037	−0.002	−0.001	−0.009	−0.038	0.011	0.107[a]							
Age	−0.046	0.127[a]	0.109[a]	0.015	−0.056	0.025	0.021	−0.051						
Smoking status	0.022	0.007	0.002	0.287[a]	0.047	0.006	−0.038	−0.004	0.010					
Alcohol consumption	0.023	0.013	0.048	0.052[a]	0.009	0.037	0.033	0.102[a]	0.109[a]	−0.075[a]				
Employment	0.012	0.004	0.056	0.068[a]	0.042	0.005	−0.069[a]	−0.036	0.093[a]	−0.117[a]	−0.045			
Education duration	0.046	0.031	−0.092[a]	−0.214[a]	0.061[a]	−0.008	0.053[a]	−0.005	−0.032	−0.191[a]	0.022	−0.143[a]		
Body mass index (BMI)	0.005	0.039	−0.001	0.068[a]	−0.030	−0.008	−0.003	−0.016	0.050[a]	0.022	−0.014	0.042	−0.176[a]	
Self-reported health	−0.037	−0.014	−0.032	−0.159[a]	−0.046	0.012	0.062[a]	0.090[a]	−0.065[a]	0.172[a]	−0.018	0.244[a]	0.191[a]	−0.189[a]

NO_2: nitrogen dioxide; $PM_{2.5}$: particulate matter with diameter <2.5 μm.
[a] Denotes $p < 0.05$ after Bonferoni correction.

These associations attenuated after adjustment for covariates but remained significant for FEV1 (0.39% increase per increase of 1 of 5 categories; 95% CI: 0.14; 0.75%) and IL-6 (2.9% decrease in log-transformed values per increase of 1 of 5 categories; 95% CI: −4.57; −1.34%) (Table 3, Fig. 1, Supplementary Material, Fig. S1). Additional inclusion of self-reported health or the other exposure variables in the model further attenuated the associations between wood stove use, FEV1, and hs-CRP (Table 3), whereas only the association with FEV1 suggested a visible exposure-response relationship in categorized analyses (Supplementary Material, Fig. S2).

The use of candles was significantly associated with increased FEV1 and IFN-γ levels as well as decreased levels of IL-6 and hs-CRP, but not associated with IL-18, IL-10, or TNF-α in crude analyses (Table 3, Supplementary Material, Fig. S1). These associations attenuated after adjustment for covariates but remained significant for IL-6 (4.22% decrease in log-transformed values per increase of 1 of 5 categories; 95% CI: −6.42; −2.01%) and hs-CRP (8.89% decrease per increase of 1 of 5 categories; 95% CI: −16.7; −1.03%) (Table 3, Fig. 1, Supplementary Material, Fig. S1), whereas a positive association with IL-18 was enhanced and became significant (0.20% increase per increase of 1 of 5 categories; 95% CI: 0.03; 0.37%). Additional inclusion of self-reported health or the other exposure variables in the model further attenuated the associations between candle use, FEV1, and hs-CRP, which lost significance (Table 3), whereas none of the associations showed a visible exposure-response relationship in categorized analyses (Supplementary Material, Fig. S2).

4. Discussion

In this comparison of hazards related to outdoor and indoor combustion-related sources of air pollution in a relatively large cohort, we found decreased lung function and increased inflammatory markers related to exposure to ambient air pollution and indoor exposure to SHS, and to some extent, kerosene heaters and gas cookers without hood. Associations with the use of wood stoves and candles mainly suggested increased lung function and decreased inflammation.

Our results support that both long- and short-term exposure to ambient air pollution can affect lung function in terms of FEV1 and inflammatory markers in terms of cytokine levels in serum. Indeed, meta-analyses have concluded that both short and long-term exposure to ambient PM in terms of $PM_{2.5}$ and/or PM_{10} is associated with decreased FEV1 in healthy adults (Edginton et al., 2019). Similarly, long-term exposure to traffic-related air pollution in terms of NO_2 has been shown to be related to reduced lung function in adults in a large European multicenter study (Adam et al., 2015). Moreover, IL-6 and TNF-α but not hs-CRP were associated with PM_{10} levels modelled at the address for up to 6 months prior to blood sampling in a Swiss cohort (Tsai et al., 2019). Similar associations were found for traffic-related NO_2 in a Swedish cohort (Panasevich et al., 2009). Moreover, in an American cohort IL-6 was associated with long-term ambient levels of $PM_{2.5}$ at the address, whereas $PM_{2.5}$ levels on the day blood draw were associated with hs-CRP and fibrinogen (Hajat et al., 2015). A meta-analysis approach found that short-term elevated levels of PM were significantly associated with TNF-α and fibrinogen across all studies and also with respect to IL-6 in studies from Asia (Tang et al., 2020). For long-term exposure, only fibrinogen was assessed without finding a significant association with PM. Accordingly, ambient air pollution in terms of PM and NO_2 seem to show relatively consistent associations with decreased lung function and inflammatory markers, especially IL-6 and TNF-α in population-based studies in line with our findings of most robust associations regarding these markers.

We found that SHS exposure was associated with decreased FEV1 and increased levels of IL-6, IL-10, IL-18, TNF-α, and hs-CRP. Although the association attenuated substantially by control for confounders, they remained significant for FEV1, IL-6, and IL-18. This is consistent with acute effects of SHS exposure in a randomized cross-over experiment

Table 3
Linear associations between lung function and inflammatory markers as related to exposure to ambient air pollutants and indoor sources of combustion product (n = 5199): participants from the Copenhagen Aging and Midlife Biobank Cohort.

	FEV1	Log IL-6	Log IL-10	Log IL-18	Log IFNγ	Log TNFα	hs-CRP
NO_2 2 years (per 10 μg/m^3)							
Crude	**−1.05 (-1.48, -0.61)**	**7.46 (4.57, 10.4)**	3.74 (−2.90, 10.4)	0.09 (−0.13, 0.31)	**4.37 (0.74, 8.01)**	**1.02 (0.24, 1.80)**	0.34 (−9.58, 10.3)
Adjusted	**−0.83 (-1.26, -0.41)**	**6.30 (3.54, 9.05)**	3.85 (−2.90, 10.6)	−0.04 (−0.26, 0.18)	**5.00 (1.35, 8.64)**	**0.95 (0.17, 1.74)**	−1.55 (−11.4, 8.28)
Further adjusted for SRH	**−0.74 (-1.16, -0.32)**	**5.95 (3.20, 8.70)**	3.64 (−3.12, 10.4)	−0.05 (−0.27, 0.17)	**4.93 (1.29, 8.58)**	**0.92 (0.13, 1.71)**	−2.83 (−12.7, 7.00)
Mutually adjusted	**−0.62 (-1.08, -0.16)**	**4.92 (1.92, 7.92)**	3.81 (−3.55, 11.2)	−0.11 (−0.34, 0.13)	**5.25 (1.31, 9.19)**	0.60 (−0.26, 1.46)	−5.39 (−16.1, 5.35)
$PM_{2.5}$ 4 days mean (per 10 μg/m^3)							
Crude	−0.58 (−1.30, 0.14)	**7.95 (3.20, 12.7)**	**12.4 (1.50, 23.3)**	**0.72 (0.37, 1.08)**	**9.00 (3.16, 14.8)**	**2.54 (1.25, 3.83)**	−4.35 (−20.6, 11.9)
Adjusted	−0.50 (−1.20, 0.21)	**7.82 (3.35, 12.4)**	**13.2 (1.98, 24.4)**	**0.51 (0.15, 0.88)**	**9.22 (3.30, 15.2)**	**2.54 (1.23, 3.85)**	−2.07 (−18.4, 14.2)
Further adjusted for SRH	−0.44 (−1.13, 0.26)	**7.59 (3.03, 12.2)**	**13.0 (1.79, 24.2)**	**0.51 (0.15, 0,87)**	**9.15 (3.22, 15.1)**	**2.52 (1.21, 3.82)**	−2.89 (−19.2, 13.4)
[a] 25 km limit	−0.07 (−0.95, 0.81)	**8.18 (2.50, 13.9)**	**14.5 (0.86, 28.2)**	**0.51 (0.06, 0.96)**	**10.9 (3.38, 18.4)**	**2.42 (0.84, 4.00)**	9.46 (−11.7, 30.7))
Mutually adjusted	−0.42 (−1.13, 0.29)	**6.89 (2.27, 11.5)**	**13.0 (1.57, 24.3)**	**0.54 (0.18, 0.91)**	**8.73 (2.74, 14.7)**	**2.49 (1.17, 3.82)**	−1.90 (−18.5, 14.7)
SHS (per 1 of 5 categories)							
Crude	**−1.63 (-1.94, -1.33)**	**11.2 (9.19, 13.2)**	**6.74 (2.09, 11.4)**	**0.38 (0.23, 0.54)**	−0.40 (−2.93, 2.12)	0.93 (0.39, 1.48)	19.8 (12.9, 26.6)
Adjusted	**−0.56 (-0.89, -0.23)**	**4.38 (2.22, 6.54)**	4.43 (−0.86, 9.72)	**0.19 (0.02, 0.36)**	0.12 (−2.74, 2.97)	0.37 (−0.25, 0.99)	4.86 (−2.84, 12.6)
Further adjusted for SRH	**−0.46 (-0.79, -0.13)**	**4.07 (1.92, 6.23)**	4.31 (−1.0, 9.61)	**0.18 (0.01, 0.36)**	0.09 (−2.77, 2.96)	0.34 (−0.28, 0.96)	3.73 (−3.97, 11.4)
Mutually adjusted	**−0.55 (-0.89, -0.22)**	**4.04 (1.86, 6.23)**	2.41 (−3.41, 7.94)	**0.18 (0.01, 0.35)**	−0.30 (−3.18, 2.58)	0.29 (−0.34, 0.91)	5.71 (−2.15, 13.6)
Gascooker (per 1 of 3 categories)							
Crude	**−0.81 (-1.50, -0.13)**	4.18 (−0.40, 8.76)	−0.36 (−10.8, 10.1)	0.09 (−0.26, 0.43)	−3.87 (−9.58, 1.84)	0.81 (−0.42, 2.03)	10.4 (−4.85, 25.7)
Adjusted	−0.57 (−1.25, 0.12)	3.54 (−0.95, 8.02)	−1.19 (−12.2, 9.82)	0.05 (−0.30, 0.40)	−2.52 (−8.44, 3.44)	0.70 (−0.57, 1.98)	9.61 (−6.31, 25.5)
Further adjusted for SRH	−0.41 (−1.09, 0.27)	2.95 (−1.52, 7.43)	−1.52 (−12.6, 9.51)	0.04 (−0.32, 0.32)	−2.65 (−8.58, 3.28)	0.65 (−0.63, 1.93)	7.67 (−8.23, 23.6)
Mutually adjusted	−0.33 (−2.02, 1.37)	0.92 (−3.82, 5.65)	0.80 (−0.15, 1.75)	−0.36 (−1.23, 0.52)	−3.91 (−10.1, 2.39)	2.16 (−100, 5.33)	5.67 (−34.2, 45.5)
Kerosene heater (no/yes)							
Crude	−0.40 (−2.09, 1.29)	**13.8 (2.51, 25.1)**	2.51 (−23.5, 28.5)	−0.17 (−1.02, 0.68)	2.97 (−11.1, 17.1)	1.72 (−1.29, 4.74)	4.27 (−33.7, 42.3)
Adjusted	−0.32 (−2.00, 1.36)	**11.2 (0.27, 22.2)**	1.17 (−26.0, 28.3)	−0.37 (−1.24, 0.49)	5.17 (−9.38, 19.7)	1.71 (−1.42, 4.83)	4.32 (−34.9, 43.5)
Further adjusted for SRH	−0.45 (−2.11, 1.21)	**11.7 (0.80, 22.7)**	1.29 (−25.9, 28.4)	−0.36 (−1.22, 0.51)	5.17 (−9.39, 19.7)	1.74 (−1.39, 4.87)	5.83 (−33.3, 44.9)
Mutually adjusted	−0.14 (−0.87, 0.58)	**13.5 (2.44, 24.6)**	2.41 (−0.35, 5.17)	0.15 (−0.22, 0.52)	6.37 (−8.23, 21.0)	0.38 (−0.97, 1.73)	11.8 (−5.07, 28.8)
Woodstove use (per 1 of 5 categories)							
Crude	**0.56 (0.31, 0.82)**	**−3.66 (-5.37, -1.95)**	−1.61 (−5.51, 2.31)	−0.05 (−0.18, 0.08)	−1.31 (−3.44, 0.81)	−0.59 (−1.04, −0.13)	−6.83 (−12.6, 1.05)
Adjusted	**0.39 (0.14, 0.75)**	**−2.90 (-4.57, -1.24)**	−0.66 (−4.76, 3.43)	0.01 (−0.13, 0.14)	−1.51 (−3.71, 0.69)	−0.46 (−0.93, 0.02)	−4.31 (−10.3, 1.66)
Further adjusted for SRH	**0.37 (0.11, 0.62)**	**−2.77 (-4.43, -1.10)**	−0.61 (−4.71, 3.49)	0.01 (−0.12, 0.14)	−1.51 (−3.71, 0.69)	−0.45 (−0.92, 0.03)	−3.88 (−9.84, 2.09) ,
Mutually adjusted	0.27 (0.00, 0.53)	**−2.00 (-3.73, -0.27)**	−5.86 (−12.7, 1.03)	−0.02 (−0.16, 0.11)	−1.03 (−3.31, 1.24)	−0.38 (−0.97, 0.11)	−3.97 (−10.2, 2.23)
Candle use (per 1 of 5 categories)							
Crude	**0.44 (0.11, 0.78)**	**−4.54 (-6.79, -2.29)**	3.22 (−1.93, 8.36)	0.15 (−0.02, 0.32)	3.03 (0.22, 5.84)	−0.27 (0.34, −11.0)	**−11.0 (-18.5, -3.45)**
Adjusted	0.31 (−0.03, 0.64)	**−4.22 (-6.42, -2.01)**	2.65 (−2.77, 8.07)	**0.20 (0.03, 0.37)**	2.46 (−0.46, 5.38)	−0.05 (−0.68, 0.58)	**−8.89 (-16.7, -1.03)**
Further adjusted for SRH	0.19 (−0.14, 0.53)	**−3.84 (-6.04, -1.64)**	2.82 (−2.62, 8.26)	**0.21 (0.04, 0.38)**	2.49 (−0.44, 5.42)	−0.02 (−0.65, 0.61)	−7.54 (−15.4, 0.32)
Mutually adjusted	0.22 (−0.12, 0.57)	**−3.78 (-6.03, -1.54)**	1.07 (−1.96, 4.09)	**0.21 (0.04, 0.39)**	2.41 (−0.54, 5.36)	0.00 (−0.64, 0.64)	−7.72 (−15.8, 0.31)

Values are % change (95% CI) derived from the coefficients of mixed regression models and expressed per 10 μg/m^3 increase for 2-years average of NO_2 at the address and ambient 4 days mean of ambient $PM_{2.5}$ or per 1 category increase for other variables. For cytokines the %change relates to log transformed values. Statistically significant ($p < 0.05$) changes are indicated by bold.

Crude models include gender and subcohort as random factors, the main adjusted model include further age, duration of education, employment status, alcohol consumption, BMI, smoking status and exposure to second hand smoke (SHS) except the SHS models. Further models included additional adjustment for self-reported health (SRH) or mutual adjustment for all exposure variables together.

NO_2: nitrogen dioxide; $PM_{2.5}$: particulate matter with diameter <2.5 μm; FEV1: % predicted forced expiratory volume in the first second; hs-CRP: high sensitivity C-reactive protein; IL-6: interleukin-6; IL-10: interleukin-10; IL-18: interleukin 18; IFN-γ: interferon gamma; TNF-α: tumor necrosis factor alpha.

[a] Participants restricted to those who lived within 25 km of the monitoring station (n = 3036).

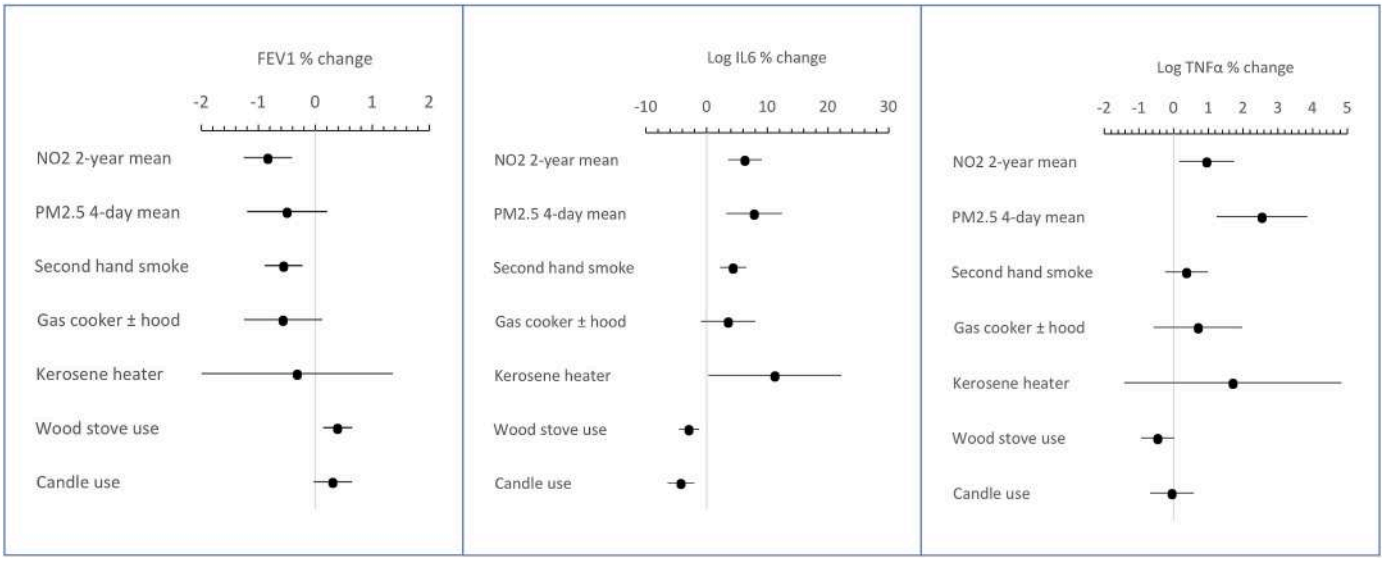

Fig. 1. Associations between exposure to ambient and indoor air pollution and lung function in terms of forced expiratory volume in the first second (FEV1 as % of predicted), interleukin-6 (IL-6) and tumor necrosis factor α (TNFa) in 5199 participants in the Copenhagen Aging and Midlife Cohort (CAMB). Associations are expressed as percent change with 95% confidence intervals in random effects regression model adjusting for mean ambient temperature as 4-day average ahead of sampling, age, smoking status, exposure to second hand smoke (except when considering that as exposure), alcohol consumption, body mass index, duration of education and employment. IL-6 and TNFa were log transformed for the analyses.
NO2: nitrogen dioxide; PM2.5: particulate matter with diameter <2.5 μm.

showing transient reduced FEV1 and increased levels of cytokines including IL-6, TNF-α, and IFN-γ (Flouris et al., 2009). Similarly, there is robust evidence for reduced FEV1 in cross-sectional and some cohort follow-up studies of populations exposed to SHS (Carey et al., 1999; Flexeder et al., 2019; Hersoug et al., 2010a). For inflammatory markers in such settings, there appears to be solid data for elevated levels of hs-CRP and fibrinogen mainly, whereas IL-6 show weaker associations (Jones et al., 2016).

Although we could not measure exposure to candlelight emissions, the levels would not be expected to be trivial from use more than 4 times per week. Thus, a Danish study measured personal exposure to $PM_{2.5}$ and black smoke for 48 h in each of the four seasons over a year (Sorensen et al., 2005). A regression analysis indicated that 1% time spent near candles was associated with 8% increase in personal $PM_{2.5}$ exposure with average levels of 17.5 and 11.9 μg/m^3 in the cold and warm part of the year, respectively. This indicates that 1 h of candle use would cause an increase of 33% in personal $PM_{2.5}$ exposure equivalent to 5.8 and 4.0 μg/m^3 from candle emission in the cold and warm part of the year, respectively. Nevertheless, we found higher FEV1 and lower levels of several cytokines related to candle use, although the associations attenuated after confounder adjustment. In contrast, short-term controlled exposure to candle emissions in human volunteers was associated with decreased lung function (Soppa et al., 2014). Similarly, reduced lung function was associated with high levels of ultrafine particles from burning candles in a cross-sectional study of 78 healthy middle-aged Danes (Karottki et al., 2014). However, lung function, self-reported respiratory symptoms, and exhaled nitric oxide as an indicator of lower airway inflammation were not associated with the use of candles in a much larger cross-sectional study with 3471 Danish participants (Hersoug et al. 2010a, 2010b). It is possible that emission of NO_2 and PM from burning candles can elicit symptoms in some people with sensitive airways who then might refrain from this, although so far, no literature appears to this have addressed it. Moreover, the use of candles might be an attribute of good health, as suggested by the correlation with good or excellent self-reported health. Similarly, adjusting for self-reported health attenuated the associations between candle use and IL-6 and hs-CRP, rendering the latter insignificant. This is also consistent with the lack of exposure-response pattern for the outcomes (Supplementary Material, Fig. S2).

The use of wood stoves in the home was associated with around 20% increased indoor or personal exposure levels of $PM_{2.5}$ and BC in both Finland and Northern New England with baseline levels of 5–7 μg/m^3 $PM_{2.5}$ (Fleisch et al., 2020; Siponen et al., 2019). Thus, wood stove use might contribute a few μg/m^3 to personal exposure, although we found higher FEV1 and lower levels of several cytokines related to such use with associations attenuating after confounder adjustment. In particular, adjustment for NO_2 at the address which was inversely correlated with wood stove use appeared important. Acute exposure to wood smoke at high levels, e.g., 300 μg/m^3 in controlled settings, has not elicited a reduction in FEV1 or increased levels of inflammatory markers (Schwartz et al., 2020). Moreover, lung function and exhaled nitric oxide as an indicator of lower airway inflammation were not associated with the use of wood stoves among 3471 Danes (Hersoug et al. 2010a, 2010b). Thus, similar to the considerations for the use of candles, the choice of using wood stoves may be related to better health. Furthermore, it is possible that prolonged indoor exposure to high levels of wood smoke such as from open cookstoves in Peru or Guatemala is required to cause an overt reduction in FEV1 or elevated inflammatory markers, including IL-6 or TNF-α (Falfan-Valencia et al., 2020; Fandino-Del-Rio et al., 2021; Pope et al., 2015).

Gas cookers, in particular unvented ones, are important indoor sources of indoor air pollution, especially NO_2, which is a strong airway irritant (Vardoulakis et al., 2020). Indeed, lung function was better among Chinese kitchen workers using electric cookers as opposed to gas cookers and a British cohort studied around the age of 35 years found that current use of a gas cooker was associated with reduced FEV1 in particular among men (Moran et al., 1999; Wong et al., 2011). In consistence with the previous studies, we found that the use of a gas

cooker without a hood was associated with lower FEV1. However, this association attenuated when adjusting for NO_2 exposure at the address, suggesting potential confounding with homes having unvented gas cookers more likely to be in areas with traffic-related air pollution. An earlier Danish cross-sectional study of 3471 subjects found that use of a gas cooker was associated with significantly reduced FEV1 among past smokers but not among never or current smokers, although information on the use of a fume hood and ambient air pollution at the address was not available (Hersoug et al., 2010a).

The use of kerosene is an important source of indoor PM, in particular in low- and middle-income countries (Vardoulakis et al., 2020). Indeed, a multi-country study focused on this issue found the use of kerosene for cooking to be associated with reduced FEV1 and increased risk of cardiorespiratory symptoms, all-cause mortality, and major cardiovascular events (Arku et al., 2020). Consistent with these findings, we found the use of a kerosene heater in the home to be associated with significantly increased levels of IL-6, whereas other associations with FEV1 and inflammatory markers were not significant, which could be related to the low statistical power with only 7% of participants reporting this.

Strengths of our study include the rather large cohort addressing associations with exposure to both outdoor and indoor pollutants from combustion sources to a range of biomarkers of effect, including lung function and inflammation central in the mechanisms of cardiopulmonary disease related to such exposures. We found significant associations for the expected relationship between exposure to ambient air pollution as well as SHS with lung function and inflammatory markers, supporting the validity of our approach. Weaknesses include that the information regarding exposure to indoor combustion sources was collected at the same time as the outcome measurements, and causal inference is thus difficult. Moreover, exposure assessment to indoor combustion sources was based on questionnaires where actual estimates of air pollution (e.g., $PM_{2.5}$ and NO_2) with indoor or personal monitoring would be much preferred. Moreover, questions on the use of candles and wood stoves only referred to the winter season. However, other studies in Denmark have found close correlations of such use in both winter and summer, albeit the latter at a lower level (Sorensen et al., 2005; Groot et al., 2021). For long-term ambient air exposure at the address, we only had modelled concentrations of NO_2 as a proxy of traffic-derived pollution and modelled PM levels as well as exposure levels at work addresses and similar would certainly have added valuable information. Nevertheless, our modelled NO_2 levels have proven associated with a wide range of health outcomes and used for health impact assessment of changed emissions in Copenhagen (Bronnum-Hansen et al., 2018; Raaschou-Nielsen et al., 2012). For short-term exposure to $PM_{2.5}$, we had to rely on one monitoring station in the study area. However, $PM_{2.5}$ levels in this part of Denmark are dominated by long-range transport, and there is a very limited local variation with similar levels found at the urban background and a rural monitoring station around 30 km away. We found similar associations between $PM_{2.5}$ and inflammatory markers when restricting the population to those living 25 km from the urban station. Although we controlled for a number of potential confounders related to the risk of cardiopulmonary disease and the associated effect markers, including age, smoking status, exposure to SHS, alcohol consumption, BMI, duration of education, and employment, residual confounding could still affect the associations.

5. Conclusion

In this comparison of hazards related to outdoor and indoor combustion-related sources of air pollution in a relatively large cohort, we found decreased lung function and increased inflammatory markers related to ambient air pollution and indoor exposure to SHS and to some extent, use of kerosene heaters and gas cooker without hood, whereas associations with use of the wood stove and candles mainly pointed at increased lung function and slightly decreased inflammation, which could be related to choices among the more healthy. These results suggest that the levels of exposure to ambient air pollution and exposure to SHS indoors are more harmful than are the levels of exposure to indoor combustion sources from candles and wood stoves in a high-income setting.

Author contributions

Youn-Hee Lim: Formal Analysis, Writing - Review & Editing, Lars-Georg Hersoug: Conceptualization, Data Curation, Rikke Lund Conceptualization, Resources, Helle Bruunsgaard: Resources, Matthias Ketzel: Resources; Jørgen Brandt: Resources, Jeanette Therming Jørgensen Writing - Review & Editing, Rudi Westendorp Writing - Review & Editing; Zorana Jovanovic Andersen: Writing - Review & Editing; Steffen Loft: Conceptualization, Formal analysis; Writing - Original Draft, Review & Editing, Funding acquisition.

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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.2022.113944.

References

Adam, M., Schikowski, T., Carsin, A.E., Cai, Y., Jacquemin, B., Sanchez, M., et al., 2015. Adult lung function and long-term air pollution exposure. Escape: a multicentre cohort study and meta-analysis. Eur. Respir. J. 45, 38–50.

Andersen, Z.J., Hvidberg, M., Jensen, S.S., Ketzel, M., Loft, S., Sørensen, M., et al., 2011. Chronic obstructive pulmonary disease and long-term exposure to traffic-related air pollution: a cohort study. Am. J. Respir. Crit. Care Med. 183, 455–461.

Andersen, Z.J., Wahlin, P., Raaschou-Nielsen, O., Ketzel, M., Scheike, T., Loft, S., 2008. Size distribution and total number concentration of ultrafine and accumulation mode particles and hospital admissions in children and the elderly in Copenhagen, Denmark. Occup. Environ. Med. 65, 458–466.

Arku, R.E., Brauer, M., Duong, M., Wei, L., Hu, B., Ah Tse, L., et al., 2020. Adverse health impacts of cooking with kerosene: a multi-country analysis within the prospective urban and rural epidemiology study. Environ. Res. 188, 109851.

Avlund, K., Osler, M., Mortensen, E.L., Christensen, U., Bruunsgaard, H., Holm-Pedersen, P., et al., 2014. Copenhagen aging and midlife biobank (camb): an introduction. J. Aging Health 26, 5–20.

Bronnum-Hansen, H., Bender, A.M., Andersen, Z.J., Sorensen, J., Bonlokke, J.H., Boshuizen, H., et al., 2018. Assessment of impact of traffic-related air pollution on morbidity and mortality in Copenhagen municipality and the health gain of reduced exposure. Environ. Int. 121, 973–980.

Carey, I.M., Cook, D.G., Strachan, D.P., 1999. The effects of environmental tobacco smoke exposure on lung function in a longitudinal study of British adults. Epidemiology 10, 319–326.

Christensen, U., Lund, R., Damsgaard, M.T., Holstein, B.E., Ditlevsen, S., Diderichsen, F., et al., 2004. Cynical hostility, socioeconomic position, health behaviors, and symptom load: a cross-sectional analysis in a Danish population-based study. Psychosom. Med. 66, 572–577.

Edginton, S., O'Sullivan, D.E., King, W., Lougheed, M.D., 2019. Effect of outdoor particulate air pollution on FEV1 in healthy adults: a systematic review and meta-analysis. Occup. Environ. Med. 76, 583–591.

Ellermann, T., Nygaard, J., Nøjgaard, J.K., Nordstrøm, C., Brandt, J., Christensen, J.H., et al., 2020. The Danish Air Quality Monitoring Programme: Annual Summary for 2018. (Scientific Report from DCE – Danish Centre for Environment and Energy). Aarhus University, Aarhus. https://dce2.au.dk/pub/SR360.pdf.

Falfan-Valencia, R., Ramirez-Venegas, A., Perez Lara-Albisua, J.L., Ramirez-Rodriguez, S. L., Marquez-Garcia, J.E., Buendia-Roldan, I., et al., 2020. Smoke exposure from

chronic biomass burning induces distinct accumulative systemic inflammatory cytokine alterations compared to tobacco smoking in healthy women. Cytokine 131, 155089.
Fandino-Del-Rio, M., Kephart, J.L., Williams, K.N., Malpartida, G., Boyd Barr, D., Steenland, K., et al., 2021. Household air pollution and blood markers of inflammation: a cross-sectional analysis. Indoor Air 31, 1509–1521.
Fleisch, A.F., Rokoff, L.B., Garshick, E., Grady, S.T., Chipman, J.W., Baker, E.R., et al., 2020. Residential wood stove use and indoor exposure to $PM_{2.5}$ and its components in Northern New England. J. Expo. Sci. Environ. Epidemiol. 30, 350–361. Flexeder C, Zock JP, Jarvis D, Verlato G, Olivieri M, Benke G, et al. 2019. Second-hand smoke exposure in adulthood and lower respiratory health during 20 year follow up in the European Community Respiratory Health Survey. Respir Res 20:33.
Flexeder, C., Zock, J.P., Jarvis, D., Verlato, G., Olivieri, M., Benke, G., et al., 2019. Second-hand smoke exposure in adulthood and lower respiratory health during 20 year follow up in the European Community Respiratory Health Survey. Respir. Res. 20, 33.
Flouris, A.D., Metsios, G.S., Carrillo, A.E., Jamurtas, A.Z., Gourgoulianis, K., Kiropoulos, T., et al., 2009. Acute and short-term effects of secondhand smoke on lung function and cytokine production. Am. J. Respir. Crit. Care Med. 179, 1029–1033.
Foverskov, E., Petersen, G.L., Pedersen, J.L.M., Rod, N.H., Mortensen, E.L., Bruunsgaard, H., Lund, R., 2019. Economic hardship over twenty-two consecutive years of adult life and markers of early ageing: physical capability, cognitive function and inflammation. Eur. J. Ageing 17, 55–67.
Groot, J., Keller, A., Pedersen, M., Sigsgaard, T., Loft, S., Nybo Andersen, A.M., 2021. Indoor home environments of Danish children and the socioeconomic position and health of their parents: a descriptive study. Environ. Int. 160, 107059. https://doi.org/10.1016/j.envint.2021, 2021.
Hajat, A., Allison, M., Diez-Roux, A.V., Jenny, N.S., Jorgensen, N.W., Szpiro, A.A., et al., 2015. Long-term exposure to air pollution and markers of inflammation, coagulation, and endothelial activation: a repeat-measures analysis in the Multi-Ethnic Study of Atherosclerosis (MESA). Epidemiology 26, 310–320.
Hersoug, L.G., Husemoen, L.L., Sigsgaard, T., Madsen, F., Linneberg, A., 2010a. Indoor exposure to environmental cigarette smoke, but not other inhaled particulates associates with respiratory symptoms and diminished lung function in adults. Respirology 15, 993–1000.
Hersoug, L.G., Husemoen, L.L., Thomsen, S.F., Sigsgaard, T., Thuesen, B.H., Linneberg, A., 2010b. Association of indoor air pollution with rhinitis symptoms, atopy and nitric oxide levels in exhaled air. Int. Arch. Allergy Immunol. 153, 403–412.
Jones, M.R., Magid, H.S., Al-Rifai, M., McEvoy, J.W., Kaufman, J.D., Hinckley Stukovsky, K.D., et al., 2016. Secondhand smoke exposure and subclinical cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis. J. Am. Heart Assoc. 5, e002965.
Karottki, D.G., Beko, G., Clausen, G., Madsen, A.M., Andersen, Z.J., Massling, A., et al., 2014. Cardiovascular and lung function in relation to outdoor and indoor exposure to fine and ultrafine particulate matter in middle-aged subjects. Environ. Int. 73, 372–381.
Ketzel, M., Berkowicz, R., Hvidberg, M., Jensen, S.S., Raaschou-Nielsen, O., 2011. Evaluation of AirGIS: a GIS-based air pollution and human exposure modelling system. Int. J. Environ. Pollut. 47, 226–238.
Khoramdad, M., Vahedian-Azimi, A., Karimi, L., Rahimi-Bashar, F., Amini, H., Sahebkar, A., 2020. Association between passive smoking and cardiovascular disease: a systematic review and meta-analysis. IUBMB Life 72, 677–686.
Lee, K.K., Bing, R., Kiang, J., Bashir, S., Spath, N., Stelzle, D., et al., 2020. Adverse health effects associated with household air pollution: a systematic review, meta-analysis, and burden estimation study. Lancet Global Health 8, e1427–e1434.
Lund, R., Mortensen, E.L., Christensen, U., Bruunsgaard, H., Holm-Pedersen, P., Fiehn, N.-E., et al., 2016. Cohort profile: the copenhagen aging and midlife biobank (CAMB). Int. J. Epidemiol. 45, 1044–1053.
Miller, M.R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., et al., 2005. Standardisation of spirometry. Eur. Respir. J. 26, 319–338.
Moran, S.E., Strachan, D.P., Johnston, I.D., Anderson, H.R., 1999. Effects of exposure to gas cooking in childhood and adulthood on respiratory symptoms, allergic sensitization and lung function in young British adults. Clin. Exp. Allergy 29, 1033–1041.
Mortensen, E.L., 1997. The Copenhagen perinatal cohort and the prenatal development project. Int. J. Risk Saf. Med. 10, 199–202.
Mortensen, E.L., Flensborg-Madsen, T., Molbo, D., Fagerlund, B., Christensen, U., Lund, R., et al., 2014. The relationship between cognitive ability and demographic factors in late midlife. J. Aging Health 26, 37–53.
Osler, M., Lund, R., Kriegbaum, M., Christensen, U., Andersen, A.-M.N., 2006. Cohort profile: the Metropolit 1953 Danish male birth cohort. Int. J. Epidemiol. 35, 541–545.
Panasevich, S., Leander, K., Rosenlund, M., Ljungman, P., Bellander, T., de Faire, U., et al., 2009. Associations of long- and short-term air pollution exposure with markers of inflammation and coagulation in a population sample. Occup. Environ. Med. 66, 747–753.
Pope, D., Diaz, E., Smith-Sivertsen, T., Lie, R.T., Bakke, P., Balmes, J.R., et al., 2015. Exposure to household air pollution from wood combustion and association with respiratory symptoms and lung function in nonsmoking women: results from the respire trial, Guatemala. Environ. Health Perspect. 123, 285–292.
Rushton, L., 2021. Health impact of environmental tobacco smoke in the home. Rev. Environ. Health 19, 291–310.
Raaschou-Nielsen, O., Andersen, Z.J., Jensen, S.S., Ketzel, M., Sorensen, M., Hansen, J., et al., 2012. Traffic air pollution and mortality from cardiovascular disease and all causes: a Danish cohort study. Environ. Health 11, 60.
Schwartz, C., Bolling, A.K., Carlsten, C., 2020. Controlled human exposures to wood smoke: a synthesis of the evidence. Part. Fibre Toxicol. 17, 49.
Siponen, T., Yli-Tuomi, T., Tiittanen, P., Taimisto, P., Pekkanen, J., Salonen, R.O., et al., 2019. Wood stove use and other determinants of personal and indoor exposures to particulate air pollution and ozone among elderly persons in a northern suburb. Indoor Air 29, 413–422.
Soppa, V.J., Schins, R.P., Hennig, F., Hellack, B., Quass, U., Kaminski, H., et al., 2014. Respiratory effects of fine and ultrafine particles from indoor sources - a randomized sham-controlled exposure study of healthy volunteers. Int. J. Environ. Res. Publ. Health 11, 6871–6889.
Sorensen, M., Loft, S., Andersen, H.V., Raaschou-Nielsen, O., Skovgaard, L.T., Knudsen, L.E., et al., 2005. Personal exposure to $PM_{2.5}$, black smoke and NO_2 in Copenhagen: relationship to bedroom and outdoor concentrations covering seasonal variation. J. Expo. Anal. Environ. Epidemiol. 15, 413–422.
Tang, H., Cheng, Z., Li, N., Mao, S., Ma, R., He, H., et al., 2020. The short- and long-term associations of particulate matter with inflammation and blood coagulation markers: a meta-analysis. Environ. Pollut. 267, 115630.
Tsai, D.H., Riediker, M., Berchet, A., Paccaud, F., Waeber, G., Vollenweider, P., et al., 2019. Effects of short- and long-term exposures to particulate matter on inflammatory marker levels in the general population. Environ. Sci. Pollut. Res. Int. 26, 19697–19704.
Vardoulakis, S., Giagloglou, E., Steinle, S., Davis, A., Sleeuwenhoek, A., Galea, K.S., et al., 2020. Indoor exposure to selected air pollutants in the home environment: a systematic review. Int. J. Environ. Res. Publ. Health 17, 8972.
Vohra, K., Vodonos, A., Schwartz, J., Marais, E.A., Sulprizio, M.P., Mickley, L.J., 2021. Global mortality from outdoor fine particle pollution generated by fossil fuel combustion: results from GEOS-Chem. Environ. Res. 195, 110754.
Wichmann, J., Folke, F., Torp-Pedersen, C., Lippert, F., Ketzel, M., Ellermann, T., et al., 2013. Out-of-hospital cardiac arrests and outdoor air pollution exposure in Copenhagen, Denmark. PLoS One 8, e53684.
Wong, T.W., Wong, A.H., Lee, F.S., Qiu, H., 2011. Respiratory health and lung function in Chinese restaurant kitchen workers. Occup. Environ. Med. 68, 746–752.
Wu, W., Jin, Y., Carlsten, C., 2019. Inflammatory health effects of indoor and outdoor particulate matter. J. Allergy Clin. Immunol. 141, 833–844.

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Polycyclic aromatic hydrocarbons (PAHs) in men and lactating women in Slovenia: Results of the first national human biomonitoring

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ABSTRACT

In the first national human biomonitoring study in the Slovenian population of adults (18–49 years), including men (n = 297) and lactating primiparous women (n = 304), exposure to polycyclic aromatic hydrocarbons (PAHs) was evaluated. Nine urinary metabolites of four parent PAHs were determined. These included 1-hydroxypyrene (1-OHPYR), 2-hydroxynaphthalene (2-OHNAP), 2-hydroxyphenanthrene (2-OHPHE), 3-hydroxyphenanthrene (3-OHPHE), 4-hydroxyphenanthrene (4-OHPHE), a combination of 2-hydroxyfluorene and 3-hydroxyfluorene (2/3-OHFLU) and a combination of 1-hydroxyphenanthrene and 9-hydroxyphenanthrene (1/9-OHPHE). For comparison, the analysed phenanthrene metabolites were reported as a sum (ΣOHPHE = 1/9-OHPHE + 2-OHPHE + 3-OHPHE + 4-OHPHE) and all the analysed PAH metabolites were reported as a sum (ΣOHPAH = 1-OHPYR + 2/3-OHFLU + 2-OHNAP + ΣOHPHE). All metabolites or their combinations were determined in more than 91% of the samples, except 4-OHPHE, which was determined in only 5% of the samples.

The highest concentration was found for 2-OHNAP. This was followed by 2/3-OHFLU and the phenanthrene metabolites, while the lowest concentration was determined for 1-OHPYR. Among the phenanthrene metabolites, the highest concentration was determined for 2-OHPHE, followed by 1/9-OHPHE and then by 3-OHPHE. Values in units of volume and values adjusted for specific gravity were significantly higher in men than in lactating primiparous women for all metabolites, whereas values in units adjusted for creatinine were generally higher in lactating primiparous women than in men. The difference between the two study groups, men and lactating primiparous women, was no longer significant in statistical models adjusted for specific gravity, suggesting that smoking, wood-burning exposure, and/or education largely explained the difference in PAH exposure in both study groups.

For most metabolites, predictors of exposure were less significant in lactating primiparous women than in men. Also, site-specific patterns of exposure were observed, with additional predictors identified in certain areas, namely, proximity to roads and release of particulate matter (PM_{10}) from industry. The time of year in which sampling took place appeared to be an important determinant in urban areas and in the case of participants who used wood for heating. Specific dietary factors could not be identified, as the study questionnaire did not include information on PAH-related diet. Despite the low number of paired partners (women and men living in the same household, n = 84), significant positive correlations for all metabolites were observed. This indicated that 31%–56% of variability in exposure could be explained by shared exposure to sources within the households (such as diet and wood-burning-related determinants).

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic pollutants present in the environment due to natural processes, such as fires, volcanic activity and biomass degradation. They also result from anthropogenic activities, such as the incomplete combustion of organic

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matter (coal, fuel oil, gas, wood) (Abdel-Shafy and Mansour, 2016; Lawal, 2017). According to the Classification, Labelling and Packaging Regulation (European Commission, 1272/2008), PAHs are carcinogenic, mutagenic and reprotoxic chemicals, classified into three categories: 1A (known human carcinogenic, mutagenic and reproductive toxicants), 1B (presumed human carcinogenic, mutagenic or reproductive toxicants) or 2 (suspected human carcinogenic, mutagenic or reproductive toxicants). Furthermore, naphthalene is classified as Group 2B (possibly carcinogenic to humans) (IARC, 2002, 2010). Fluorene, pyrene and phenanthrene have also been evaluated for carcinogenicity by IARC and classified as Group 3 (not classifiable as to its carcinogenicity to humans). Moreover, some PAH congeners are listed as priority pollutants and considered carcinogenic to humans by the United States Environmental Protection Agency (US EPA, 1993) and endocrine-disrupting chemicals by the World Health Organization (WHO, 2013). The toxicity of these chemicals has been well reviewed and summarised in the literature (Honda and Suzuki, 2020; Krzyszczak and Czech, 2021; Ramesh et al., 2004; Sun et al., 2021; Zhang et al., 2021). Epidemiological occupational studies of workers exposed to mixtures containing PAHs have shown an increased risk of skin and lung cancers as well as bladder and gastrointestinal cancers (ATSDR, 1995; Campo et al., 2014). An association between exposure to PAHs and a higher incidence of cardiovascular diseases, diabetes and breast cancer in women was reported (Hu et al., 2018; Korsh et al., 2015; Stallings-Smith et al., 2018). Recently, available data on the effects and consequences of exposure to PAHs in childhood and adulthood were summarised by Drwal et al. (2019).

Humans are potentially exposed to PAHs through a variety of pathways. For non-smokers and non-occupationally exposed individuals, the main route of exposure is assumed to be food consumption (EFSA, 2008; Zhang et al., 2014). Barbecuing with charcoal plus wood chips or consuming grilled food has been found to result in the formation of PAHs in most foods (Cheng et al., 2019; Rose et al., 2015).

In smokers, tobacco smoke has been reported to contribute significantly to PAH exposure (Aquilina et al., 2010; Chetiyanukornkul et al., 2004). Exposure may also occur through skin contact while using many consumer products that contain PAHs, as well as due to inhalation of PAHs in ambient air, especially near high-traffic roads or industrial facilities, and indoor exposure in the case of open fire appliances, candle burning and heating and cooking using gas, wood or fuel-oil (Lawal, 2017).

Recently, more attention has been paid to PAH exposure in the general population by utilising human biomonitoring (HBM) surveys using appropriate biomarkers of exposure (Choi et al., 2015, 2017). The Human Biomonitoring project for Europe, HBM4EU (https://www.hbm4eu.eu/about-hbm4eu/), a joint effort of 30 countries, the European Environment Agency and the European Commission, coordinated by the German Environment Agency (Umweltbundesamt, UBA) and funded by the European Commission under Horizon 2020, placed PAHs high on the agenda (adding it to the List 1 priority groups of chemicals), taking into account both national and European Union level policy needs to better understand human exposure and health outcomes due to exposure to PAHs (Ougier et al., 2021; Vorkamp et al., 2021) Furthermore, some studies have reported that exposure to PAHs does not decrease over time (Pollock et al., 2021; Schoeters et al., 2017). In various HBM schemes, urinary 1-hydroxypyrene (1-OHPYR) was principally used as the most common biomarker of PAH exposure (Choi et al., 2015, 2017; Jongeneelen, 1994; Strickland et al., 1996). Its general use was based on the fact that pyrene was present in all PAH mixtures at relatively high concentrations (2–10%) of the total PAHs, and in certain environments, the pyrene content of the total PAHs was fairly constant (Mucha et al., 2006). However, other urine metabolites of PAHs have been routinely applied in HBM studies, such as metabolites of fluorene, phenanthrene and naphthalene (CDC, 2019; Choi et al., 2015; Health Canada, 2017; Rosofsky et al., 2017; Urbancova et al., 2020).

Creatinine (CRT)-based normalisation is preferentially used to better predict the excretion of most exogenous chemicals, including urine PAH biomarkers; however, the estimation might be biased because CRT can vary between individuals, depending on sex, age, body mass index, non-fat body mass, etc. (Barr et al., 2005). Some recent studies have reported the inadequacy of urine CRT as a method for normalising urinary biomarkers (Hoet et al., 2016; Runkel et al., 2020; Snoj Tratnik et al., 2019; Stajnko et al., 2017). Instead, normalisation using specific gravity (SG) has been recommended using the formula published in Stajnko et al. (2017) and Suwazono et al. (2005).

The present work shows the first assessment of PAH exposure in a Slovenian adult population using the official national HBM programme, which was mandatory in view of the National Chemicals Act (Official Gazette of the Republic of Slovenia, No. 16/2008). The aims of the study were (1) to obtain the baseline levels of PAH exposure in the Slovenian adult population of men and lactating primiparous women; (2) to evaluate geographical variations and potential sources of possible PAH exposures in both study groups; and (3) to compare the results with those reported in other related research worldwide. The following PAH metabolites in urine were determined: 1-hydroxypyrene (1-OHPYR), 2/3-hydroxyfluorene (2/3-OHFLU), 2-hydroxynaphthalene (2-OHNAP), 2-hydroxyphenanthrene (2-OHPHE), 3-hydroxyphenanthrene (3-OHPHE), 4-hydroxyphenanthrene (4-OHPHE) and 1/9-hydroxyphenanthrene (1/9-OHPHE).

2. Methods

2.1. Study population and study areas

Urinary PAH metabolites were analysed in a subsample of participants of a broader Slovenian HBM programme (SLO HBM I) conducted between 2008 and 2014. The aim of this former programme was to determine the levels of trace elements and selected persistent organic pollutants in a childbearing population (18–49 years) and to estimate infant exposure via breast milk. Initially, 1084 participants – including men and lactating primiparous women – were recruited from 12 statistical regions in Slovenia. The recruitment of the original study population is described in detail by Snoj Tratnik et al. (2019) and Runkel et al. (2020). Later, additional funding and the availability of archived urine samples enabled the analysis of PAH metabolites in 601 adults (304 lactating primiparous women and 297 men), who subsequently gave their written informed consent for additional analyses to assess the aims of the present study. Throughout the text, the term “women” is mainly used instead of the term “lactating primiparous women”, except where it was intentionally quoted as such to emphasise this specific study group.

The geographic distribution of the subsample of participants is shown in Fig. 1. The living environments of the study participants were divided into rural, urban and suburban (Table 1). In the rural environment, the main activities are agricultural, while in the urban and suburban areas, there are numerous sources of pollution, mainly from traffic and past and/or present industrial activities, such as metallurgical plants, a cement factory and waste incineration, power plants, a glass factory, former mines and iron works, etc. (Snoj Tratnik et al., 2019). In Slovenia, biomass is used as a heating source more often in rural areas than in urban or suburban areas.

2.2. Ethical considerations

The first national HBM study in Slovenia was approved by the Republic of Slovenia National Medical Ethics Committee with the following numbers of accordance: 42/12/07, 53/07/09 and 0120–431/2018/4. The study was conducted per the Code of Ethics of the World Medical Association (Declaration of Helsinki). Informed written consent was obtained from all participants. Participation was voluntary, and the participants were permitted to withdraw from the study at any stage. All the samples and accompanying data were pseudo-anonymised, with

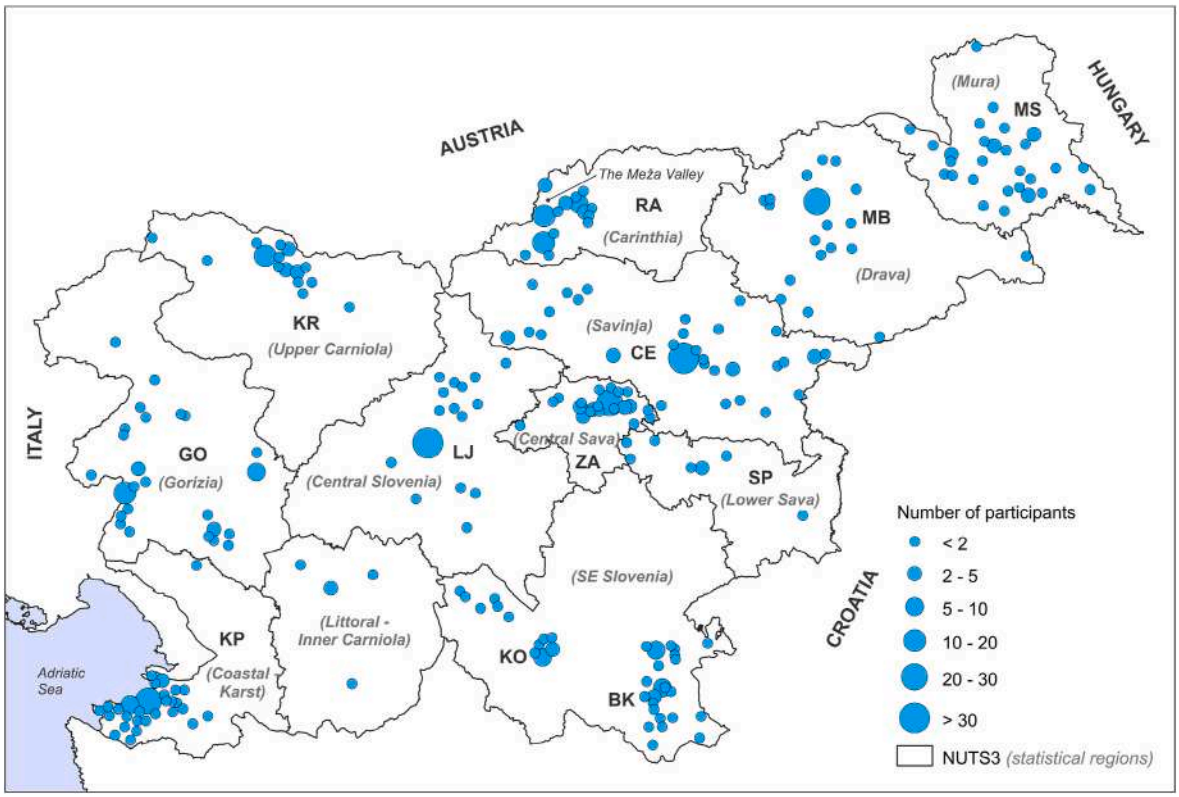

Fig. 1. Geographical distribution of the participants in the first Slovenian HBM survey of PAHs. Circles depict the total number of participants recruited in each individual settlement in all 12 statistical regions of Slovenia (in parenthesis) and organised in the following study areas: MS = Murska Sobota (Mura Region), MB = Maribor (Drava Region), CE = Celje (Savinja Region), RA = the Meža Valley (Carinthia Region), ZA = Zasavje (Central Sava Region), SP = Savinjsko–Posavska (Savinja Region and Lower Sava Region), LJ = Ljubljana (Central Slovenia), BK = Bela Krajina (Southeast Slovenia), KO = Kočevje and Cerknica (Southeast Slovenia and Littoral Inner Carniola), KR = Jesenice (Upper Carniola Region), GO = Idrija in Posočje (Gorizia Region) and KP = Koper (Coastal-Karst Region) (https://en.wikipedia.org/wiki/Statistical_regions_of_Slovenia).

Table 1
General characteristics of the study population, participating in HBM of PAHs.

Population group	n	Living environment (%)			Age (years)	Level of education (%)			Smoking (%)		Type of heating (%)		Season of sampling			
		R	U	Sub	Mean (min–max)	I	II	III	YES	NO	Wood	Other	Winter	Spring	Summer	Autumn
Total	601	38	30	32	30 (18–49)	8	44	48	9	91	40	60	29	25	20	26
Female	304	40	29	31	29 (20–39)	6	38	56	14	86	40	60	23	25	24	28
Men	297	36	31	33	31 (18–49)	11	50	39	5	95	40	60	34	25	16	25
Study area																
Murska Sobota (MS)	46	75	20	5	29 (20–48)	12	53	35	11	89	64	36	16	29	20	35
Maribor (MB)	48	23	25	52	31 (23–39)	0	40	60	6	94	21	79	29	23	17	31
Savinjsko–Posavska (SP)	48	70	6	24	30 (21–40)	11	46	43	6	94	68	32	32	30	17	21
Celje (CE)	49	15	48	37	32 (20–40)	15	48	37	8	92	17	83	23	38	16	23
Zasavje (ZA)	62	20	41	39	30 (21–44)	7	50	43	6	94	38	62	35	31	11	23
The Meža valley (RA)	56	44	27	29	33 (18–42)	16	47	37	4	96	47	53	68	6	4	22
Jesenice (KR)	47	33	39	28	31 (21–49)	6	60	34	17	83	35	65	23	19	26	32
Idrija in Posočje (GO)	52	37	35	28	30 (21–40)	12	43	45	16	84	47	53	16	45	27	12
Koper (KP)	61	36	26	38	30 (21–38)	8	34	58	7	93	20	80	24	18	24	33
Bela Krajina (BK)*	44	61	9	30	29 (22–36)	7	54	39	25	75	70	30	55	9	13	23
Kočevje and Cerknica (KO)*	28	37	22	41	28 (21–34)	7	45	48	4	96	69	31	11	61	25	3
Ljubljana (LJ)*	60	20	47	33	30 (23–40)	0	18	82	3	97	12	88	7	8	40	45

Notes: n = number of participants; Type of living environment: R – rural, U – urban, Sub – suburban; min – minimal value, max – maximum value; Level of education: I – primary or apprenticeship (Low), II – secondary or high school (Medium), III – university or higher (High); * The first period of sampling (2008–2009).

access granted to only authorised personnel.

2.3. Recruitment and sampling

Recruitment of the study participants and collection of the biological samples were described in detail by Snoj Tratnik et al. (2019). In summary, recruitment of the participants who met the inclusion criteria was performed through maternity hospitals, parenting schools, gynaecologists and/or regional units of the National Institute of Public Health (NIPH) in a single study area. In addition to childbearing age and the mother's first and singleton pregnancy, the participants had to have resided in one of the selected areas for at least five years and had to be healthy. Only healthy pregnant women were selected, and women with pregnancy complications were not recruited. In each study area, 50

women and 50 men were selected for participation. Sampling was carried out by the NIPH regional units mainly 6–8 weeks after each mother's delivery (the actual range was 2 weeks–7 months, but 73% of the women were within 6–8 weeks of delivery). The samples were transported to the central laboratory within 4–6 h after being obtained, where they were aliquoted into subsamples for specific analysis. In cases in which some of the samples were transported to the central laboratory the next day, partial aliquoting was performed in the regional laboratory. Sample transport and storage at 2–8 °C was guaranteed before aliquoting. Before the analysis, the urine samples were stored for long term at −20 °C. Recruitment and sampling spanned six years in two separate periods: 2008–2009 (pilot phase, including the LJ, KO and BK study areas) and 2011–2014 (final phase, including all the other areas), regardless of the season of the year. All the materials used in the sampling and laboratory analysis were checked for contamination through blank sample measurements.

2.4. Questionnaire

The participants completed questionnaires to obtain data about (a) general information (age, body weight – for women pre-pregnancy weight, body height, education level and occupation), (b) basic home characteristics (residential environment [rural, urban, suburban], type of heating source, type of water supply [public, private, bottled], vicinity of industry and age of residence), (c) health conditions (medicine, dietary supplements and number of dental amalgam fillings) and (d) lifestyle (smoking and hobbies) and nutritional habits (food consumption frequency, daily water intake, alcohol and coffee/tea intake and use of glazed pots). An additional set of questions regarding the pregnancy and lactation period was included in the maternal questionnaire (birth weight, birth length, intake of alcohol and smoking during pregnancy, and feeding).

2.5. Chemical and biochemical analyses

PAH metabolites were determined in urine samples at the VITO laboratory (Belgium) (Onyemauwa et al., 2009; Ramsauer et al., 2011). Briefly, urine samples were spiked with a mixture of 10, ^{13}C-labelled internal standards and sodium acetate buffer containing β-glucuronidase and sulfatase and hydrolysed overnight at 37 °C, and then acetonitrile was added before injection. The following urinary PAH metabolites were measured: 1-hydroxypyrene (pyrene metabolite; 1-OHPYR), 2-hydroxynaphthalene (naphthalene metabolite; 2-OHNAP), a combination of 2- and 3-hydroxyfluorene (fluorene metabolites; 2/3-OHFLU), 2-, 3- and 4-hydroxyphenanthrene and a combination of 1- and 9-hydroxyphenanthrene (phenanthrene metabolites; 2-OHPHE, 3-OHPHE, 4-OHPHE and 1/9-OHPHE). Instrumental analysis was performed using ultra-performance liquid chromatography (UPLC)-tandem mass spectrometry (MS) using a Waters H-class Acquity UPLC system (Waters, Milford, MA, USA). The UPLC system consists of an Acquity quaternary solvent manager, an Acquity sample manager and an Acquity column heater manager. The PAH metabolites were separated on an Acquity UPLC HSS T3 column (150 mm × 2.1 mm; 1.8 μm). The column temperature was kept constant at 40 °C. The UPLC system was coupled to a Waters Xevo TQ-S tandem mass spectrometer, which was operated in the negative electrospray ionisation mode. By recording the mass spectrum upon direct infusion of the compound into the mass spectrometer, both parameters were optimised to obtain maximum sensitivity with the highest amount of product ions available. Based on these results, the characteristic precursor and product ions were selected for detection in the multiple reaction monitoring (MRM) mode. For each compound, the two highest precursor/product ion transitions were used. Positive identification of the PAH metabolites was based on the LC retention time match and their specific MRM transitions. The PAH metabolites were quantified using the internal standard method by adding isotopically labelled analogues to the sample. The relative response factors of the compounds in relation to the corresponding internal standard were calculated. Thus, the reported concentrations were corrected for recovery. External quality control was assured through successful participation in the External Quality Assessment Scheme (EQUAS) for PAH metabolites in urine, organised by the Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Germany. This EQUAS was organised within the frame of HBM4EU as part of the quality assurance programme for biomonitoring analyses, following protocols HBM4EU-SOP-QA-001 to 004, which are available through the HBM4EU website (www.hbm4eu.eu). The limit of quantification (LOQ) was 0.015 μg/L for 1-OHPYR, 0.080 μg/L for 2-OHNAP, 0.030 μg/L for 2/3-OHFLU, 0.015 μg/L for 2-OHPHE, 0.015 μg/L for 3-OHPHE, 0.015 for 4-OHPHE and 0.030 μg/L for 1/9-OH-PHE. The amount of CRT in the urine samples was determined by a standardised routine method (compensated Jaffé reaction), and SG was determined using a refractometer as described in Snoj Tratnik et al. (2019).

2.6. Acquisition of ancillary data

The associations between the measured metabolite levels and the potential sources of PAH exposure were assessed using three groups of potential predictor variables. The first group of potential predictor variables included the nominal heat output (kW) of small combustion appliances burning biomass in different buffer zones (50 m, 250 m, 500 m, 1000 m, 2500 m and 5000 m) from the participants' homes and was summarised based on information available in the Chimney Sweeper Information System of Slovenia (https://www.dis.si/evidim/). The second set of potential predictor variables included the release of particulate matter (PM_{10}) from industry and road traffic in the vicinity of participants' homes and was obtained from spatially distributed emissions datasets (5 × 5 km) available as part of the European Pollutant Release and Transfer Register. The third set of potential predictor variables included the distance (in metres) between participants' homes and the three road types (motorway/highway, major roads and regional roads) and the length of all roads (in metres) within three buffer zones (50 m, 100 m and 500 m) around participants' homes. These data were obtained from the Surveying and Mapping Authority of the Republic of Slovenia as part of the Public Infrastructure Cadastre.

2.7. Statistical analyses

Descriptive statistics of the observed variables included determination of the geometric mean (GM) and median values, range (min–max values), percentiles 5th, 10th, 50th, 90th and 95th (P5, P10, P50, P90 and P95, respectively) and 95% confidence interval (CI_{95}) of the GM, P90 and P95 for each metabolite or their combination or sum. The fluorene metabolites (2-OHFLU and 3-OHFLU) and phenanthrene metabolites (1-OHPHE and 9-OHPHE) were reported as a combination of individual isomers, for example, a combination of 2- and 3-hydroxyfluorene (2/3-OHFLU) and a combination of 1- and 9-hydroxyphenanthrene (1/9-OHPHE), respectively, since it was not possible to evaluate them separately using this instrumental technique. Other analysed metabolites were 1-OHPYR, 2-OHNAP, 2-OHPHE, 3-OHPHE and 4-OHPHE. For comparison, all the analysed phenanthrene metabolites were reported as a sum (ΣOHPHE = 1/9-OHPHE + 2-OHPHE + 3-OHPHE + 4-OHPHE) and all the analysed PAH metabolites were reported as a sum (ΣOHPAH = 1-OHPYR + 2/3-OHFLU + 2-OHNAP + ΣOHPHE).

Values below the LOQ were assigned the values of LOQ/2, except in the case of 4-OHPHE, where P95 was also below the LOQ. Further analysis was not performed for this metabolite. A comparison of the PAH metabolite levels among the study areas or the defined population groups was made using one-way ANOVA. Multiple linear regression models were applied to evaluate the association between the metabolite levels and the potential predictor variables (independent variables), which were obtained through the participants' questionnaire responses

and ancillary data to identify possible predictors of exposure. The following variables were considered: sex (study group), smoking (yes/no or number of cigarettes), environmental tobacco smoke (second-hand smoke), education (low: primary or apprenticeship; medium: secondary or high school; high: university or higher), source of heating, nominal heat output of wood-burning appliances and the place of residence. In addition, the distance between the participants' residence and the three types of roads in different buffer zones around the participants' home, industrial and road transport releases of PM_{10} were considered. Furthermore, to a limited extent, diet was also studied using univariate and multiple linear regression of individual metabolites (or the sum of metabolites). In addition, simple linear regression was done to explore the correlation in metabolite levels between paired partners living in the same households.

Statistical evaluation (one-way ANOVA, multiple linear regression) was performed using log-transformed data for ΣOHPAH with probability values (p-values) of <0.05 recognised as significant. The results were presented in units of volume (μg/L), in units adjusted for CRT (μg/g CRT) and in units adjusted for SG (μg/L SG). According to Suwazono et al. (2005), SG means of 1.013 for women and 1.019 for men were used as the SG standards in the calculation of units adjusted for SG. Urine samples with CRT levels above 50 mg/L were considered normal (Lauwerys and Hoet, 2001). Table 2 presents the results in units of volume for better comparability with other available studies. In addition, the results expressed in units adjusted for CRT and adjusted for SG are summarised in the Supplementary Materials (Table S1). In the exposure predictor analyses, adjustment for SG was used. All statistical analyses were performed using STATA12/SE (USA) statistical software.

3. Results and discussion

3.1. General characteristics of the study population

The mean age for all the statistical areas included in this study was 29 years for women and 31 years for men (Table 1). The average body height was 167 cm for women and 181 cm for men; the average body

Table 2
Levels of the PAH metabolites in the urine of the study population of men and lactating primiparous women of the same age range (18–49 years) in Slovenia, μg/L.

Biomarker	Population group	N	N<LOQ	GM [95%CI]	Min-max	P5	P10	P50	P90 [95%CI]	P95 [95%CI]
1−OHPYR	Total	601	50	0.053 [0.050–0.057]	<LOQ−0.888	<LOQ	0.016	0.058	0.146 [0.133–0.164]	0.180 [0.162–0.199]
	Men	297	6	0.069 [0.063–0.074]	<LOQ−0.642	0.018	0.026	0.068	0.158 [0.140–0.179]	0.188 [0.167–0.213]
	Women	304	44	0.041 [0.037–0.046]	<LOQ−0.888	<LOQ	0.010	0.044	0.124 [0.107–0.147]	0.168 [0.142–0.203]
2/3−OHFLU	Total	600	5	0.203 [0.190–0.218]	<LOQ−4.43	0.051	0.068	0.200	0.563 [0.507–0.626]	0.864 [0.740–1.03]
	Men	296	0	0.265 [0.243–0.289]	40–4.43	0.078	0.108	0.260	0.698 [0.607–0.818]	1.19 [0.859–1.68]
	Women	304	5	0.157 [0.143–0.173]	<LOQ−2.28	0.042	0.056	0.157	0.400 [0.352–0.459]	0.692 [0.554–0.960]
2−OHNAP	Total	598	3	2.13 [1.97–2.30]	<LOQ−42.1	0.456	0.656	2.11	7.06 [6.23–8.01]	9.60 [8.34–11.1]
	Men	294	3	2.57 [2.30–2.87]	<LOQ−42.1	0.656	0.866	2.66	7.82 [6.72–9.33]	11.7 [9.55–14.6]
	Women	304	0	1.77 [1.59–1.97]	0.186–20.6	0.412	0.544	1.69	5.87 [4.96–7.17]	8.30 [6.76–11.0]
1/9−OHPHE	Total	601	25	0.102 [0.97–0.108]	<LOQ−1.11	0.030	0.042	0.102	0.252 [0.229–0.277]	0.340 [0.301–0.392]
	Men	297	5	0.123 [0.114–0.133]	<LOQ−1.11	0.042	0.056	0.120	0.294 [0.258–0.338]	0.370 [0.316–0.437]
	Women	304	20	0.085 [0.079–0.02]	<LOQ−0.714	<LOQ	0.034	0.088	0.192 [0.177–0.227]	0.284 [0.236–0.341]
2−OHPHE	Total	601	20	0.079 [0.074–0.085]	<LOQ−1.23	0.018	0.026	0.084	0.218 [0.198–0.244]	0.270 [0.245–0.302]
	Men	297	2	0.111 [0.102–0.121]	<LOQ−1.21	0.028	0.042	0.114	0.256 [0.227–0.288]	0.336 [0.293–0.388]
	Women	304	18	0.057 [0.052–0.063]	<LOQ−1.23	<LOQ	0.020	0.058	0.158 [0.137–0.184]	0.190 [0.165–0.219]
3−OHPHE	Total	601	33	0.063 [0.059–0.068]	<LOQ−1.04	<LOQ	0.020	0.066	0.168 [0.152–0.185]	0.226 [0.201–0.254]
	Men	297	4	0.087 [0.080–0.094]	<LOQ−1.04	0.026	0.034	0.086	0.208 [0.183–0.238]	0.262 [0.226–0.306]
	Women	304	29	0.047 [0.042–0.052]	<LOQ−0.898	<LOQ	0.016	0.050	0.122 [0.107–0.140]	0.158 [0.138–0.185]
4−OHPHE	Total	601	571	<LOQ	<LOQ−0.048	<LOQ	<LOQ	<LOQ	<LOQ [<LOQ−<LOQ]	<LOQ [<LOQ−0.019]
	Men	297	276	<LOQ	<LOQ−0.048	<LOQ	<LOQ	<LOQ	<LOQ [<LOQ−<LOQ]	0.018 [<LOQ−0.024]
	Women	304	295	<LOQ	<LOQ−0.036	<LOQ	<LOQ	<LOQ	<LOQ [<LOQ−<LOQ]	<LOQ [<LOQ−<LOQ]
SUM−OHPHE	Total	601	n.r.	0.254 [0.239–0.270]	<LOQ−2.95	0.068	0.060	0.272	0.622 [0.569–0.684]	0.762 [0.710–0.869]
	Men	297	n.r.	0.334 [0.310–0.359]	0.048–2.95	0.108	0.136	0.340	0.704 [0.633–0.785]	0.870 [0.773–0.985]
	Women	304	n.r.	0.194 [0.179–0.212]	0.026–2.65	0.050	0.072	0.204	0.458 [0.406–0.534]	0.564 [0.501–0.646]
SUM−OHPAH	Total	598	n.r.	2.78 [2.60–2.98]	<LOQ−42.7	0.660	0.912	2.81	8.14 [7.27–9.11]	11.1 [9.72–12.8]
	Men	294	n.r.	3.43 [3.13–3.77]	0.340–42.7	0.856	1.26	3.42	9.31 [8.04–10.9]	12.5 [10.5–15.3]
	Women	304	n.r.	2.27 [2.06–2.50]	0.292–22.3	0.570	0.776	2.13	6.71 [5.72–7.90]	9.99 [8.02–13.1]

Notes: n = number of participants; 1−OHPYR = 1−hydroxypyrene; 2/3−OHFLU = a combination of 2−hydroxyfluorene and 3−hydroxyfluorene; 2−OHNAP = 2−hydroxynaphtalene; SUM−OHPHE = Sum of 1/9−hydroxyphenanthrene, 2−hydroxyphenanthrene, 3−hydroxyphenanthrene and 4−hydroxyphenanthrene; SUM−OHPAH = Sum of all analysed urine metabolites of PAHs; LOQ = Limit of Quantification; GM = Geometric mean, min−max = minimum and maximum values; Percentiles, 5th, 10th, 50th, 90th, 95th (P5, P10, P50, P90, P95); CI = Confidence Interval; n.r. = not relevant.

weight was 64.7 kg for women and 84.8 kg for men. Analysis of the type of living environment showed that 60–75% of the participants in SP (Savinja and Lower Sava Regions), MS (Mura Region) and BK (Southeast Slovenia) lived in a rural setting. In CE (Savinja Region), LJ (Central Slovenia) and ZA (Central Sava Region), more than 40% of the participants lived in urban centres, and 52% of the participants in MB (Drava Region) lived in suburban areas. In the other study areas, the proportion of participants was more or less evenly distributed between rural, urban and suburban environments. The level of education was higher in the urban environment, where more than 50% of the participants had a university level of education or higher. The analysis showed that the recruitment and subsequent sample collection was more or less equally distributed among winter (29%), spring (25%), summer (20%) and autumn (26%) across the entire study population. However, in some of the study areas, this was not the case. In the KO and GO study areas, the sampling for most of the participants was done in spring; in LJ, it was done in summer and autumn, and in RA and BK, it was done in winter. Wood was the predominant heating source in the study areas with a predominant rural living environment (SP, MS and BK) and in KO, which was primarily selected as a rural environment. In RA and GO, in addition to using wood, other heating sources (mostly gas) were present in about equal proportion. Indeed, according to living environment classification, 72% of participants residing in rural locations used biomass as a primary source, 45% in suburban and only 20% in urban environments (data not presented). The proportion of participants who were smokers was 9%, which was low compared to the Slovenian adult population (18.9%) (Statistical Office of Slovenia, https://www.stat.si/statweb). One of the reasons for this discrepancy might be that smoking (before pregnancy and/or current) was one of the exclusion criteria, but it was omitted during the recruitment phase because the participants' response was generally very low. The proportion of smokers in the selected study group was higher in women (14% of the women smoked before being pregnant) than in men (5%). The percentage differed slightly between the study areas, except BK, where it was 25%. Moreover, the proportion of participants exposed to second-hand smoke was relatively low (up to 23%).

3.2. Levels of urinary PAH metabolites in the study population

The levels of urinary PAH metabolites in the study population, that is, the values of 1-OHPYR, 2/3-OHFLU, 2-OHNAP, 1/9-OHPHE, 2-OHPHE and 3-OHPHE, are presented in Table 2. The sum of the phenanthrene metabolites (ΣOHPHE) and the sum of all the analysed PAH metabolites (ΣOHPAH) are also presented. All individual metabolites, or their combination in the case of 2/3-OHFLU and 1/9-OHPHE, were determined in a high proportion of urine samples, between 91.7% and 99.5%, except for 4-OHPHE, which was determined only in 5% of the samples. However, the LOQ for 4-OHPHE in our study was 0.015 μg/L, which was in line with the LOQ of the other measured phenanthrene metabolites. A similar pattern of detection and urinary levels of 4-OHPHE, which were lower than levels of the other phenanthrene metabolites, have also been reported in some other studies (Murawski et al., 2020; Verheyen et al., 2021).

In the present study, among all the metabolites analysed, the highest levels were determined for 2-OHNAP (determined in 99.5% of the samples); they were more than 10-fold higher than the levels of fluorene and phenanthrene metabolites and more than 40-fold higher than the levels of 1-OHPYR. The 2-OHNAP levels varied from below the LOQ at 0.08 μg/L to 42 μg/L and were generally lower in women (GM = 1.77 μg/L) than in men (2.57 μg/L) ($p < 0.001$). The proportion of 2-OHNAP was 78% in women and 75% in men.

The second-highest concentration was found for 2/3-OHFLU, determined in 99.2% of the samples and ranged from below the LOQ at 0.015 μg/L to 4.43 μg/L. The levels were generally lower in women (GM = 0.157 μg/L) than in men (GM = 0.265 μg/L) ($p < 0.001$). The phenanthrene metabolites 1/9-OHPHE, 2-OHPHE and 3-OHPHE were determined in 96.8%, 96.7% and 94.5% of the samples, respectively. Their values ranged from below the LOQ at 0.015 μg/L to 1.23 μg/L and to 1.04 μg/L for 2-OHPHE and 3-OHPHE, respectively, and from the LOQ at 0.030 μg/L to 1.11 μg/L for 1/9-OHPHE. Considering the sum of phenanthrene metabolites (ΣOHPHE), the levels were generally higher in men (GM = 0.334 μg/L) than in women (GM = 0.194 μg/L) ($p < 0.001$). The lowest concentration (except for 4-OHPHE) was found for 1-OHPYR (determined in 91.7% of the samples), ranging from below the LOQ at 0.015 μg/L to 0.888 μg/L. The levels were generally lower in women (GM = 0.041 μg/L) than in men (GM = 0.069 μg/L) ($p < 0.001$). The present results may indicate that the assessment of exposure to PAHs may be underestimated if it is based solely on 1-OHPYR.

In general, the values expressed in units of volume and the values adjusted for SG (Supplementary Materials, Table S1) were both significantly higher in men than in women for all the metabolites, while the values normalised to CRT were generally higher in women as expected due to the sex-related difference in CRT levels (Hoet et al., 2016). The difference between the study groups was no longer significant in the statistical models adjusted for SG, indicating that smoking, wood-burning related exposure and/or education largely explain the difference in PAH exposure between men and women, rather than the presumed difference in the physiology between the sexes. This is discussed further in the "Predictors of exposure and geographic variability" section.

There were no available HBM health-based guidance values (HBM-HBGVs) for PAHs that would help interpret the HBM results and put the results in a health risk context. Recently, the need for deriving HBM-HBGVs has been well recognised under the HBM4EU initiative (Ganzleben et al., 2017), and the HBM-HBGVs for some chemicals (e.g. bisphenol A, phthalates, cadmium) have already been derived so far (Apel et al., 2020; David et al., 2021; Lange et al., 2021; Ougier et al., 2021). The HBM-HBGVs for PAHs are still in preparation within the HBM4EU initiative. Until then, we can only compare the levels of internal exposure to PAHs in the Slovenian adult population (men of reproductive age and lactating primiparous women) with levels reported in other relevant HBM studies, as shown in Table 3 and briefly discussed below.

3.3. Comparison of levels of urinary PAH metabolites with other countries

To put the obtained results in an international context, we compared the urinary levels of PAH metabolites with levels reported in other HBM studies and programmes in adults, such as men, pregnant women, mothers and the general population from different countries (Table 3). In this table, the levels of urinary PAH metabolites (geometric mean, mean or median) were expressed in units of volume and/or in units adjusted to CRT, as reported in the literature used for comparison.

As in the present study, urinary 2-OHNAP levels were the most frequently determined, with the highest levels among all analysed PAH metabolites in the studies listed in Table 3. In the present study, in lactating primiparous women, urinary 2-OHNAP levels were found to be lower or close to those found in other countries, such as Spain (Fernández et al., 2021), the Czech Republic (Urbancova et al., 2020), the USA (CDC, 2019), Canada (Health Canada, 2017; Nethery et al., 2012; Wheeler et al., 2014) and Korea (Kwon et al., 2019). However, they were higher than in pregnant women from Denmark (Rosofsky et al., 2017) and China (Lou et al., 2019). In men, the urinary levels of 2-OHNAP were lower than those reported in the NHANES (CDC, 2019), the CHMS (Health Canada, 2017) and the KoNEHS programmes (Kwon et al., 2019). Typically, naphthalene can be found in a variety of microenvironments, including environments in which people smoke, as well as in grilled/smoked food (Jo and Lee, 2011; Preuss et al., 2004). Also, higher levels of urinary 2-OHNAP may be affected by concomitant exposure to other PAHs in the mixture (Preuss et al., 2004; Ramesh et al., 2004). Some authors have suggested using 1-OHNAP in combination with 2-OHNAP as the strongest predictors of inhalation exposure,

Table 3
Comparison of the urinary PAH metabolites levels (geometric mean, mean or median) in units of volume, μg/L or/and adjusted to creatinine, μg/g CRT) in other HBM studies with men, women, pregnant women, mothers and general adult population.

Country/Study (year of study)	Group, age (years)	Sample size (n)	Value	Unit	1-OHPYR	2/3-OHFLU or noted otherwise	2-OHNAP	Σ-OHPHE (Σ-OHPHE = 2 + 3 + 4 + 1 + 9-OHPHE) or noted otherwise	Reference
Slovenia HBM-I (2007–2014)	Men, 18−49	297	GM	μg/L	0.069 [0.063–0.074]	0.265 [0.243–0.289]	2.57 [2.30–2.87]	0.334 [0.310–0.359]	This study
				μg/g CRT	0.051 [0.047–0.056]	0.199 [0.181–0.218]	1.99 [1.79–2.22]	0.249 [0.230–0.269]	
	Lactating primipara, 18−49	304		μg/L	0.041 [0.037–0.046]	0.157 [0.143–0.173]	1.77 [1.59–1.97]	0.194 [0.179–0.212]	
				μg/g CRT	0.065 [0.059–0.072]	0.249 [0.229–0.271]	2.82 [2.57–3.09]	0.307 [0.286–0.331]	
Belgium FLEHS II 2007–2011	Adults, 20−40	191	GM	μg/L	0.101 [0.091–0.111]				Schoeters et al., 2012
Canada, Ottawa (ON), 2010	Pregnant women, 20−47	62	GM	μg/L			2.92 ± 2.08		Wheeler et al. (2014)
Canada, Hamilton (ON) 2007	Pregnant women, 19−42	57	GM	μg/g CRT	0.136	0.264	2.61	0.827	Nethery et al. (2012)
China 2015	Pregnant women, 19−42	188	GM	μg/g CRT	0.130	0.140	1.32	0.340[a]	Lou et al. (2019)
Czech R. 2016–2017	Pregnant women, 18−44	330	Median	μg/g CRT	0.120	0.230[b]	5.15	1.11	Urbancova et al. (2020)
Denmark SF/ Milieu 2011–2014	Pregnant women, 18−40	56	GM	μg/L	0.059	0.135	1.16	0.256	Rosofsky et al. (2017)
Poland 2007	Pregnant women, 20−40	104	Mean	μg/g CRT	0.43 ± 0.30			2.88 ± 43	Polanska et al., 2014
Spain 2015	Lactating women	110	GM	μg/L	0.12	0.30	7.1	0.41	F. Fernández et al. (2021)
				μg/g CRT	0.13	0.31	7.2	0.43	
Spain 2003–2005	Pregnant women, 16+	204	Median	μg/L	0.071				Llop et al. (2008)
Spain BIOAMBIENT. ES 2009–2010	Men, 16−65	522	GM	μg/g CRT	0.105 [0.089–0.125]			0.132 [0.110–0.159]	Bartolomé et al. (2015)
	Women, 16−65	435		μg/g CRT	0.133 [0.117–0.151]			0.128 [0.103–0.158]	
CHMS 4th Report 2014–2015	Men, 3−79	1251	GM	μg/L	0.10 [0.086–0.12]	0.43 [0.341–0.52]	4.5 [4.0–5.0]	0.383 [0.333–0.447]	Health Canada, 2017
				μg/g CRT	0.082 [0.067–0.099]	0.331 [0.27–0.42]	3.5 [3.0–4.0]	0.303 [0.257–0.351]	
	Women, 3−79	1257		μg/L	0.090 [0.082–0.099]	0.342 [0.297–0.40]	4.7 [3.9–5.6]	0.365 [0.330–0.414]	
				μg/g CRT	0.094 [0.083–0.11]	0.355 [0.31–0.41]	4.8 [4.2–5.5]	0.382 [0.338–0.429]	
KoNEHS Cycle 3, 2015–2017	Men, 20+	1517	GM	μg/L	0.169 [0.151–0.189]		3.52 [3.21–3.80]		Kwon et al. (2019)
	Women, 20+	1793		μg/L	0.122 [0.110–0.136]		2.46 [2.30–2.63]		
NHANES 4th Report 2011–2014	Men	1315	GM	μg/L	0.134 [0.124–0.146]	0.295 [0.268–0.324]	4.14 [3.92–4.59]	0.297 [0.269–0.329][c]	CDC, 2019
				μg/g CRT	0.135 [0.126–0.144]	0.296 [0.270–0.322]	4.16 [3.83–4.51]	0.130 [0.122–0.137][c]	
	Women	1335		μg/L	0.129 [0.121–0.138]	0.231 [0.208–0.256]	4.29 [3.96–4.65]	0.245 [0.224–0.269][c]	
				μg/g CRT	0.173 [0.161–0.185]	0.309 [0.280–0.339]	5.74 [5.39–6.11]	0.137 [0.128–0.146][c]	

Notes: 1−OHPYR = 1−hydroxypyrene; 2/3−OHFLU = a combination of 2−hydroxyfluorene and 3−hydroxyfluorene; 2−OHNAP = 2−hydroxynaphtalene; ΣOHPHE = Sum of 1/9−hydroxyphenanthrene, 2−hydroxyphenanthrene, 3−hydroxyphenanthrene and 4−hydroxyphenanthrene; CRT = creatinine; GM = Geometric mean.
[a] ΣOHPHE = 1-OHPHE + 2-OHPHE + 3-OHPHE + 4-OHPHE.
[b] Only 2-OHFLU.
[c] ΣOHPHE = 2-OHPHE + 3-OHPHE.

including tobacco smoke and occupational exposure to naphthalene (Castorina et al., 2010; Kuusimäki et al., 2004). However, 1-OHNAP is also a metabolite of carbaryl (n-methylcarbamate insecticide), and while naphthalene metabolises to 1- and 2-hydroxynaphthalene in approximately equal proportions, carbaryl only metabolises to 1-hydroxynaphthalene. Consequently, a ratio of 1-OHNAP to 2-OHNAP was suggested to differentiate between exposure to carbaryl and naphthalene (Castorina et al., 2010; Meeker et al., 2007). The German HBM Commission even derived a background exposure level for 1-OHNAP at 30 μg/L, in addition to the background exposure level for 2-OHNAP at 20 μg/L, for orientation in assessing human exposure to naphthalene (Schulz et al., 2011; Wilhelm et al., 2008). Furthermore, in the general non-smoking

Canadian populations, aged between 20–79 years, the reference value (P95) for 2-OHNAP was set at 14 μg/L, while a provisional reference value (P95) for 1-OHNAP in the group aged between 3 and 79 years was set at 4.3 μg/L (Khoury et al., 2018). For comparison, the P95 of 2-OHNAP in our study amounted to 9.6 μg/L, and it was higher in men at 11.7 μg/L than in women at 8.30 μg/L. As naphthalene has been classified as a potential carcinogen for humans (IARC, 2002) more attention should be paid to it in HBM surveys by potentially including and evaluating urinary 1-OHNAP and 2-OHNAP in HBM studies.

The levels of 2/3-OHFLU in lactating primiparous women in our study were found comparable to those reported in other countries, but lower than in Spain (Fernández et al., 2021), the USA (CDC, 2019) and Canada (Health Canada, 2017; Nethery et al., 2012). However, they were higher than in Denmark (Rosofsky et al., 2017) and China (Lou et al., 2019). In men, the urinary levels of 2/3-OHFLU were comparable to those reported in the NHANES programme and lower than those reported in the CHMS programme.

In our study, the levels of ΣOHPHE in lactating primiparous women were mainly lower than those in the studies listed in Table 3, except in women from the Spain BIOAMBIENT.ES programme (Bartolomé et al., 2015). In men, the levels of ΣOHPHE were comparable to those in the CHMS programme but higher than those in the BIOAMBIENT.ES programme. The highest levels of ΣOHPHE were found in Poland in 104 non-smoking pregnant women in a study conducted by Polanska et al. (2014). It should be noted that a sum of the urinary metabolites of phenanthrene cannot be directly compared between these studies since the sum of the individual metabolites of phenanthrene or their combination could vary. For example, in the NHANES study, only a sum of two hydroxylated metabolites of phenanthrene was considered, namely 2-OHPHE and 3-OHPHE (CDC, 2019), while in Lou et al. (2019), a sum of 1-OHPHE + 2-OHPHE + 3-OHPHE + 4-OHPHE was considered. The levels of 1-OHPYR in our study were the lowest compared to those reported in the studies listed in Table 3.

However, this comparison should be taken with caution because different study designs and analytical measurement methods may not give directly comparable results. Observed differences between countries could potentially be explained, among other factors, by differences in industrial activities, road vicinity, wood-burning practices and lifestyle habits, including diets and smoking. Moreover, the urine samples from women in this study were collected 6–8 weeks after pregnancy, which may be a confounding factor in comparison with other studies on pregnant or non-pregnant women. The changes that occur during pregnancy have been reported to influence absorption, distribution, metabolism and excretion of chemicals, including partition between maternal and foetus body tissues, which may influence the concentrations of PAH biomarkers in biological samples (Costantine, 2014; Pariente et al., 2016). It has also been reported that PAHs accumulate in the placenta and are, due to their lipophilicity, excreted in breast milk (Dong et al., 2018; Drwal et al., 2019). All these lifestyle and other factors should definitely be taken into account when comparing and evaluating different groups of pregnant or lactating women (Fernández et al., 2021).

3.4. Predictors of exposure and geographic variability

Multiple linear regression showed that the identified determinants of PAH exposure differed between the study groups and, to some extent, between metabolites. The significant determinants for most metabolites and for their sum were smoking status (or number of cigarettes), season of sampling (higher in winter/spring than summer/autumn), wood combustion in the participant's home and/or nominal heat output from small-scale wood-burning appliances within 250 m of the participant's home. In addition, higher education was associated with lower metabolite levels for ΣOHPAH, 2/3-OHFLU, and 2-OHNAP. For 1-OHPYR, it was also found that higher road density in the 500-m buffer zone was associated with higher metabolite levels in the urine samples. Associations with traffic-related variables were not observed for the other metabolites. The results of the multiple linear regression are presented in Table 4 and discussed below.

We also analysed the differences between the study areas and ran the statistical models for the sum of the PAH metabolites separately for each study area (due to the limited sample size, we did not stratify for the study group; i.e., sex). The highest GM for the sum of analysed PAH metabolites was observed in the MS area (4.44 μg/L SG), followed by KO (3.88 μg/L SG) and RA (3.84 μg/L SG) areas (Fig. 2). According to the P95 values, the ranking was slightly different; that is, the MS area had the highest values, followed by CE, KR, and RA study areas (Fig. 2). Marked differences were observed in PAH determinants among the study areas (Supplementary material, Table S2). Despite the limited sample size, the area-specific models had higher R^2 values than the general models shown in Table 4.

According to the bivariate analysis, current smoking (no vs yes) in men was positively associated with all the metabolite levels. For women, the smoking status was available for the period before pregnancy and during pregnancy. However, the smoking status during pregnancy could not be associated with PAH metabolite levels because only one woman (in the subpopulation group of 304 women, selected for PAH HBM) declared herself to be a smoker. Pre-pregnancy smoking (no vs yes) in women was positively associated with 2/3-OHFLU and ΣOHPAH.

The statistical models (Table 4) confirmed the association between PAH exposure and smoking in men; each cigarette smoked per day increased the PAH metabolite level in urine in the range of 4%–12%, depending on the metabolite. The highest increase was observed for 2/3-OHFLU, which was in line with the literature, where fluorene was reported to be the second most common representative of PAHs in cigarette smoke (Ding et al., 2005). Furthermore, the urinary metabolites of fluorene have been proposed as the most specific and selective biomarker for differentiating exposure to PAHs between smokers and non-smokers (Chetiyanukornkul et al., 2004; St.Helen et al., 2012). In the literature, it has also been shown that urinary phenanthrene metabolites are associated with recent potential exposure to PAHs in ambient air, especially cigarette smoke and/or polluted air in the living environment due to burning wood or fuel oil, cooking on a gas stove, etc. (Boström et al., 2002; Ohura et al., 2004; Tissari et al., 2007). In women, only 1-OHPYR was observed to be significantly associated with smoking (3% increase in the 1-OHPYR level for each cigarette). In comparison to the smoking status (no vs yes), the number of cigarettes smoked per day gave a better prediction in the models, as observed through higher R^2 for all the metabolites and especially for the phenanthrene metabolites. The number of cigarettes smoked daily was assessed as the predominant and the only statistically significant source of PAH exposure in the BK study area (Fig. 2 and Table 4). That area also had the highest proportion of smoking participants in comparison to the other study areas (Table 1). SP was another area where the number of cigarettes smoked daily was the only significant PAH determinant; in that area, only 6% of the participants were smokers. Exposure to second-hand smoke (reported for 44 women and 28 men) was also tested for its association with PAH metabolite levels in non-smokers. The only statistically significant association was for 2/3-OHFLU in men ($p = 0.035$). However, accounting for second-hand smoke in the regression models, no significant association was found for any of the metabolites; therefore, it was not included in the final models presented in this paper. To more appropriately evaluate the potential association between tobacco smoke and urinary PAHs in the Slovenian population, employing an additional biochemical parameter, such as urinary cotinine concentration, should be considered in future studies (Den Hond et al., 2015).

The sampling season was significantly associated with the level of all the PAH metabolites in the urine samples (marginally significant for the phenanthrene metabolites). Using a bivariate test, the difference between the participants sampled in winter and spring and those sampled in summer and autumn was the most pronounced, with the latter having lower levels (overall $p < 0.01$ for the sum of the PAH metabolites). This

Table 4
Predictors of exposure to PAHs based on urinary PAH metabolites for the Slovenian study population of men and lactating primiparous women in the same age range (18–49 years).

			Estimate of change (95% CI), multiplicative factor		
			Total population	Women, lactating	Men
1−OHPYR	*Model R^2*		*0.39 ($p < 0.001$)*	*0.37 ($p < 0.001$)*	*0.34 ($p < 0.001$)*
			n = 532	*n = 266*	*n = 266*
Sex	Women		1.00	–	–
	Men		1.06 (0.93–1.20)	–	–
Education	Low		1.00	1.00	1.00
	Medium		0.95 (0.76–1.18)	1.17 (0.79–1.73)	0.85 (0.67–1.08)
	High		0.84 (0.67–1.04)	0.93 (0.63–1.37)	0.85 (0.66–1.09)
No. cigarettes			**1.05 (1.03−1.07)****	**1.03 (1.00−1.06)***	**1.07 (1.04−1.10)****
Season	Winter/Spring		1.00	1.00	1.00
	Summer/Autumn		0.92 (0.82–1.04)	0.94 (0.79–1.13)	0.93 (0.81–1.08)
Wood burning	No		1.00	1.00	1.00
	Yes		**1.41 (1.23−1.62)****	**1.41 (1.14−1.74)****	**1.37 (1.16−1.62)****
Heating power in radius 250m (MW)			1.06 (0.98–1.14)	1.00 (0.88–1.14)	**1.12 (1.02−1.22)***
Road density, buffer zone 500 m			**1.02 (1.00−1.04)***	**1.03 (1.01−1.06)***	1.01 (0.99–1.03)
2/3−OHFLU		*Model R^2*	*0.44 ($p < 0.001$)*	*0.41 ($p < 0.001$)*	*0.43 ($p < 0.001$)*
			n = 531	*n = 266*	*n = 265*
Sex		Women	1.00	–	–
		Men	1.08 (0.96–1.22)	–	–
Education		Low	1.00	1.00	1.00
		Medium	0.92 (0.75–1.13)	1.00 (0.71–1.42)	0.89 (0.70–1.13)
		High	**0.79 (0.64−0.97)***	0.86 (0.61–1.21)	**0.76 (0.59−0.97)***
No. cigarettes			**1.06 (1.04−1.08)****	1.02 (1.00–1.05)	**1.12 (1.09−1.15)****
Season		Winter/Spring	1.00	1.00	1.00
		Summer/Autumn	**0.89 (07.80−0.99)***	0.89 (0.76–1.04)	0.95 (0.82–1.10)
Wood burning		No	1.00	1.00	1.00
		Yes	**1.42 (1.25−1.61)****	**1.42 (1.18−1.71)****	**1.33 (1.13−1.58)****
Heating power in radius 250m (MW)			1.07 (0.99–1.15)#	1.02 (0.91–1.15)	**1.12 (1.03−1.23)***
Road density, buffer zone 500 m			1.01 (1.00–1.03)	1.02 (0.99–1.04)	1.01 (0.99–1.03)
2−OHNAP		*Model R^2*	*0.33 ($p < 0.001$)*	*0.38 ($p < 0.001$)*	*0.27 ($p < 0.001$)*
			n = 531	*n = 266*	*n = 265*
Sex		Women	1.00	–	–
		Men	0.89 (0.77–1.03)	–	–
Education		Low	1.00	1.00	1.00
		Medium	0.80 (0.62–1.02)	0.92 (0.63–1.36)	0.73 (0.52–1.02)
		High	**0.62 (0.48−0.80)****	0.70 (0.48–1.03)#	**0.59 (0.41−0.83)***
No. cigarettes			**1.03 (1.01−1.06)***	1.00 (0.98–1.03)	**1.07 (1.03−1.11)****
Season		Winter/Spring	1.00	1.00	1.00
		Summer/Autumn	**0.83 (0.73−0.95)***	0.86 (0.72–1.02)#	0.85 (0.70–1.04)
Wood burning		No	1.00	1.00	1.00
		Yes	1.14 0.98–1.33)	1.13 (0.92–1.40)	1.10 (0.87–1.39)
Heating power in radius 250m (MW)			**1.10 (1.01−1.20)***	1.04 (0.91–1.18)	**1.17 (1.03−1.32)***
Road density, buffer zone 500 m			1.00 (0.98–1.02)	1.00 (0.97–1.03)	0.99 (0.96–1.02)
1/9−OHPHE		*Model R^2*	*0.25 ($p < 0.001$)*	*0.29 ($p < 0.001$)*	*0.17 ($p < 0.001$)*
			n = 539	*n = 271*	*n = 268*
Sex		Women	1.00	–	–
		Men	1.07 (0.95–1.20)	–	–
Education		Low	1.00	1.00	1.00
		Medium	0.90 (0.74–1.10)	1.06 (0.77–1.46)	0.82 (0.64–1.06)
		High	0.88 (0.72–1.08)	1.06 (0.77–1.45)	0.79 (0.60–1.03)#
No. cigarettes			**1.02 (1.01−1.04)***	1.00 (0.98–1.03)	**1.05 (1.02−1.08)***
Season					
Season		Winter/Spring	1.00	1.00	1.00
		Summer/Autumn	0.97 (0.87–1.07)	1.05 (0.90–1.21)	0.94 (0.81–1.10)
Wood burning		No	1.00	1.00	1.00
		Yes	**1.25 (1.12−1.40)****	1.16 (1.00–1.35)#	**1.33 (1.14−1.55)****
Heating power in radius 250m (MW)			1.03 (0.96–1.11)	1.03 (0.92–1.14)	1.04 (0.94–1.14)
2−OHPHE	*Model R^2*		*0.58 ($p < 0.001$)*	*0.54 ($p < 0.001$)*	*0.51 ($p < 0.001$)*
			n = 539	*n = 271*	*n = 268*
Sex	Women		1.00	–	–
	Men		1.07 (0.96–1.19)	–	–
Education	Low		1.00	1.00	1.00
	Medium		1.03 (0.86–1.23)	1.21 (0.89–1.64)	0.93 (0.75–1.15)
	High		0.95 (0.79–1.14)	1.09 (0.80–1.48)	0.87 (0.69–1.09)
No. cigarettes			**1.02 (1.00−1.04)***	1.01 (0.98–1.03)	**1.04 (1.02−1.07)****
Season	Winter/Spring		1.00	1.00	1.00
	Summer/Autumn		0.96 (0.87–1.05)	0.92 (0.80–1.06)	1.04 (0.91–1.18)
Wood burning	No		1.00	1.00	1.00
	Yes		**1.18 (1.07−1.30)****	1.14 (0.98–1.31)#	**1.20 (1.05−1.37)***
Heating power in radius 250m (MW)			**1.08 (1.01−1.15)***	1.03 (0.93–1.14)	**1.12 (1.04−1.22)***
3−OHPHE	*Model R^2*		*0.48 ($p < 0.001$)*	*0.48 ($p < 0.001$)*	*0.38 ($p < 0.001$)*

(*continued on next page*)

Table 4 (*continued*)

2−OHPHE	*Model R^2*	*0.58 ($p < 0.001$)*	*0.54 ($p < 0.001$)*	*0.51 ($p < 0.001$)*
		n = 539	*n = 271*	*n = 268*
		n = 539	*n = 271*	*n = 268*
Sex	Women	1.00	–	–
	Men	1.12 (1.00–1.26)#	–	–
Education	Low	1.00	1.00	1.00
	Medium	0.93 (0.76–1.13)	1.12 (0.80–1.57)	0.82 (0.65–1.03)#
	High	0.87 (0.71–1.06)	0.99 (0.72–1.38)	0.80 (0.63–1.02)#
No. cigarettes		**1.03 (1.01−1.05)***	1.01 (0.99–1.04)	**1.05 (1.02−1.08)****
Season	Winter/Spring	1.00	1.00	1.00
	Summer/Autumn	0.97 (0.88–1.08)	0.95 (0.82–1.11)	1.05 (0.92–1.21)
Wood burning	No	1.00	1.00	1.00
	Yes	**1.26 (1.13−1.41)****	1.15 (0.99–1.35)#	**1.34 (1.16−1.54)****
Heating power in radius 250m (MW)		1.04 (0.97–1.12)	0.99 (0.89–1.11)	**1.10 (1.01−1.20)***
SUM−OHPHE	*Model R^2*	*0.47 ($p < 0.001$)*	*0.46 ($p < 0.001$)*	*0.37 ($p < 0.001$)*
		n = 532	*n = 266*	*n = 266*
Sex	Women	1.00	–	–
	Men	1.09 (0.98–1.22)	–	–
Education	Low	1.00	1.00	1.00
	Medium	0.95 (0.79–1.13)	1.14 (0.84–1.54)	0.86 (0.69–1.07)
	High	0.89 (0.74–1.07)	1.06 (0.79–1.43)	0.81 (0.64–1.01)#
No. cigarettes		**1.02 (1.01−1.04)***	1.01 (0.99–1.03)	**1.04 (1.02−1.07)****
Season	Winter/Spring	1.00	1.00	1.00
	Summer/Autumn	0.97 (0.88–1.07)	0.98 (0.86–1.13)	1.01 (0.89–1.15)
Wood burning	No	1.00	1.00	1.00
	Yes	**1.30 (1.16−1.45)****	**1.24 (1.06−1.46)****	**1.32 (1.13−1.54)****
Heating power in radius 250m (MW)		1.04 (0.97–1.11)	1.00 (0.90–1.11)	1.07 (0.99–1.16)#
Road density, buffer zone 500 m		1.01 (1.00–1.03)#	1.02 (1.00–1.04)#	1.01 (0.99–1.03)
SUM−OHPAH	*Model R^2*	*0.41 ($p < 0.001$)*	*0.43 ($p < 0.001$)*	*0.35 ($p < 0.001$)*
		n = 538	*n = 271*	*n = 267*
Sex	Women	1.00	–	–
	Men	0.93 (0.82–1.05)	–	–
Education	Low	1.00	1.00	1.00
	Medium	0.83 (0.67–1.02)#	0.94 (0.67–1.33)	**0.76 (0.58−0.98)***
	High	**0.66 (0.53−0.82)****	0.74 (0.53–1.04)#	**0.62 (0.47−0.82)****
No. cigarettes		**1.03 (1.01−1.05)****	1.01 (0.98–1.03)	**1.07 (1.04−1.10)****
Season	Winter/Spring	1.00	1.00	1.00
	Summer/Autumn	**0.86 (0.77−0.96)***	0.88 (0.75–1.03)#	0.88 (0.75–1.03)
Wood burning	No	1.00	1.00	1.00
	Yes	**1.18 (1.06−1.33)***	1.17 (0.99–1.37)#	1.16 (0.99–1.36)#
Heating power in radius 250m (MW)		**1.08 (1.00−1.16)***	1.03 (0.92–1.15)	**1.13 (1.03−1.25)***
Road density, buffer zone 500 m		Ns	ns	ns

Notes: Margin of statistical significance: #p < 0.1, *p < 0.05, **p ≤ 0.001, ns – not significant. Variables with significance of p < 0.05 in at least one group are bolded. All models are adjusted for specific gravity in urine. 1−OHPYR = 1−hydroxypyrene; 2/3−OHFLU = a combination of 2−hydroxyfluorene and 3−hydroxyfluorene; 2−OHNAP = 2−Hydroxynaphtalene; SUM−OHPHE = Sum of 1/9-hydroxyphenanthrene, 2−Hydroxyphenanthrene, 3−Hydroxyphenanthrene and 4−Hydroxyphenanthrene; SUM−OHPAH = Sum of all analysed urine metabolites of PAHs.

classification was then used in the multiple regression models, where the difference was confirmed for 2/3-OHFLU, 2-OHNAP and ΣOHPAH in men only (Table 4). The observed difference most probably reflects the increased pollution in ambient air due to the heating season; therefore, wood as a source for heating in a participant's home was another significant determinant of PAH exposure. Bivariate analysis showed strongly significantly higher levels of all the PAH metabolites in the participants who reported using wood as a (major or partial) heating source than in those who claimed not to use wood as a heating source (overall $p < 0.001$ for ΣOHPAH). In the multiple regression models, the associations were confirmed for all the metabolites, except 2-OHNAP, independent of the sampling season. For 1-OHPYR and 2/3-OHFLU, the estimated coefficients were slightly higher in women than in men, while for the phenanthrene metabolites, men showed higher coefficients and more significant associations with wood as a source of heating (Table 4). In the area-specific models, the sampling season appeared to be the only significant determinant in the two urban areas, LJ and CE. In the KO and ZA areas (also marginally significant in RA), the participants using wood as a heating source had significantly higher ΣOHPAH levels, although the areas had different proportions of participants who declared the use of wood as a heating source.

Similar correlations between urine concentrations of 1-OHPYR and exposure to PAHs in ambient air (mainly in controlled occupational settings) were reported in the literature (Klöslová et al., 2016). As shown by Boström et al. (2002) and Egeghy et al. (2005), using a wood stove or a fireplace, as well as using gas-powered equipment (and smoking cigarettes), were associated with elevated exposure to PAHs. Several other studies also showed that urinary metabolites of fluorene were associated with exposure to polluted air due to the burning of wood, coal, fuel oil and gas and were highly variable based on the season (Becker et al., 2003; Bulejko et al., 2016; Martellini et al., 2012; Sochacka-Tatara et al., 2018).

In addition to wood burning in the participants' homes, the calculated heat output in the radius of 250 m around the participants' homes appeared to be significantly associated with the PAH metabolite levels in men, but not in women. Among the tested distances (50 m, 250 m, 500 m, 1000 m, 2500 m and 5000 m), the radius of 250 m appeared to be the most significant for the associations (overall $p < 0.001$ for ΣOHPAH). The associations were also significant and positive for the radii distances of 50 m and 500 m ($p = 0.015$ and 0.005), but not for the distances of 1000 m ($p = 0.689$), 2500 m ($p = 0.298$) and 5000 m ($p = 0.004$), where the association was negative. In the area-specific models (Table 4), the nominal heat output of small combustion appliances was found to be significant in the KO and KR study areas.

Road density in the buffer zone of 500 m appeared to be significantly correlated with 1-OHPYR for the total population and in women but not in men. A marginally significant association was observed for ΣOHPHE in the total population and in women. Similarly, as in the case of wood-

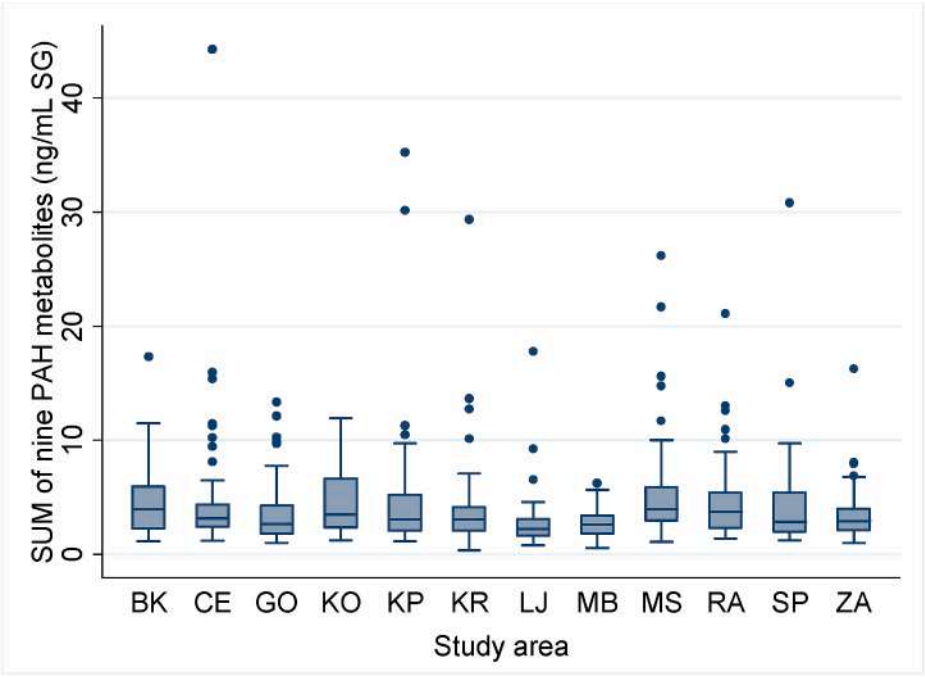

Fig. 2. The distribution (median, P25–P75, min–max values and outliers) of all the analysed urinary PAH metabolites, expressed as the sum of nine metabolites (Σ_9OHPAH) in values adjusted for specific gravity (SG) in the Slovenian study population of men and lactating primiparous women of the same age range (18–49 years) in Slovenian regions (see Table 1).

burning appliances, different buffer zones were applied to the regression models (50 m, 100 m and 500 m); however, the buffer zone of 500 m was the only one that was statistically significant (p = 0.487, 0.193 and 0.026, respectively).

In the urban environment of the KP study area, the association with industrial PM_{10} release was shown to be the only significant determinant. Industrial PM_{10} releases were also found to have a significant association with PAH metabolite levels in the ZA and KR study areas (the industrial zones) but not in any other general models presented in Table 4. These results showed that outdoor air quality seemed to be significant. The environmental dispersion of PAHs was found to be associated with atmospheric PM_{10} (and $PM_{2.5}$) aerosols in many studies (Barrado et al., 2012; Galarneau et al., 2014; Slezakova et al., 2013). In addition, urinary metabolites of fluorene and phenanthrene were found to be a potential indicator of recent exposure to PAH outdoors, especially near high-traffic roads or industrial facilities (Delgado-Saborit et al., 2011; Gatti et al., 2017; Thai et al., 2016).

Regarding the study areas, the MS study area was found to have somehow a different pattern, with the only significant determinant observed in the model, e.g. the intake of "other meat". In the questionnaire, we asked the participants to indicate the consumption frequencies of different types of meat, such as poultry and game meat, and meat other than these two categories ("other meat"). The obtained results might reflect the typical food consumed in this eastern Slovenian region (Mura Region) known to be higher in PAHs, such as grilled, fried or smoked food, and pumpkin seed oil (Cheng et al., 2019; Rose et al., 2015). At the time of conducting this research, the questionnaire did not specifically ask about dietary habits regarding the quantity and frequency of consumption of this kind of food. However, such questions were already included in the HBM questionnaire in a study conducted with children and will also be considered in future HBM studies in adults.

Education level (low, medium, high) was included in the models as an indicator of socio-economic status; in general, this also represented lifestyle habits (including food consumption habits). Moreover, more educated people generally prefer healthier dietary choices over less healthy ones. Indeed, the statistical models showed that participants with higher education had lower levels of 2/3-OHFLU, 2-OHNAP and ΣOHPAH metabolites and also marginally significantly lower levels of 1/9-OHPHE and ΣOHPHE (Table 4). The study group-specific models showed significant differences only in men. Although education was presumed to reflect different aspects of exposure from smoking, wood-burning and dietary habits, the observed negative association between educational levels and the PAH metabolite levels in our study most probably reflects dietary habits. Inclusion of smoking and wood-burning-related variables into statistical models showed an association with PAH metabolites independently of education.

Moreover, pairwise analysis of the metabolite levels between the paired partners (women and men living in the same household) (n = 84) in our study population revealed significant positive correlations between the pairs for all metabolites: r = 0.56 for ΣOHPAH, r = 0.36 for 1-OHPYR, r = 0.54 for 2/3-OHFLU, r = 0.56 for 2-OHNAP, r = 0.36 for 1/9−OHPHE, r = 0.33 for 2-OHPHE, r = 0.31 for 3-OHPHE and r = 0.38 for ΣOHPHE (all $p < 0.001$). Although the number of pairs was small, these results indicate that between 31% and 56% of variability in exposure could be explained by shared exposure sources within the households (such as diet and wood-burning-related determinants).

3.5. *Limitations and strengths of the study*

This study has some limitations. It was not designed specifically to assess PAH exposure; therefore, it lacks some PAH-specific questionnaire data (such as grilled/smoked food, pumpkin seed oil consumption, etc.). A major limitation is self-reported smoking status and/or smoking history (for men, former smokers were not identified) and an unbalanced group of smokers and non-smokers for comparison. The relatively small sample size per study area, differences in the spatial resolution and the collection of environmental (ancillary) data may have introduced bias in assessing their association with the biomarker levels.

One of the major strengths of this study is that it is the first to assess PAH exposure in a nationally representative population of adults of reproductive age, including lactating primiparous women and their male partners or other males in the same age range. The fact that the participants were recruited in 12 statistical regions of Slovenia allowed

us to assess geographic variability and identify local characteristics related to PAH exposure. Despite the lack of relevant dietary information and the uncertainty of self-reported data, the availability of ancillary data from the Chimney Sweeper Information System, the European Pollutant Release and Transfer Register, and the Public Infrastructure Cadastre, as well as the possibility of linking them to the individual participant's data, provides important information for predicting PAH exposure sources in the general and area-specific statistical models.

4. Conclusions

The first assessment of PAH exposure in the Slovenian adult population of men and lactating primiparous women was performed. Nine urinary metabolites of pyrene, phenanthrene, naphthalene and fluorene were determined. The obtained results provided the first insight into the PAH exposure of this study group, potential sources of exposure to PAHs and its spatial variability. They also provided a baseline from which future time trends could be observed and assessed, as well as a comparison with other related research worldwide. They will also contribute to the national and international requirements related to the environment and public health, as well as risk assessment and management decisions, especially in terms of a European HBM network, dedicated mainly to these goals (Louro et al., 2019; Smolders et al., 2008).

Spatial (geographical) differences were noted, as were the differences between the two study groups (men and lactating primiparous women) and the differences based on the main exposure predictors (tobacco smoke, domestic wood-burning and environmental exposure, such as nominal heat output, road vicinity or their density in a certain radius from the participants' homes and ambient PM_{10} levels). Evaluating the differences in smoking status appeared to be helpful in demonstrating the importance of not smoking during pregnancy. Indeed, informing the participants that the PAH metabolite levels were generally lower in non-smokers could contribute significantly to promoting a healthy lifestyle. We also found some spatial differences in dietary exposure, which might relate to traditional differences in dietary habits between the eastern and western parts of Slovenia. In addition, a shared exposure to sources within the households was confirmed for paired partners.

However, to confirm these results and assumptions, specific and more detailed questionnaires on dietary habits will be used in all future HBM studies. In future HBM cycles, care will also be taken to overcome the limitations encountered in this study, such as increasing the number of participants per study area and including non-pregnant women. Furthermore, it is worth pointing out that, in addition to evaluating the PAH exposure predictors, individual genetic variability in the PAH-metabolising enzymes (e.g. cytochrome P450) might influence PAH exposure and possible toxicity (Alexandrie et al., 2000; Li et al., 2018; Marinković et al., 2013) and should also be investigated in future studies.

Declaration of competing interests

The authors report no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.113943.

References

Abdel-Shafy, H.I., Mansour, M.S.M., 2016. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egypt. J. Pet. 25, 107–123. https://doi.org/10.1016/j.ejpe.2015.03.011.

Agency for Toxic Substances and Disease Registry (ATSDR), 1995. Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs). U. S. Department of Health and Human Services, Public Health Service, Atlanta, GA. https://wwwn.cdc.gov/TSP/ToxProfiles/ToxProfiles.aspx?id=122&tid=25.

Alexandrie, A.K., Warholm, M., Carstensen, U., Axmon, A., Hagmar, L., Levin, J.O., Östman, C., Rannug, A., 2000. CYP1A1 and GSTM1 polymorphisms affect urinary 1-hydroxypyrene levels after PAH exposure. Carcinogenesis 21, 669–676. https://doi.org/10.1093/carcin/21.4.669.

Apel, P., Rousselle, C., Lange, R., Sissoko, F., Kolossa-Gehring, M., Ougier, E., 2020. Human biomonitoring initiative (HBM4EU) – strategy to derive human biomonitoring guidance values (HBM-GVs) for health risk assessment. Int. J. Hyg Environ. Health 230, 113622. https://doi.org/10.1016/j.ijheh.2020.113622.

Aquilina, N.J., Delgado-Saborit, J.M., Meddings, C., Baker, S., Harrison, R.M., Jacob, P., Wilson, M., Yu, L., Duan, M., Benowitz, N.L., 2010. Environmental and biological monitoring of exposures to PAHs and ETS in the general population. Environ. Int. 36, 763–771. https://doi.org/10.1016/j.envint.2010.05.015.

Barr, D.B., Wilder, L.C., Caudill, S.P., Gonzalez, A.J., Needham, L.L., Pirkle, J.L., 2005. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ. Health Perspect. 113, 192–200. https://doi.org/10.1289/ehp.7337.

Barrado, A.I., Garcia, S., Castrillejo, Y., Perez, R.M., 2012. Hydroxy-PAH levels in atmospheric PM10 aerosol samples correlated with season, physical factors and chemical indicators of pollution. Atmos. Pollut. Res. 3, 81–87. https://doi.org/10.5094/APR.2012.007.

Bartolomé, M., Ramos, J.J., Cutanda, F., Huetos, O., Esteban, M., Ruiz-Moraga, M., Calvo, E., Pérez-Gómez, B., González, O., Castaño, A., Aleixandre, J.L., Aragonés, N., Cañas, A.I., Cervantes-Amat, M., Cortés, M.V., Jimenez, J.A., López-Abente, G., López-Herranz, A., Mayor, J., Méndez, C.F., Molina, M.A., Navarro, C., Pollán, M., Pastor, R., Román, J., 2015. Urinary polycyclic aromatic hydrocarbon metabolites levels in a representative sample of the Spanish adult population: the BIOAMBIENT.ES project. Chemosphere 135, 436–446. https://doi.org/10.1016/j.chemosphere.2014.12.008.

Becker, K., Schulz, C., Kaus, S., Seiwert, M., Seifert, B., 2003. German environmental survey 1998 (GerES III): environmental pollutants in the urine of the German population. Int. J. Hyg Environ. Health 206, 15–24. https://doi.org/10.1078/1438-4639-00188.

Boström, C.E., Gerde, P., Hanberg, A., Jernström, B., Johansson, C., Kyrklund, T., Rannug, A., Törnqvist, M., Victorin, K., Westerholm, R., 2002. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ. Health Perspect. 110, 451–488. https://doi.org/10.1289/ehp.110-1241197.

Bulejko, P., Adamec, V., Schüllerová, B., Skeřil, R., 2016. Levels, sources, and health risk assessment of polycyclic aromatic hydrocarbons in Brno, Czech Republic: a 5-year study. Environ. Sci. Pollut. Res. 23, 20462–20473. https://doi.org/10.1007/s11356-016-7172-5.

Campo, L., Fustinoni, S., Consonni, D., Pavanello, S., Kapka, L., Siwinska, E., Mielzyńska, D., Bertazzi, P., 2014. Urinary carcinogenic 4–6 ring polycyclic aromatic hydrocarbons in coke oven workers and in subjects belonging to the general population: Role of occupational and environmental exposure. Int. J. Hyg. Environ. Health 2017 (2–3), 231–238. https://doi.org/10.1016/j.ijheh.2013.06.005.

Castorina, R., Bradman, A., Fenster, L., Barr, D.B., Bravo, R., Vedar, M.G., Harnly, M.E., Mckone, T.E., Eisen, E.A., Eskenazi, B., 2010. Comparison of current-use pesticide and other toxicant urinary metabolite levels among pregnant women in the CHAMACOS cohort and NHANES. Environ. Health Perspect. 118, 856–863. https://doi.org/10.1289/ehp.0901568.

Centers for Disease Control and Prevention (CDC), 2019. Fourth National Report on Human Exposure to Environmental Chemicals: Updated Tables, vol. 1. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention., Atlanta, GA. https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Jan2019-508.pdf.

Cheng, J., Zhang, X., Ma, Y., Zhao, J., Tang, Z., 2019. Concentrations and distributions of polycyclic aromatic hydrocarbon in vegetables and animal-based foods before and after grilling: implication for human exposure. Sci. Total Environ. 690, 965–972. https://doi.org/10.1016/j.scitotenv.2019.07.074.

Chetiyanukornkul, T., Toriba, A., Kizu, R., Hayakawa, K., 2004. Urinary 2-hydroxyfluorene and 1-hydroxypyrene levels in smokers and nonsmokers in Japan and Thailand. Polycycl. Aromat. Comp. 24 (4–5), 467–474. https://doi.org/10.1080/10406630490471483.

Choi, J., Aarøe Mørck, T., Joas, A., Knudsen, L.E., 2015. Major national human biomonitoring programs in chemical exposure assessment. AIMS Environ. Sci. 2, 782–802. https://doi.org/10.3934/environsci.2015.3.782.

Choi, J., Aarøe Mørck, T., Polcher, A., Knudsen, L.E., Joas, A., 2017. Review of the State of the Art of Human Biomonitoring for Chemical Substances and its Application to Human Exposure Assessment for Food Safety. EFSA Support, vol. 12. Publ, p. 724E. https://doi.org/10.2903/sp.efsa.2015.en-724.

Costantine, M.M., 2014. Physiologic and pharmacokinetic changes in pregnancy. Front. Pharmacol. 5, 65. https://doi.org/10.3389/fphar.2014.00065.

David, M., Gerofke, A., Lange, R., Kolossa-Gehring, M., Apel, P., 2021. The European Human Biomonitoring Initiative (HBM4EU): human biomonitoring guidance values (HBM-GVs) for the aprotic solvents N-methyl-2-pyrrolidone (NMP) and N-ethyl-2-pyrrolidone (NEP). Int. J. Hyg Environ. Health 238, 113856. https://doi.org/10.1016/j.ijheh.2021.113856.

Delgado-Saborit, J.M., Stark, C., Harrison, R.M., 2011. Carcinogenic potential, levels and sources of polycyclic aromatic hydrocarbon mixtures in indoor and outdoor environments and their implications for air quality standards. Environ. Int. 37, 383–392. https://doi.org/10.1016/j.envint.2010.10.011.

Den Hond, E., Govarts, E., Willems, H., Smolders, R., Casteleyn, L., Kolossa-Gehring, M., Schwedler, G., Seiwert, M., Fiddicke, U., Castaño, A., Esteban, M., Angerer, J., Koch, H.M., Schindler, B.K., Sepai, O., Exley, K., Bloemen, L., Horvat, M., Knudsen, L.E., Joas, A., Joas, R., Biot, P., Aerts, D., Koppen, G., Katsonouri, A., Hadjipanayis, A., Krskova, A., Maly, M., Mørck, T.A., Rudnai, P., Kozepesy, S., Mulcahy, M., Mannion, R., Gutleb, A.C., Fischer, M.E., Ligocka, D., Jakubowski, M., Fátima Reis, M., Namorado, S., Gurzau, A.E., Lupsa, I.R., Halzlova, K., Jajcaj, M., Mazej, D., Tratnik, J.S., López, A., Lopez, E., Berglund, M., Larsson, K., Lehmann, A., Crettaz, P., Schoeters, G., 2015. First steps toward harmonized human biomonitoring in Europe: demonstration project to perform human biomonitoring on a European scale. Environ. Health Perspect. 123, 255–263. https://doi.org/10.1289/ehp.1408616.

Ding, Y.S., Trommel, J.S., Yan, X.J., Ashley, D., Watson, C.H., 2005. Determination of 14 polycyclic aromatic hydrocarbons in mainstream smoke from domestic cigarettes. Environ. Sci. Technol. 39, 471–478. https://doi.org/10.1021/es048690k.

Dong, X., Wang, Q., Peng, J., Wu, M., Pan, B., Xing, B., 2018. Transfer of polycyclic aromatic hydrocarbons from mother to fetus in relation to pregnancy complications. Sci. Total Environ. 636, 61–68. https://doi.org/10.1016/j.scitotenv.2018.04.274.

Drwal, E., Rak, A., Gregoraszczuk, E.L., 2019. Review: polycyclic aromatic hydrocarbons (PAHs)—action on placental function and health risks in future life of newborns. Toxicology 411, 133–142. https://doi.org/10.1016/j.tox.2018.10.003.

European Food Safety Authority EFSA, 2008. Polycyclic aromatic hydrocarbons in food – scientific opinion of the panel on contaminants in the food chain. EFSA J. 724, 1–114. https://doi.org/10.2903/j.efsa.2008.724.

Egeghy, P.P., Quackenboss, J.J., Catlin, S., Ryan, P.B., 2005. Determinants of temporal variability in NHEXAS-Maryland environmental concentrations, exposures, and biomarkers. J. Expo. Anal. Environ. Epidemiol. 15, 388–397. https://doi.org/10.1038/sj.jea.7500415.

Fernández, S.F., Pardo, O., Pastor, A., Yusà, V., Vento, M., Cernada, M., Gormaz, M., Kuligowski, J., Sánchez, A., Torres, E., Correcher, P., Núñez, A., Parra, A., Ramón, A., 2021. Biomonitoring of polycyclic aromatic hydrocarbons in the urine of lactating mothers: urinary levels, association with lifestyle factors, and risk assessment. Environ. Pollut. 268, 115646. https://doi.org/10.1016/j.envpol.2020.115646.

Galarneau, E., Makar, P.A., Zheng, Q., Narayan, J., Zhang, J., Moran, M.D., Bari, M.A., Pathela, S., Chen, A., Chlumsky, R., 2014. PAH concentrations simulated with the AURAMS-PAH chemical transport model over Canada and the USA. Atmos. Chem. Phys. 14, 4065–4077. https://doi.org/10.5194/acp-14-4065-2014.

Ganzleben, C., Antignac, J.P., Barouki, R., Castaño, A., Fiddicke, U., Klánová, J., Lebret, E., Olea, N., Sarigiannis, D., Schoeters, G.R., Sepai, O., Tolonen, H., Kolossa-Gehring, M., 2017. Human biomonitoring as a tool to support chemicals regulation in the European Union. Int. J. Hyg Environ. Health 220, 94–97. https://doi.org/10.1016/j.ijheh.2017.01.007.

Gatti, M.G., Bechtold, P., Campo, L., Barbieri, G., Quattrini, G., Ranzi, A., Sucato, S., Olgiati, L., Polledri, E., Romolo, M., Iacuzio, L., Carrozzi, G., Lauriola, P., Goldoni, C. A., Fustinoni, S., 2017. Human biomonitoring of polycyclic aromatic hydrocarbons and metals in the general population residing near the municipal solid waste incinerator of Modena. Italy. Chemosphere 186, 546–557. https://doi.org/10.1016/j.chemosphere.2017.07.122.

Health Canada, 2017. Fourth Report on Human Biomonitoring of Environmental Chemicals in Canada. Results of the Canadian Health Measures Survey Cycle, vol. 4, p. 239p, 2014–2015, Ottawa, Ontario K1A 0K9.

Hoet, P., Deumer, G., Bernard, A., Lison, D., Haufroid, V., 2016. Urinary trace element concentrations in environmental settings: is there a value for systematic creatinine adjustment or do we introduce a bias? J. Expo. Sci. Environ. Epidemiol. 26, 296–302. https://doi.org/10.1038/jes.2015.23.

Honda, M., Suzuki, N., 2020. Toxicities of polycyclic aromatic hydrocarbons for aquatic animals. Int. J. Environ. Res. Publ. Health 17, 1363. https://doi.org/10.3390/ijerph17041363.

Hu, C., Hou, J., Zhou, Y., Sun, H., Yin, W., Zhang, Y., Wang, X., Wang, G., Chen, W., Yuan, J., 2018. Association of polycyclic aromatic hydrocarbons exposure with atherosclerotic cardiovascular disease risk: a role of mean platelet volume or club cell secretory protein. Environ. Pollut. 233, 45–53. https://doi.org/10.1016/j.envpol.2017.10.042.

International agency for research on cancer (IARC), 2002. Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene/IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2002), 82, p. 601p. Lyon, France. https://monographs.iarc.who.int/wp-content/uploads/2018/06/mono82.pdf.

International agency for research on cancer (IARC), 2010. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures, 2005), 92 IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 868p (Lyon, France).

Jo, W.K., Lee, J.H., 2011. Naphthalene and benzene levels in microenvironments associated with potential exposure: new and old apartments with moth repellents, and cabins of passenger cars. Int. J. Environ. Anal. Chem. 91, 1412–1424. https://doi.org/10.1080/03067310903359468.

Jongeneelen, F.J., 1994. Biological monitoring of environmental exposure to polycyclic aromatic hydrocarbons; 1-hydroxypyrene in urine of people. Toxicol. Lett. 72, 205–211. https://doi.org/10.1016/0378-4274(94)90030-2.

Khoury, C., Werry, K., Haines, D., Walker, M., Malowany, M., 2018. Human biomonitoring reference values for some non-persistent chemicals in blood and urine derived from the Canadian Health Measures Survey 2009–2013. Int. J. Hyg Environ. Health 221, 684–696. https://doi.org/10.1016/j.ijheh.2018.03.003.

Klöslová, Z., Drímal, M., Balog, K., Koppová, K., Dubajová, J., 2016. The relations between polycyclic aromatic hydrocarbons exposure and 1-OHP levels as a biomarker of the exposure. Cent. Eur. J. Publ. Health 24, 302–307. https://doi.org/10.21101/cejph.a4179.

Korsh, J., Shen, A., Aliano, K., Davenport, T., 2015. Polycyclic aromatic hydrocarbons and breast cancer: a review of the literature. Breast Care 10, 316–318. https://doi.org/10.1159/000436956.

Krzyszczak, A., Czech, B., 2021. Occurrence and toxicity of polycyclic aromatic hydrocarbons derivatives in environmental matrices. Sci. Total Environ. 788, 147738. https://doi.org/10.1016/j.scitotenv.2021.147738.

Kuusimäki, L., Peltonen, Y., Mutanen, P., Peltonen, K., Savela, K., 2004. Urinary hydroxy-metabolites of naphthalene, phenanthrene and pyrene as markers of exposure to diesel exhaust. Int. Arch. Occup. Environ. Health 77, 23–30. https://doi.org/10.1007/s00420-003-0477-y.

Kwon, Y.M., Joo, Y., Park, C.-H., Kim, S.Y., Choi, K., Lee, C., Yu, S.D., Yoo, J., 2019. Exposure levels and influence factors of PAHs and benzene metabolites in the urine of the general Korean adult population – Korean National Environmental Health Survey (2009-2017). J. Environ. Health Sci. 45, 529–540. https://doi.org/10.5668/JEHS.2019.45.5.529.

Lange, R., Apel, P., Rousselle, C., Charles, S., Sissoko, F., Kolossa-Gehring, M., Ougier, E., 2021. The European Human Biomonitoring Initiative (HBM4EU): human biomonitoring guidance values for selected phthalates and a substitute plasticizer. Int. J. Hyg Environ. Health 234, 113722. https://doi.org/10.1016/j.ijheh.2021.113722.

Lauwerys, R.R., Hoet, P., 2001. Industrial Chemical Exposure, third ed. CRC Press, Boca Raton, p. 664p. https://doi.org/10.1201/9781482293838.

Lawal, A.T., 2017. Polycyclic aromatic hydrocarbons. A review. Cogent Environ. Sci. 3, 1339841. https://doi.org/10.1080/23311843.2017.1339841.

Li, N., Mu, Y., Liu, Z., Deng, Y., Guo, Y., Zhang, X., Li, X., Yu, P., Wang, Y., Zhu, J., 2018. Assessment of interaction between maternal polycyclic aromatic hydrocarbons exposure and genetic polymorphisms on the risk of congenital heart diseases. Sci. Rep. 8, 3075. https://doi.org/10.1038/s41598-018-21380-3.

Llop, S., Ballester, F., Estarlich, M., Ibarluzea, J., Manrique, A., Rebagliato, M., Esplugues, A., Iñiguez, C., 2008. Urinary 1-hydroxypyrene, air pollution exposure and associated life style factors in pregnant women. Sci. Total Environ. 407, 97–104. https://doi.org/10.1016/j.scitotenv.2008.07.070.

Lou, X.Y., Wu, P.R., Guo, Y., 2019. Urinary metabolites of polycyclic aromatic hydrocarbons in pregnant women and their association with a biomarker of oxidative stress. Environ. Sci. Pollut. Res. 26, 27281–27290. https://doi.org/10.1007/s11356-019-05855-y.

Louro, H., Heinälä, M., Bessems, J., Buekers, J., Vermeire, T., Woutersen, M., van Engelen, J., Borges, T., Rousselle, C., Ougier, E., Alvito, P., Martins, C., Assunção, R., Silva, M.J., Pronk, A., Schaddelee-Scholten, B., Del Carmen Gonzalez, M., de Alba, M., Castaño, A., Viegas, S., Humar-Juric, T., Kononenko, L., Lampen, A., Vinggaard, A.M., Schoeters, G., Kolossa-Gehring, M., Santonen, T., 2019. Human biomonitoring in health risk assessment in Europe: current practices and recommendations for the future. Int. J. Hyg Environ. Health 222, 727–737. https://doi.org/10.1016/j.ijheh.2019.05.009.

Marinković, N., Pašalić, D., Potočki, S., 2013. Polymorphisms of genes involved in polycyclic aromatic hydrocarbons' biotransformation and atherosclerosis. Biochem. Med. 23, 255–265. https://doi.org/10.11613/BM.2013.032.

Martellini, T., Giannoni, M., Lepri, L., Katsoyiannis, A., Cincinelli, A., 2012. One year intensive PM 2.5 bound polycyclic aromatic hydrocarbons monitoring in the area of Tuscany, Italy. Concentrations, source understanding and implications. Environ. Pollut. 164, 252–258. https://doi.org/10.1016/j.envpol.2011.12.040.

Meeker, J.D., Barr, D.B., Serdar, B., Rappaport, S.M., Hauser, R., 2007. Utility of urinary 1-naphthol and 2-naphthol levels to assess environmental carbaryl and naphthalene exposure in an epidemiology study. J. Expo. Sci. Environ. Epidemiol. 17, 314–320. https://doi.org/10.1038/sj.jes.7500502.

Mucha, A.P., Hryhorczuk, D., Serdyuk, A., Nakonechny, J., Zvinchuk, A., Erdal, S., Caudill, M., Scheff, P., Lukyanova, E., Shrkiryak-Nyzhnyk, Z., Chislovska, N., 2006. Urinary 1-hydroxypyrene as a biomarker of PAH exposure in 3-year-old Ukrainian

children. Environ. Health Perspect. 114, 603–609. https://doi.org/10.1289/ehp.7898.
Murawski, A., Roth, A., Schwedler, G., Schmied-Tobies, M.I.H., Rucic, E., Pluym, N., Scherer, M., Scherer, G., Conrad, A., Kolossa-Gehring, M., 2020. Polycyclic aromatic hydrocarbons (PAH) in urine of children and adolescents in Germany – human biomonitoring results of the German Environmental Survey 2014–2017 (GerES V). Int. J. Hyg Environ. Health 226, 113491. https://doi.org/10.1016/j.ijheh.2020.113491.
Nethery, E., Wheeler, A.J., Fisher, M., Sjödin, A., Li, Z., Romanoff, L.C., Foster, W., Arbuckle, T.E., 2012. Urinary polycyclic aromatic hydrocarbons as a biomarker of exposure to PAHs in air: a pilot study among pregnant women. J. Expo. Sci. Environ. Epidemiol. 22, 70–81. https://doi.org/10.1038/jes.2011.32.
Ohura, T., Amagai, T., Fusaya, M., Matsushita, H., 2004. Polycyclic aromatic hydrocarbons in indoor and outdoor environments and factors affecting their concentrations. Environ. Sci. Technol. 38, 77–83. https://doi.org/10.1021/es030512o.
Onyemauwa, F., Rappaport, S.M., Sobus, J.R., Gajdošová, D., Wu, R., Waidyanatha, S., 2009. Using liquid chromatography-tandem mass spectrometry to quantify monohydroxylated metabolites of polycyclic aromatic hydrocarbons in urine. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 877, 1117–1125. https://doi.org/10.1016/j.jchromb.2009.02.067.
Ougier, E., Ganzleben, C., Lecoq, P., Bessems, J., David, M., Schoeters, G., Lange, R., Meslin, M., Uhl, M., Kolossa-Gehring, M., Rousselle, C., Vicente, J.L., 2021. Chemical prioritisation strategy in the European human biomonitoring initiative (HBM4EU) – development and results. Int. J. Hyg Environ. Health 236, 113778. https://doi.org/10.1016/j.ijheh.2021.113778.
Pariente, G., Leibson, T., Carls, A., Adams-Webber, T., Ito, S., Koren, G., 2016. Pregnancy-associated changes in pharmacokinetics: a systematic review. PLoS Med. 13, e1002160 https://doi.org/10.1371/journal.pmed.1002160.
Polanska, K., Hanke, W., Dettbarn, G., Sobala, W., Gromadzinska, J., Magnus, P., Seidel, A., 2014. The determination of polycyclic aromatic hydrocarbons in the urine of non-smoking Polish pregnant women. Sci. Total Environ. 487, 102–109. https://doi.org/10.1016/j.scitotenv.2014.04.006.
Pollock, T., Karthikeyan, S., Walker, M., Werry, K., St-Amand, A., 2021. Trends in environmental chemical concentrations in the Canadian population: biomonitoring data from the Canadian Health Measures Survey 2007–2017. Environ. Int. 155, 106678. https://doi.org/10.1016/j.envint.2021.106678.
Preuss, R., Koch, H.M., Wilhelm, M., Pischetsrieder, M., Angerer, J., 2004. Pilot study on the naphthalene exposure of German adults and children by means of urinary 1- and 2-naphthol levels. Int. J. Hyg Environ. Health 207, 441–445. https://doi.org/10.1078/1438-4639-00313.
Ramesh, A., Walker, S.A., Hood, D.B., Guillén, M.D., Schneider, K., Weyand, E.H., 2004. Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. Int. J. Toxicol. 23, 301–333. https://doi.org/10.1080/10915810490517063.
Ramsauer, B., Sterz, K., Hagedorn, H.W., Engl, J., Scherer, G., McEwan, M., Errington, G., Shepperd, J., Cheung, F., 2011. A liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination of phenolic polycyclic aromatic hydrocarbons (OH-PAH) in urine of non-smokers and smokers. Anal. Bioanal. Chem. 399, 877–889. https://doi.org/10.1007/s00216-010-4355-7.
Rose, M., Holland, J., Dowding, A., Petch, S.R.G., White, S., Fernandes, A., Mortimer, D., 2015. Investigation into the formation of PAHs in foods prepared in the home to determine the effects of frying, grilling, barbecuing, toasting and roasting. Food Chem. Toxicol. 78, 1–9. https://doi.org/10.1016/j.fct.2014.12.018.
Rosofsky, A., Janulewicz, P., Thayer, K.A., McClean, M., Wise, L.A., Calafat, A.M., Mikkelsen, E.M., Taylor, K.W., Hatch, E.E., 2017. Exposure to multiple chemicals in a cohort of reproductive-aged Danish women. Environ. Res. 154, 73–85. https://doi.org/10.1016/j.envres.2016.12.011.
Runkel, A.A., Snoj-Tratnik, J., Mazej, D., Horvat, M., 2020. Urinary phthalate concentrations in the Slovenian population: an attempt to exposure assessment of family units. Environ. Res. 186, 109548. https://doi.org/10.1016/j.envres.2020.109548.
Schoeters, G., Colles, A., Den Hond, E., Croes, K., Vrijens, J., Baeyens, W., Nelen, V., Van De Mieroop, E., Covaci, A., Bruckers, L., Van Larebeke, N., Sioen, I., Morrens, B., Loots, I., 2012. The flemish environment and health study (FLEHS) - Second survey (2007–2011): Establishing reference values for biomarkers of exposure in the flemish population. Biomarkers and Human Biomonitoring, vol. 1. The Royal Society of Chemistry, pp. 135–165.
Schoeters, G., Govarts, E., Bruckers, L., Den Hond, E., Nelen, V., De Henauw, S., Sioen, I., Nawrot, T.S., Plusquin, M., Vriens, A., Covaci, A., Loots, I., Morrens, B., Coertjens, D., Van Larebeke, N., De Craemer, S., Croes, K., Lambrechts, N., Colles, A., Baeyens, W., 2017. Three cycles of human biomonitoring in flanders – time trends observed in the flemish environment and health study. Int. J. Hyg Environ. Health 220, 36–45. https://doi.org/10.1016/j.ijheh.2016.11.006.
Schulz, C., Wilhelm, M., Heudorf, U., Kolossa-Gehring, M., 2011. Update of the reference and HBM values derived by the German human biomonitoring commission. Int. J. Hyg Environ. Health 215, 26–35. https://doi.org/10.1016/j.ijheh.2011.06.007.
Slezakova, K., Pires, J.C.M., Castro, D., Alvim-Ferraz, M.C.M., Delerue-Matos, C., Morais, S., Pereira, M.C., 2013. PAH air pollution at a Portuguese urban area: carcinogenic risks and sources identification. Environ. Sci. Pollut. Res. 20, 3932–3945. https://doi.org/10.1007/s11356-012-1300-7.
Smolders, R., Koppen, G., Schoeters, G., 2008. Translating biomonitoring data into risk management and policy implementation options for a European Network on Human Biomonitoring. Environ. Heal. Glob. Access Sci. Source 7, S2. https://doi.org/10.1186/1476-069X-7-S1-S2.
Snoj Tratnik, J., Falnoga, I., Mazej, D., Kocman, D., Fajon, V., Jagodic, M., Stajnko, A., Trdin, A., Šlejkovec, Z., Jeran, Z., Osredkar, J., Sešek-Briški, A., Krsnik, M., Kobal, A. B., Kononenko, L., Horvat, M., 2019. Results of the first national human biomonitoring in Slovenia: trace elements in men and lactating women, predictors of exposure and reference values. Int. J. Hyg Environ. Health 222, 563–582. https://doi.org/10.1016/j.ijheh.2019.02.008.
Sochacka-Tatara, E., Majewska, R., Perera, F.P., Camann, D., Spengler, J., Wheelock, K., Sowa, A., Jacek, R., Mróz, E., Pac, A., 2018. Urinary polycyclic aromatic hydrocarbon metabolites among 3-year-old children from Krakow, Poland. Environ. Res. 164, 212–220. https://doi.org/10.1016/j.envres.2018.02.032.
StHelen, G., Goniewicz, M.L., Dempsey, D., Wilson, M., Jacob, P., Benowitz, N.L., 2012. Exposure and kinetics of polycyclic aromatic hydrocarbons (PAHs) in cigarette smokers. Chem. Res. Toxicol. 25, 952–964. https://doi.org/10.1021/tx300043k.
Stajnko, A., Falnoga, I., Tratnik, J.S., Mazej, D., Jagodic, M., Krsnik, M., Kobal, A.B., Prezelj, M., Kononenko, L., Horvat, M., 2017. Low cadmium exposure in males and lactating females – estimation of biomarkers. Environ. Res. 152, 109–119. https://doi.org/10.1016/j.envres.2016.09.025.
Stallings-Smith, S., Mease, A., Johnson, T.M., Arikawa, A.Y., 2018. Exploring the association between polycyclic aromatic hydrocarbons and diabetes among adults in the United States. Environ. Res. 166, 588–594. https://doi.org/10.1016/j.envres.2018.06.041.
Strickland, P., Kang, D., Sithisarankul, P., 1996. Polycyclic aromatic hydrocarbon metabolites in urine as biomarkers of exposure and effect. Environ. Health Perspect. 104, 927–932. https://doi.org/10.1289/ehp.96104s5927.
Sun, K., Song, Y., He, F., Jing, M., Tang, J., Liu, R., 2021. A review of human and animals exposure to polycyclic aromatic hydrocarbons: health risk and adverse effects, photo-induced toxicity and regulating effect of microplastics. Sci. Total Environ. 773, 145403. https://doi.org/10.1016/j.scitotenv.2021.145403.
Suwazono, Y., Åkesson, A., Alfvén, T., Järup, L., Vahter, M., 2005. Creatinine versus specific gravity-adjusted urinary cadmium concentrations. Biomarkers 10, 117–126. https://doi.org/10.1080/13547500500159001.
Thai, P.K., Heffernan, A.L., Toms, L.M.L., Li, Z., Calafat, A.M., Hobson, P., Broomhall, S., Mueller, J.F., 2016. Monitoring exposure to polycyclic aromatic hydrocarbons in an Australian population using pooled urine samples. Environ. Int. 88, 30–35. https://doi.org/10.1016/j.envint.2015.11.019.
Tissari, J., Hytönen, K., Lyyränen, J., Jokiniemi, J., 2007. A novel field measurement method for determining fine particle and gas emissions from residential wood combustion. Atmos. Environ. 41, 8330–8344. https://doi.org/10.1016/j.atmosenv.2007.06.018.
Urbancova, K., Dvorakova, D., Gramblicka, T., Sram, R.J., Hajslova, J., Pulkrabova, J., 2020. Comparison of polycyclic aromatic hydrocarbon metabolite concentrations in urine of mothers and their newborns. Sci. Total Environ. 723, 138116. https://doi.org/10.1016/j.scitotenv.2020.138116.
US EPA, 1993. Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons, vol. 600. US Environmental Protection Agency, Research Triangle Park, pp. 1–20. NC. EPA-600/R-93/089. Off. Res. Dev.
Verheyen, V.J., Remy, S., Govarts, E., Colles, A., Rodriguez Martin, L., Koppen, G., Voorspoels, S., Bruckers, L., Bijnens, E.M., Vos, S., Morrens, B., Coertjens, D., De Decker, A., Franken, C., Den Hond, E., Nelen, V., Covaci, A., Loots, I., De Henauw, S., Van Larebeke, N., Teughels, C., Nawrot, T.S., Schoeters, G., 2021. Urinary polycyclic aromatic hydrocarbon metabolites are associated with biomarkers of chronic endocrine stress, oxidative stress, and inflammation in adolescents: FLEHS-4 (2016–2020). Toxics 9, 245. https://doi.org/10.3390/toxics9100245.
Vorkamp, K., Castaño, A., Antignac, J.P., Boada, L.D., Cequier, E., Covaci, A., Esteban López, M., Haug, L.S., Kasper-Sonnenberg, M., Koch, H.M., Pérez Luzardo, O., Osīte, A., Rambaud, L., Pinorini, M.T., Sabbioni, G., Thomsen, C., 2021. Biomarkers, matrices and analytical methods targeting human exposure to chemicals selected for a European human biomonitoring initiative. Environ. Int. 146, 106082. https://doi.org/10.1016/j.envint.2020.106082.
Wheeler, A.J., Dobbin, N.A., Héroux, M.E., Fisher, M., Sun, L., Khoury, C.F., Hauser, R., Walker, M., Ramsay, T., Bienvenu, J.F., LeBlanc, A., Daigle, É., Gaudreau, E., Belanger, P., Feeley, M., Ayotte, P., Arbuckle, T.E., 2014. Urinary and breast milk biomarkers to assess exposure to naphthalene in pregnant women: an investigation of personal and indoor air sources. Environ. Heal. Glob. Access Sci. Source 13, 30. https://doi.org/10.1186/1476-069X-13-30.
Wilhelm, M., Hardt, J., Schulz, C., Angerer, J., 2008. New reference value and the background exposure for the PAH metabolites 1-hydroxypyrene and 1- and 2-naphthol in urine of the general population in Germany: basis for validation of human biomonitoring data in environmental medicine. Int. J. Hyg Environ. Health 211, 447–453. https://doi.org/10.1016/j.ijheh.2007.09.002.
Zhang, X., Yang, L., Zhang, H., Xing, W., Wang, Y., Bai, P., Zhang, L., Hayakawa, K., Toriba, A., Wei, Y., Tang, N., 2021. Assessing approaches of human inhalation exposure to polycyclic aromatic hydrocarbons: a review. Int. J. Environ. Res. Publ. Health 18, 1–14. https://doi.org/10.3390/ijerph18063124.
World Health Organization (WHO), United Nations Environment Programme, Inter-Organization Programme for the Sound Management of Chemicals, 2013. State of the science of endocrine disrupting chemicals 2012 : summary for decision-makers. Bergman, Åke, Heindel, Jerrold J. et al.. World Health Organization, Geneva. https://apps.who.int/iris/handle/10665/78102.
Zhang, Y., Ding, J., Shen, G., Zhong, J., Wang, C., Wei, S., Chen, C., Chen, Y., Lu, Y., Shen, H., Li, W., Huang, Y., Chen, H., Su, S., Lin, N., Wang, X., Liu, W., Tao, S., 2014. Dietary and inhalation exposure to polycyclic aromatic hydrocarbons and urinary excretion of monohydroxy metabolites – a controlled case study in Beijing, China. Environ. Pollut. 184, 515–522. https://doi.org/10.1016/j.envpol.2013.10.005.

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Prevalence of opportunistic pathogens in a school building plumbing during periods of low water use and a transition to normal use

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ABSTRACT

The spread of opportunistic pathogens via building water supply and plumbing is of public health concern. This study was conducted to better understand microbial water quality changes in a LEED-certified school building during low water use (Summer) and normal water use (Autumn). The copper plumbed building contained water saving devices, a hot water recirculation system, and received chloraminated drinking water from a public water system. Three separate sampling events were conducted during the summer break inside the building and another three sampling events were conducted after the school returned to session. Using quantitative PCR, *Legionella* spp. were detected in all water samples, followed by *Mycobacterium* spp. (99%). *Mycobacterium avium* (75%) and *Acanthamoeba* spp. (17.5%) throughout the building water system. *Legionella pneumophila* and *Naegleria fowleri* were not detected in any of the samples. The mean concentrations of *Legionella* spp., *Mycobacterium* spp., *Mycobacterium avium*, and *Acanthamoeba* spp. detected in water samples were 3.9, 5.7, 4.7, and 2.8 $\log_{10}$ gene copies per 100 ml, respectively. There was a statistically significantly difference in the mean concentrations of *Legionella* spp., *Mycobacterium* spp. and *M. avium* gene markers in water samples between school breaks and when school was in session. Cultivable *Legionella* were also detected in water samples collected during periods of low water use. This study highlights the need for routine proactive water quality testing in school buildings to determine the extent of drinking water quality problems associated with plumbing and direct action to remediate microbial colonization.

1. Introduction

Most youth spend a considerable amount of time in schools and thus, access to safe drinking water for various activities in this setting is crucial. A majority of schools in the United States (U.S.) receive water supply from public water systems which are subject to federal and state regulations to ensure safety. Public water systems monitor for contaminants and treat water by filtration and disinfection in order to meet U.S. Environmental Protection Agency (EPA) drinking water standards. Public water systems have sole responsibility for the regulated safety of drinking water up to the consumer property line (Patel et al., 2020). After that point, it has fallen on consumers to maintain water safety. However, active monitoring of microbial water quality after entering a building is not mandatory under current regulations except for healthcare facilities. Therefore, sampling and monitoring of opportunistic pathogens including strains of *Legionella*, *Mycobacterium*, *Pseudomonas* and *Acanthamoeba* in building plumbing are not routine despite the significant water quality alterations that can occur, particularly within large buildings.

Current public health measures primarily focus on the control of enteric pathogens such as pathogenic *Escherichia coli* (*E. coli*), *Salmonella enterica*, *Campylobacter jejuni*, *Cryptosporidium* and *Giardia*, and enteric viruses in drinking water. Unlike traditional fecal contamination, opportunistic pathogens are found naturally in freshwater environments, but major reservoirs are man-made water systems particularly building plumbing and hot water systems. Therefore, their presence in water environments is not necessarily correlated with the presence of indicator microorganisms used in current water quality monitoring

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(Whiley et al., 2014). Opportunistic pathogens, particularly *Legionella*, are now considered to cause a higher health burden than enteric pathogens in the U.S. and other developed countries due to drinking water exposures (Benedict et al., 2017). Over the last decade, the leading cause of disease outbreaks due to potable water has shifted significantly from gastrointestinal pathogens to *Legionella pneumophila* (Beer et al., 2015). For example, analysis of the U.S. Centers for Disease Control and Prevention (CDC) waterborne outbreak data from 2013 to 2014 showed that 57% of the drinking water associated outbreaks were caused by *Legionella*. Although less frequently, other opportunistic pathogens such as non-tuberculous mycobacteria, *Pseudomonas aeruginosa* and free-living amoeba (*Naegleria fowleri*) have been reported to be associated with drinking water nosocomial diseases or outbreaks (Bédard et al., 2015; Costa et al., 2015; Kline et al., 2004; LaBombardi et al., 2002; Yoder et al., 2012).

There are reports of colonization of opportunistic pathogens in warm water systems in buildings such as healthcare facilities, nursing homes and large apartment buildings (Barna et al., 2016; Borella et al., 2005; Edagawa et al., 2008; Kruse et al., 2016). In these plumbing systems, opportunistic pathogen proliferation is often influenced by many factors, such as aged plumbing, low water flow rate or stagnation, lack of disinfectant residual, in-building water storage tanks, physicochemical constituents of the water and poor management (Inkinen et al., 2014). In particular, the increasing occurrence of low-flows in building plumbing presents an emerging public health concern because opportunistic pathogens can easily proliferate under low-flow conditions. In the U.S., reduced water usage and higher residence times are occurring because (i) low-flow water fixtures are being installed in order to comply with plumbing codes initiated by the Energy Policy Act of 1992, and (ii) water conservation practices are being promoted, for example, by the U.S. Green Building Council (USGBC). Green buildings without a proper water management, can exacerbate the problem of opportunistic pathogens in drinking water by increasing water residence times, decreasing disinfectant residuals and lowering hot water temperatures in building plumbing (Ley et al., 2020; Rhoads et al., 2016). However, information remains limited about the water quality and the prevalence of opportunistic pathogens inside green buildings, particularly when the degree of water quality may seasonally and spatially differ across building fixtures.

In communities where water that is entering school buildings meets municipal water quality standards, building plumbing or other building-specific drinking water infrastructure and quality assurance practices may affect water quality (Cradock et al., 2019). Although legionellosis is considered a rare cause of community-acquired pneumonia in children, an analysis of all cases of legionellosis reported to the CDC from 1990 through 2005 noted a trend toward younger ages, highlighting the importance of considering *Legionella* species as important pathogens in all age groups (Neil and Berkelman, 2008). However, there is a paucity of studies addressing the presence of opportunistic pathogens in school or academic building plumbing to ascertain trends or possible hotspots of contamination. Testing drinking water systems for the presence of opportunistic pathogens is a proactive approach to assess and reduce the risk of exposure to these organisms. Therefore, the objectives of this study were to (1) profile the incidence and prevalence of opportunistic pathogens in drinking water in a green school building and their changes with water use frequency, and (2) evaluate the relationships of critical water quality parameters and the detection of opportunistic pathogens in the taps. The results of this investigation permit a preliminary assessment of the possible exposure to opportunistic pathogens in school via the potable water supply.

2. Materials and methods

2.1. Description of the testing site and plumbing characteristics

In this study, we focused on a single-story school building in Indiana, U.S. that was LEED certified by the USGBC. Additional information about the building can be found in a previous publication (Ra et al., 2020). Drinking water was provided to the school campus from a public water system through a single water meter. The public water system used water from two different sources depending on the overall water demand (75% from a wellfield and 25% from a river). Chloramines were used as the disinfectant. After passing through the water meter, the chloraminated water entered a campus loop piping system with 20.3 cm diameter, which circled the building. Once in the loop, the water either entered the school building or irrigation system and athletic field buildings located on the campus. Water meter records published in April 2018 by the utility in the same area as the school is located were obtained. No water use records were available specifically for the school building where the sampling was conducted.

In the school building, water passed through a water softener before entering into one of four water heaters. Hot water exiting each water heater entered one of four hot water recirculation systems or flowed directly into cold water piping. Copper pipes were used in the building. The distance from the point-of-entry to the furthest water outlet was longer than 152 m for both cold and hot taps. Water samples were collected from both cold and hot water sampling locations throughout the building.

2.2. School campus water use during the study period and water sampling

A timeline of key events at the school is shown in Fig. 1. Three separate sampling events were conducted during the summer break inside the building and another three sampling events were conducted after the school returned to session. During summer break, some water use occurred in the north part of the building, e.g., four summer camps which involved 50 to 250 students and church services every Sunday. In contrast, the south part of the building (classroom side) was mostly unused during the summer break. When school returned to session, 830 students and staff began inhabiting the school building five days a week and thus increasing water use of the building.

Water sampling began between 7:00 and 7:15 a.m. Drinking water samples were first collected from a tap where the water enters the building (influent) and then 19 locations of hot and cold water tap in the building (Table 1). All samples were immediately collected; no flushing was conducted. Field and trip blanks were also included and analyzed for each sampling event. Two 1-liter water samples were collected from each site.

2.3. Water quality analysis

2.3.1. Physicochemical analysis

Water physicochemical parameters were measured at the time of collection. Water temperature, pH and dissolved oxygen (DO) was measured using an Orion Star A329 portable pH meter (Thermo Scientific). Total chlorine, free chlorine, monochloramine and free ammonia were analyzed onsite using HACH® 131 pocket colorimeter (DPD method). Method detection limit (MDL) for total chlorine was 0.05 mg/L, free ammonia was 0.02 mg/L, and monochloramine was 0.04 mg/L. Total organic carbon (TOC) and dissolved organic carbon (DOC) was measured using a Shimadzu TOC-L CPH/CPN in accordance with USEPA method 415.1. Metal concentrations were quantified by inductively coupled plasma -optical emission spectrometry (iCAP 7400 Duo ICP-OES, Thermo Scientific) and an autosampler (ASX-280, CETAC Teledyne).

2.3.2. Microbiological analysis

2.3.2.1. Heterotrophic plate counts (HPC). HPC was measured using a membrane filtration technique (Method 9215B) as outlined in Standard Methods for the Examination of Water and Wastewater (American

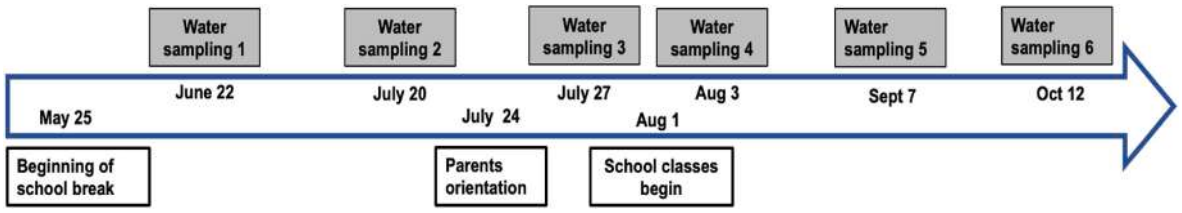

Fig. 1. Timeline of major school and water sampling events.

Table 1
Water sampling locations in the school building including influent, water systems in the utility room, several hot- and cold-water taps.

Sampling location	Site ID
Utility Room	
Building entry point sampling tap (Influent)	BE
After water softener sampling tap	AS
Before water heater sampling tap	BWH
Hot water recirculation loop-a (120 °F temperature)	HWRa
Hot water recirculation loop-b (140 °F temperature)	HWRb
After water heater sampling tap	AWH
Most used building portion during summer break	
Bathroom outside utility room - cold sink faucet	B1C
Bathroom outside utility room - hot sink faucet	B1H
Shower head ADA compliant	SH1
Shower head – combined all stands	SH2
Farthest bathroom - cold sink faucet	B2C
Farthest bathroom - hot sink faucet	B2H
Student's kitchen - cold sink faucet	SKC
Student's kitchen - hot sink faucet	SKH
Least used building portion during summer break	
Teacher's kitchen - cold sink faucet	TKC
Teacher's kitchen - hot sink faucet	TKH
Men's bathroom - cold sink faucet	B3C
Men's bathroom - hot sink faucet	B3H
Drinking water fountain lower	WF1
Drinking water fountain higher ADA compliant	WF2

Public Health Association, American Water Works Association and Water Environment Federation). Briefly, water samples were filtered through sterile, hydrophilic mixed cellulose ester membranes (GN-6 Metricel; Pall Corporation) and the filtered bacteria was grown on Difco mHPC media (Becton, Dickinson and Company). Samples were incubated for 48 ± 2 h at 35 ± 2 °C, and the colonies were enumerated visually.

2.3.2.2. Legionella cultivation and DNA sequencing. *Legionella* cultivation assay was conducted for water samples collected on July 20 (during school summer break) and October 12 (during school in session). Samples of 500 ml of water were concentrated by membrane filtration using a 0.45 μm pore size polycarbonate filters (Millipore, Burlington, MA). Bacteria were washed from the filter using 10 ml of sterile Page's saline. *Legionella* spp. were enumerated by standard culture technique according to ISO 11731-2 (2004). Briefly, sample concentrates (100 μl) were plated onto buffered charcoal yeast extract (BCYE) agar supplemented with glycine, vancomycin, polymyxin and cycloheximide (GVPC) after heat treatment (50 °C for 30 min). Plates were incubated at (36 ± 1) °C for up to 10 days. Presumptive *Legionella* isolates were verified by a genus-specific PCR and DNA sequencing (Wullings and van der Kooij, 2006). Genus-specific primers LEG-225 (5′-AAGATTAGCCTGCGTCCGAT-3′) and LEG-858 (5′-GTCAACTTATCGCGTTTGCT-3′) were used. The PCR products were cloned using pGEM-T Easy Vector system (Promega, USA), and transformed into *E. coli* JM109 high efficiency competent cells. The plasmid DNA was purified using Wizard Plus SV Minipreps DNA Purification System (Promega, Madison, WI), and the resulting DNA insert was sequenced in both the forward and reverse directions using primers T7 and SP6. Samples were sequenced using an Applied Biosystem 3730*xl* DNA Analyzer at the Research Technology Support Facility, Michigan State University.

2.3.2.3. Detection of opportunistic pathogens by quantitative PCR (qPCR). Approximately 1 L of water sample was filtered through a 0.45 μm pore size polycarbonate filters (Millipore, Burlington, MA). DNA was extracted directly from filters using a protocol as described in EPA Methods 1611. Briefly, the filter was transferred to a 2 ml semi-conical screw cap micro-centrifuge tube containing 0.3 g of acid-washed, 212–300 mm glass beads (Sigma-Aldrich, #G-1277) and 600 mL AE buffer (Qiagen, Valencia, CA, USA) added. The tubes were sealed, bead milled at 5000 reciprocations/min for 60 s and centrifuged at 12,000×*g* for 1 min to pellet silica beads and debris. The supernatants were transferred to clean, low retention micro-centrifuge tubes and centrifuged for an additional 5 min. The resulting clarified supernatants were transferred to another clean, low retention micro-centrifuge tubes and stored at −80 °C until qPCR analysis.

Legionella spp., *Legionella pneumophila*, *Mycobacterium* spp., *Mycobacterium avium*, *Acanthamoeba* spp. and *Naegleria fowleri* were enumerated by qPCR using previously published methods (Bruijnesteijn van Coppenraet et al., 2004; Chern et al., 2014; Mull et al., 2013; Nazarian et al., 2008; Qvarnstrom et al., 2006). All qPCR primers and probes used in this study and qPCR parameters are listed in Table S1. A duplex qPCR assay was used for *Legionella* detection. Amplification reaction mixtures (total final volume of 25 μl) contained 5 μl template DNA, 5 μl of 5 × Perfecta Multiplex qPCR ToughMix (QuantaBio), 500 nM of each primer and 200 nM of probe. The thermal cycling protocol was as follows: 15 min at 95 °C for initial denaturation, followed by 45 cycles of two steps consisting of 15 s at 95 °C and 60 s at 60 °C. For all other qPCR assays, amplification reaction mixtures (total final volume of 25 μl) contained 5 μl template DNA, 12.5 μl of 2 × Perfecta qPCR ToughMix (QuantaBio), 400 nM of each primer and 200 nM of probe. The thermal cycling protocol was as follows: 15 min at 95 °C for initial denaturation, followed by 45 cycles of three steps consisting of 30 s at 95 °C, 40 s at 55 °C, and 30 s at 72 °C.

All qPCR reactions were performed using a StepOne Plus™ real-time PCR sequence detector (Applied Biosystems, Foster City, CA). For each assay, a 10-fold diluted standard curve of at least six points, a non-template control, and samples were tested in triplicate. Genomic DNA of *Legionella pneumophila* strain Philadelphia-1 (ATCC 33152D-5) and *Mycobacterium avium* (ATCC 25291), respectively was used to generate standard curves. For *Acanthamoeba* and *Naegleria fowleri* qPCR, standard curves were established with plasmid DNA generated using Acanthamoeba polyphaga (ATCC 30461) and *Naegleria fowleri* (ATCC 30863), respectively.

2.4. Statistical analysis

All pathogen gene copy (GC) concentration datasets were determined to be non-normally distributed.

Samples which were below the detection limit of the qPCR assay were negatively censored to half of the detection limit (24.6 gene copies/100 mL). *Acantamoeba* abundances were excluded from statistical analysis due to its infrequent detection. Kruskal-Wallis tests were used to compare opportunistic pathogen abundances between sampling time and water line temperature groups. Spearman's rank correlation was used to determine the associations between pathogen abundances and physiochemical parameters. Best fit trend lines were generated

using simple linear regression. Predictive modeling was done using multiple linear regression including all parameters measured in this study. The model producing the highest adjusted R squared was selected as the chosen model. Multiple linear regression was done in SAS 9.4. All other statistical tests were done in Prism 9 (GraphPad).

3. Results

3.1. Physicochemical water quality variation in school plumbing

The water quality characteristics of the building plumbing are summarized in Table 2. The mean temperature of water entering building (influent) varied from 26.1 ^{O}C (Summer) to 24.2 ^{O}C (Fall). The water temperature in the building ranged from 14.5 to 30.2 ^{O}C and 19.7 to 47.3 ^{O}C for the cold-water lines and hot-water lines, respectively. The pH of water entering the building was consistent across sampling events (7.6–7.9). However, the water pH levels were often higher in the building, ranging from 7.2 to 8.5 and 7.5 to 8.4 in the cold- and hot-water samples, respectively.

Disinfectant, HPC, and copper levels entering and throughout the building varied. The total chlorine concentration of water samples entering the building varied from non-detectable (ND) (Indiana state law: ND < 0.2 mg/L as Cl_2) to 0.43 mg/L as Cl_2. Total chlorine levels above 0.2 mg/L as Cl_2 was only detected in 2 of 6 influent samples: June (0.20 mg/L as Cl_2) and August (0.43 mg/L as Cl_2). After entering the building, chlorine was only detectable for 12% of the total fixture samples during the study. Monochloramine levels in influent were slightly greater than total chlorine. In the building, monochloramine was detectable for 73.1% of the total fixture samples. Average HPC measurements were consistently higher in the building plumbing as compared to the influent. Copper levels in the building plumbing were elevated, averaging 1167.9 μg/L in cold-water lines and 690.8 μg/L in hot-water lines. Additional information about the chemical water quality of the building can be found in a previous publication (Ra et al., 2020).

Table 2
Physicochemical properties of water collected from the school.

Parameter	During summer break			After summer break		
	Influent (n = 3)	Cold water line (n = 27)	Hot water line (n = 27)	Influent (n = 3)	Cold water line (n = 27)	Hot water line (n = 27)
Temperature, ^{O}C						
Mean ± SD	26.1 ± 1.1	21.9 ± 3.2	29.3 ± 7.4	24.2 ± 3.4	21.8 ± 3.9	29.8 ± 8.6
Range	25.2–27.3	15.8–26.4	21.5–47.3	20.4–27.1	14.5–30.2	19.7–46.3
pH						
Mean ± SD	7.8 ± 0.1	7.8 ± 0.3	8.0 ± 0.2	7.8 ± 0.1	7.9 ± 0.2	8.0 ± 0.2
Range	7.6–7.9	7.2–8.5	7.5–8.4	7.7–7.9	7.7–8.2	7.7–8.2
DO, mg/L						
Mean ± SD	9.02 ± 0.1	6.94 ± 2.1	5.64 ± 1.42	8.40 ± 0.87	7.43 ± 1.2	6.58 ± 1.42
Range	8.94–9.13	2.58–10.15	3.12–8.86	7.44–9.15	4.42–9.21	3.2–8.98
Total Cl_2, mg/L						
Mean ± SD	0.17 ± 0.03	0.11 ± 0.27	0.10 ± 0.21	0.20 ± 0.22	0.02 ± 0.06	0.02 ± 0.03
Range	0.14–0.2	ND-1.4	ND-1.0	ND-0.43	ND-0.3	ND-0.13
NH_2Cl, mg/L						
Mean ± SD	0.22 ± 0.11	0.08 ± 0.13	0.04 ± 0.03	0.47 ± 0.44	0.07 ± 0.09	0.09 ± 0.14
Range	0.14–0.34	ND-0.49	ND-0.1	0.07–0.94	ND-0.41	ND-0.7
Free NH_3,mg/L						
Mean ± SD	0.2 ± 0.25	0.11 ± 0.12	0.17 ± 0.15	0.06 ± 0.07	0.08 ± 0.07	0.06 ± 0.04
Range	ND-0.48	ND-0.41	ND-0.84	ND-0.13	ND-0.21	ND-0.16
TOC, mg/L						
Mean ± SD	1.87 ± 0.12	2.3 ± 1.56	3.44 ± 0.22	2.01 ± 0.19	2.32 ± 1.3	2.32 ± 0.36
Range	1.74–1.97	1.46–6.71	2.9–3.79	1.84–2.22	1.49–6.8	1.6–3.43
DOC, mg/L						
Mean ± SD	1.85 ± 0.11	2.1 ± 1.46	3.33 ± 0.22	1.97 ± 0.15	1.87 ± 0.2	2.23 ± 0.31
Range	1.74–1.96	1.4–6.52	2.58–3.61	1.8–2.08	1.45–2.18	1.72–3.27
HPC, Log_{10} CFU/100 ml						
Mean	2.17 ± 2.34	4.1 ± 4.63	3.11 ± 3.46	2.06 ± 2.07	3.87 ± 4.39	5.86 ± 6.57
Range	1.04–2.6	1.12–5.33	ND-4.1	1.26–2.39	ND-5.07	ND-7.29
Copper, μg/L						
Mean ± SD	414.7 ± 80.0	1356.0 ± 665.5	688.8 ± 272.8	67.7 ± 12.3	979.8 ± 651.5	692.9 ± 343.5
Range	347.0–503.0	55.3–2440.0	196.0–1320.0	56.9–81.1	ND-2290.0	ND-1320.0
Iron, μg/L						
Mean ± SD	16.1 ± 19.6	3.0 ± 4.2	19.9 ± 6.9	2.3 ± 4.0	1.4 ± 1.9	4.5 ± 6.7
Range	ND-38.0	ND-16.6	ND-26.2	ND-6.9	ND-5.7	ND-19.9
Aluminum, μg/L						
Mean ± SD		11.0 ± 32.7	8.4 ± 8.0		5.1 ± 7.4	3.9 ± 4.3
Range	ND	ND-131.0	ND-18.7	ND-18.3	ND-19.7	ND-13.4
Manganese, μg/L						
Mean ± SD		0.9 ± 1.5	0.4 ± 0.5	0.7 ± 0.6	0.6 ± 0.6	0.4 ± 0.5
Range	ND-1.8	ND-5.2	ND-1.6	ND-1.1	ND-1.9	ND-1.1
Nickel, μg/L						
Mean ± SD	1.7 ± 1.6	8.9 ± 10.8	7.1 ± 19.1	1.2 ± 1.1	5.2 ± 7.1	2.0 ± 2.2
Range	ND-3.2	ND-55.9	ND-93.0	ND-2.0	ND-20.8	ND-8.3
Zinc, μg/L						
Mean ± SD	26.2 ± 4.3	224.8 ± 262.1	144.5 ± 251.5	74.3 ± 89.4	160.0 ± 282.9	65.3 ± 108.7
Range	21.3–28.8	3.8–827.0	3.5–1020.0	18.2–177.5	ND-1180.0	ND-580.0
Lead, μg/L						
Mean ± SD		2.2 ± 8.5				
Range	ND	ND-40.9	ND	ND	ND-35.1	ND

DO: Dissolved oxygen; Cl_2: Chlorine; NH_2Cl: Monochloramine; NH_3: Free ammonia; TOC: Total organic carbon; DOC: Dissolved organic carbon; HPC: Heterotrophic Plate Count; ND: non-detect.

3.2. Enumeration of Legionella by cultivation and DNA sequencing

First flush water samples collected on July 20 (during summer break) and Oct 12 (after school began) were subjected to *Legionella* cultivation assay. Colonies of *Legionella* spp. were enumerated from 5 of 20 (5%) sampling sites during the school summer break (Table 3). Presumptive *Legionella* colonies ranged from 130 to nearly 6000 CFU/100 ml. Higher numbers of colonies were found in water exiting the water softener. All samples collected when school was in session were negative for *Legionella* by cultivation assay. Cloning and sequencing of the PCR products resulted in 28 16S rRNA gene sequences from five samples. All 16S rRNA gene sequences exhibited 99.8–100% similarity to *L. feeleii* and *L. donaldsonii* sequences deposited in the GenBank database (Table 3).

3.3. Molecular survey of opportunistic pathogens in school building plumbing

The frequencies of detection and concentrations of *Legionella*, mycobacteria and two species of amoeba using qPCR are presented in Table 4. *Legionella* spp. were detected using qPCR in all water samples (n = 120), whereas *Legionella pneumophila* was not detected in any water samples collected during the study. *Mycobacterium* spp. were detected using qPCR in 119 of 120 water samples. The average concentrations of *Mycobacterium* spp. detected in water samples were approximately 2.0 logs higher than the average concentrations of *Legionella* spp. *Mycobacterium avium* was detected in 75% of water samples collected mainly in the building plumbing, with detected concentrations ranged from 2.2 to 6.3 $\log_{10}$ GC/100 mL. *Naegleria fowleri* was not detected in any of the samples collected during the study. However, *Acanthamoeba* spp. was detected in 21 of 120 water samples with detected concentrations ranged from 1.7 to 5.8 $\log_{10}$ GC/100 mL.

Correlations between HPC and the detection of opportunistic pathogens by qPCR were explored. No significant correlation was found between HPC and *Legionella* spp. ($p = 0.4957$), *Mycobacterium* spp. ($p = 0.6045$), or *Mycobacterium avium* ($p = 0.6153$) molecular detection.

Table 4
Overall opportunistic pathogens detected by qPCR for 20 sampling sites.

Target organism	Occurrence rate (%)		Concentration Log $_{10}$ (Gene copies per 100 ml)	
	Sampling sites (n = 20)	Water samples (n = 120)	Range	Average for positive samples
Legionella spp.	100	100	1.7–5.2	3.9
Legionella pneumophila	0	0	N/A	N/A
Mycobacterium spp.	100	99.2	<1.4–7.3	5.7
Mycobacterium avium	95	75	<1.4–6.3	4.7
Naegleria fowleri	0	0	N/A	N/A
Acanthamoeba spp.	70	17.5	<1.4–5.8	2.8

3.4. Concentrations of opportunistic pathogen gene markers showed spatial and temporal variation

During the school summer break sampling events, the mean concentrations of *Legionella* spp., *Mycobacterium* spp. and *M. avium* gene markers in water samples were 4.2, 6.1 and 4.2 $\log_{10}$ GC/100 mL, respectively. After school began, the mean concentrations of *Legionella* spp., *Mycobacterium* spp. and *M. avium* gene markers in water samples decreased to 3.7, 5.2 and 3.5 $\log_{10}$ GC/100 mL, respectively. These differences were statistically significant with the Kruskal-Wallis test for *Legionella* spp. ($p < 0.0001$), *Mycobacterium* spp. ($p < 0.0001$), and *M. avium* ($p = 0.0073$) (Fig. S1).

Fig. 2 compares the mean concentrations of opportunistic pathogen gene markers in water samples collected from different locations in the building. In influent samples, the concentrations of *Legionella* spp. and *Mycobacterium* spp. ranged from 2.5 to 3.9 $\log_{10}$ GC/100 mL and 4.6 to 5.9 $\log_{10}$ GC/100 mL, respectively. When comparing the influent concentrations to the taps during the same sampling event, 84% and 83% of water samples collected at the taps in the building had higher

Table 3
Legionella concentrations in potable water as determined with cell culture method and *Legionella* identification by 16S rRNA sequence analysis.

Sampling date/Site ID	20-Jul			12-Oct		
	Cultivable *Legionella* ($\log_{10}$ CFU per 100 ml)	Best match % to species (GenBank accession numbers)	qPCR ($\log_{10}$ GC per 100 ml)	Cultivable *Legionella* spp. (Log10 CFU per 100 ml)	Best match % to species (GenBank accession numbers)	qPCR (Log10 GC per 100 ml)
BE	nd	-	3.9	nd	-	3.6
AS	3.8	99.8–100% to *L. feeleii* (MN865866, EF474027) and *L. donaldsonii* (Z49724, KM504126)	3.9	nd	-	3.4
BWH	3.7		4.4	nd	-	3.9
HWRa	3.0		4.4	nd	-	3.7
HWRb	nd	-	4.5	nd	-	3.6
AWH	nd	-	4.4	nd	-	3.7
B1C	nd	-	2.6	nd	-	3.7
B1H	3.0	99.8–100% to *L. feeleii* (MN865866, EF474027) and *L. donaldsonii* (Z49724, KM504126)	4.4	nd	-	3.8
SH1	nd	-	3.9	nd	-	2.9
SH2	2.1	99.8–100% to *L. feeleii* (MN865866, EF474027) and *L. donaldsonii* (Z49724, KM504126)	4.4	nd	-	3.3
B2C	nd	-	5.2	nd	-	4.3
B2H	nd	-	4.5	nd	-	4.3
SKC	nd	-	4.5	nd	-	2.7
SKH	nd	-	4.4	nd	-	3.6
TKC	nd	-	3.8	nd	-	2.5
TKH	nd	-	4.4	nd	-	3.7
B3C	nd	-	4.7	nd	-	3.9
B3H	nd	-	4.6	nd	-	4.1
WF1	nd	-	4.5	nd	-	2.9
WF2	nd	-	4.5	nd	-	3.6

nd: non-detect.

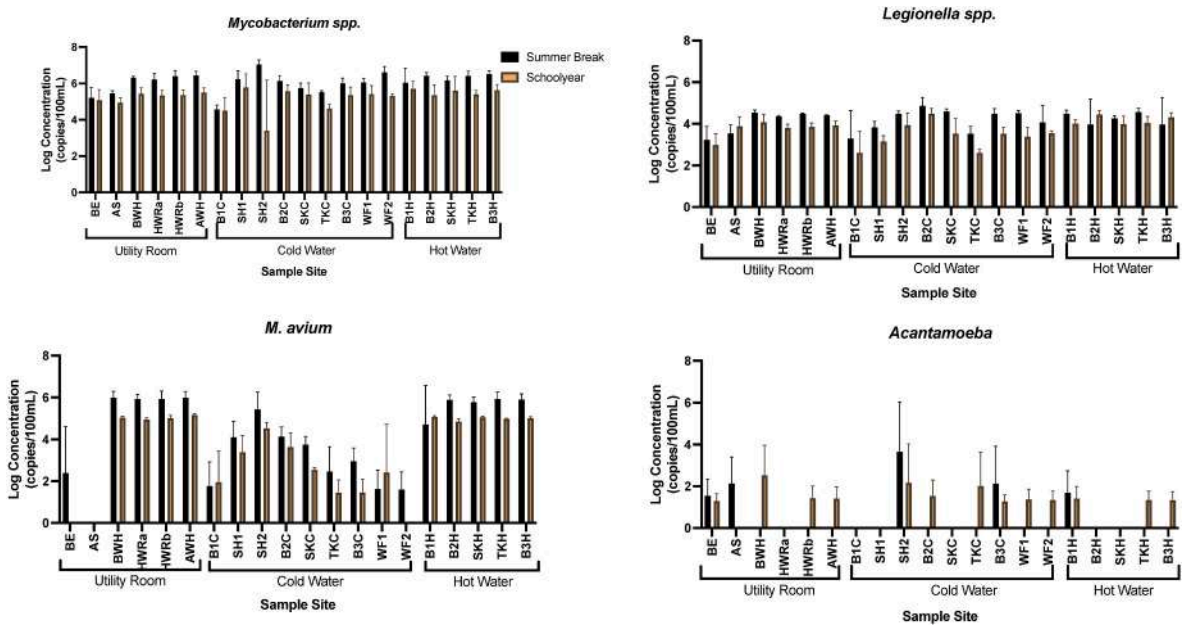

Fig. 2. Spatial and temporal distributions of molecular markers of *Legionella* spp., *Mycobacterium* spp., *Mycobacterium avium* and *Acanthamoeba* spp. in building plumbing. Bars reflect all measurements collected at each tap during the study.

concentrations of *Legionella* spp. and *Mycobacterium* spp., respectively. *M. avium* was detected in only one influent sample during summer sampling event at gene copies of 4.9 $\log_{10}$ GC/100 mL. The detection rates and concentrations of *M. avium* increased substantially in the building plumbing. The occurrence of *Acanthamoeba* spp. in building plumbing was sparse with no clear spatial and temporal patterns.

Fig. 3 compares the $\log_{10}$ gene copies of *Legionella* spp., *Mycobacterium* spp. and *M. avium* in hot- and cold-water lines. Overall, opportunistic pathogens were detected with high abundance in hot-water lines. During summer break, *Legionella* spp. gene copies were not significantly different between hot- and cold-water lines ($p = 0.3606$). After school began, *Legionella* spp. gene copies were significantly higher in hot-water lines ($p = 0.0302$). In contrast, *Mycobacterium* spp. gene copies were significantly higher in hot-water lines than those in cold-water lines during summer break ($p = 0.0299$). After school began, there was no significant difference in *Mycobacterium* spp. concentrations between hot- and cold-water lines ($p > 0.9999$). *M. avium* concentrations were significantly higher in hot-water lines during summer break ($p < 0.0001$) and after school began ($p < 0.0001$). *M. avium* was detected in all hot-water line samples and 60% of cold-water line samples.

3.5. Association of opportunistic pathogens and abiotic water factors

Correlation analysis was performed to determine if there were positive or negative associations between the detection of opportunistic pathogens and measured water quality characteristics within each sampling site (Table 5A). *Legionella* spp. gene copy numbers were significantly correlated with pH and negatively correlated with dissolved oxygen. Negative correlations were found between *Mycobacterium* spp. and dissolved oxygen, and monochloramine. Gene copy numbers of *Mycobacterium* spp. were positively correlated with total organic carbon. Gene copy numbers of *M. avium* were significantly positively correlated with pH, free ammonia, total organic carbon, and dissolved organic carbon, but were negatively correlated with dissolved oxygen.

Metal concentrations in water were also tested for correlations with gene copy numbers of the three opportunistic pathogen groups (Table 5B). Positive correlations were noted between iron and gene copy

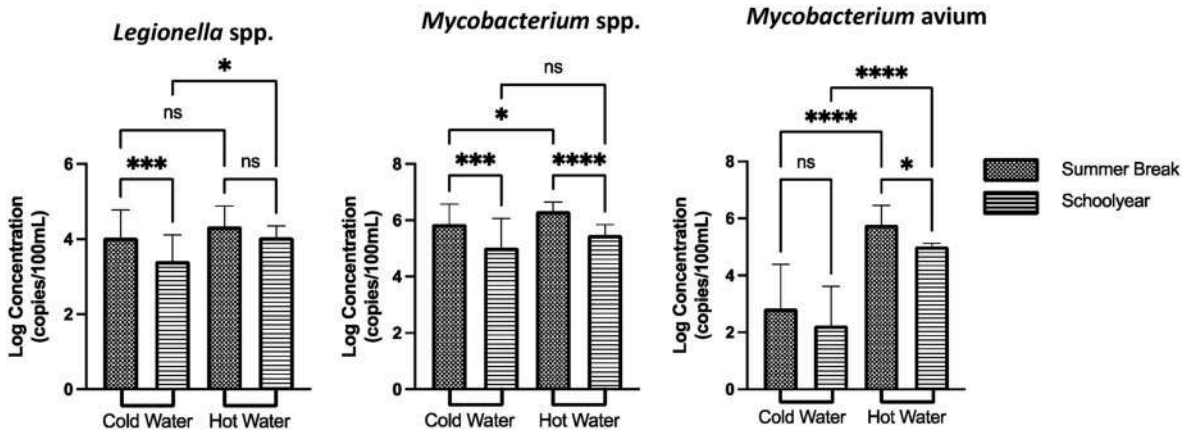

Fig. 3. Average gene copy numbers of *Legionella* spp., *Mycobacterium* spp., and *M. avium* in hot- and cold-water samples from taps in the building and Kruskal-Wallis test outcomes. Error bars denote standard deviation. Statistical significance is indicated for alpha = 0.05, alpha = 0.01, alpha = 0.001, and alpha < 0.0001 by one, two, three, and four asterisks respectively. Non-significant statistical comparisons are denoted with "ns".

Table 5(A)
Spearman correlation analysis for physicochemical and microbial properties.

rho p-value	*Legionella spp.*	*Myco spp.*	*M. avium*	Temp.	pH	DO	Total Cl_2	NH_2Cl	NH4	TOC	DOC
Legionella spp.	1 -										
Myco spp.	0.461 <0.0001	1 -									
M. avium	0.434 <0.0001	0.533 <0.0001	1 -								
Temp.	0.108 0.2525	-0.119 0.2081	0.392 <0.0001	1 -							
pH	0.212 0.0233	0.015 0.8775	0.273 0.0033	0.366 0.0001	1 -						
DO	-0.278 0.0028	-0.303 0.0010	-0.426 <0.0001	-0.156 0.0974	0.158 0.0941	1 -					
Total Cl_2	-0.127 0.1768	0.039 0.6793	-0.016 0.8639	0.207 0.0274	0.097 0.3059	0.045 0.6379	1 -				
NH_2Cl	-0.133 0.1591	-0.202 0.0313	-0.123 0.1915	-0.003 0.9789	0.231 0.0134	0.272 0.0034	0.163 0.0828	1 -			
Free NH_3	-0.008 0.9337	0.083 0.3831	0.271 0.0035	0.114 0.2279	0.056 0.5574	-0.241 0.0097	0.274 0.0032	0.179 0.0566	1 -		
TOC	0.063 0.5082	0.217 0.0207	0.555 <0.0001	0.461 <0.0001	0.4 <0.0001	-0.115 0.2249	0.102 0.2804	-0.056 0.5524	0.210 0.0250	1 -	
DOC	0.130 0.1693	0.182 0.0523	0.600 <0.0001	0.544 <0.0001	0.455 <0.0001	-0.133 0.1579	0.130 0.1673	-0.033 0.7308	0.152 0.1075	0.886 <0.0001	1 -

	+	0.80 – 1.0	Very Strong		-	-0.80 - -1.0	Very Strong
	+	0.60-0.79	Strong		-	-0.6 - -0.79	Strong
	+	0.40 – 0.59	Moderate		-	-0.40 - -0.59	Moderate
	+	0.20 – 0.39	Weak		-	-0.20 - -0.39	Weak
	+	0.00 – 0.19	Very Weak		-	0.00 - -0.19	Very Weak

Myco: *Mycobacterium*; DO: Dissolved oxygen; Cl_2: Chlorine; NH_2Cl: Monochloramine; NH_3: Free ammonia; TOC: Total organic carbon; DOC: Dissolved organic carbon

numbers of *Legionella* spp., *Mycobacterium* spp. and *M. avium*. Gene copy numbers of *Legionella* spp. were also positively correlated with nickel and zinc in water. Negative correlations were found between manganese and gene copy numbers of *M. avium*.

3.6. Predictive modeling of Legionella and Mycobacterium in school plumbing

A one-way ANOVA was run for the gene copy concentrations for *Legionella* spp., *Mycobacterium* spp., and *M. avium*. The mean copy concentrations were significantly different for all three groups. A multiple linear regression was run for each pathogen to determine if any important predictors of pathogen abundance might be missing from the analyses. All physiochemical parameters and gene copy concentrations for *Legionella* spp., *Mycobacterium* spp., and *M. avium* measured in this study were included as possible predictors in the model assessment. The model of best fit based on the adjusted R square was chosen. Chosen models are shown in Table 6. The best fitting model for detected abundances of *Legionella* spp. in this dataset included time of sampling (summer break or schoolyear), pH, and total organic carbon. The model predicting *Legionella* abundances had the lowest adjusted R^2 of the analyzed pathogens, suggesting important predictors may be missing. *Mycobacterium* spp. were included in the final model for prediction of *M. avium*, suggesting *Mycobacterium* spp. may be good indicators of *M. avium* under these conditions. Models of *Mycobacterium* spp. and *M. avium* yielded higher adjusted R^2 values indicating a better representation of important predictors of their abundance. For higher resolution predictive models of opportunistic pathogens in school drinking water systems, a larger sample size would be needed.

4. Discussion

While previous research has studied the prevalence of opportunistic pathogens, particularly *Legionella* in hot-water networks, the spatial and temporal distribution of these pathogens in building plumbing is still not well understood. There have been very few studies conducted in the U.S. that examined the prevalence of opportunistic pathogens at potable water taps, other than at healthcare facilities. This study provides new quantitative information about the distribution of opportunistic pathogens of public health concern and water quality insights for a 7-year-old green school building, where prior water quality testing had not been conducted. The high frequencies of detection of *Legionella* spp. and *Mycobacterium* spp. in first-draw water samples using molecular techniques indicate their persistence and regrowth in building plumbing systems. This suggests that even when the municipal water distribution system is highly chloraminated, conditions in plumbing may still permit the persistence and proliferation of bacteria harbored in pipe biofilm.

In this study, high densities of cultivable *Legionella* spp. were found in water samples collected in July during summer break, particularly in water existing the softener with over 3.8 Log_{10} CFU per 100 ml.

Table 5(B)
Spearman correlation analysis for metal concentrations and microbial properties.

rho p-value	*Legionella* spp.	*Myco* spp.	*M. avium*
Legionella spp.	1 -		
Myco spp.	0.461 <0.0001	1 -	
M. avium	0.434 <0.0001	0.533 <0.0001	1 -
Copper	0.175 0.0628	0.115 0.2226	-0.104 0.2712
Iron	0.265 0.0044	0.207 0.0272	0.468 <0.0001
Aluminum	-0.008 0.9324	0.054 0.5767	0.152 0.1064
Manganese	-0.086 0.3627	-0.171 0.0685	-0.186 0.0479
Nickel	0.214 0.0223	0.111 0.2412	-0.073 0.4396
Zinc	0.256 0.006	0.152 0.1077	-0.009 0.9252
	+	0.80-1.0	Very Strong
	+	0.60-0.79	Strong
	+	0.40-0.59	Moderate
	+	0.20-0.39	Weak
	+	0.00-0.19	Very Weak
	-	0.00- -0.19	Very Weak
	-	-0.20 - -0.39	Weak
	-	-0.40 - -0.59	Moderate
	-	-0.60 - -0.79	Strong
	-	-0.80 - -1.0	Very Strong

Table 6
Models of best fit for three opportunistic pathogens in school water sources.

Opportunistic Pathogen	Variables in Model of Best Fit	Adjusted R Square
Legionella	Time of sampling, pH, total organic carbon	0.2213
Mycobacterium spp.	Time of sampling, water temperature, pH, monochloramine	0.5100
Mycobacterium avium	Water temperature, dissolved oxygen, total chlorine, total organic carbon, *Mycobacterium* spp.	0.5305

Although *Legionella* spp. appear ubiquitous in potable water, their cultivable concentrations in treated water are generally low. For example, in a study of two chloraminated water systems in Virginia, U.S., cultivable *Legionella* spp. were detected in only one of 56 water samples at 2.3 Log_{10} CFU per 100 ml (Wang et al., 2012). In a recent study of a 40-year-old building, cultivable *Legionella* was not detected in plumbing that supplied monochloramine-treated water, whereas cultivable *Legionella* was detected in various chlorine-treated water systems with densities ranging from 1.2 to 4.5 Log_{10} CFU per 100 ml (Buse et al., 2020). The 16S rRNA gene sequences of isolates closely related to both *L. feeleii* and *L. donaldsonii* were identified in all water samples positive for *Legionella* by cultivation. Previous phylogenetic analysis of *Legionella* based on 16S rRNA gene sequences showed that *L. feeleii* and *L. donaldsonii* are clustered although they are relatively distant from other species (Hookey et al., 1996). Further whole-genome sequencing of environmental isolates may allow us to better distinguish *Legionella* species. The L. *donaldsonii* 16S rRNA gene sequences detected in this study showed greatest similarity to gene sequences of clinical isolate Glasgow 86/35785 (Hookey et al., 1996) and *L. donaldsonii* isolated from patients with cancer in Houston, U.S (Han et al., 2015). Previous studies reported the detection of *L donaldsonii* in water treatment plants in Taiwan (Huang et al., 2009) and domestic hot and cold water in Spain (Salinas et al., 2021). *L. feeleii* is an important human pathogen that caused an outbreak of Pontiac fever in 1981 in an automobile plant in Canada (Herwaldt et al., 1984). However, only a few studies reported the detection of *L. feeleii* in water samples (Buse et al., 2020; Nazarian et al., 2008; Vaccaro et al., 2021).

Little is known about the effects of water softeners on the microbial quality of potable water. Early studies have found that microbial growth can occur in water after ion exchange resins treatment (Flemming, 1987; Stamm et al., 1969). In contrast, a study using modern and compact domestic water softeners shows no evidence of the growth of coliform and *Pseudomonas* in water after treatment (Parsons, 2000). In this study, HPC levels increased by 2.8 logs and 1.5 logs in water after existing the softener during summer break and after school returned to session, respectively. Additionally, cultivable *Legionella* spp. was detected in the same site but not in the influent during a sampling event in summer. However, no significant impacts on gene copy number variations were observed. Although water softeners are mainly used for non-potable purposes such as dishwashers and water heaters, opportunistic pathogens can establish and proliferate as part of the native drinking water microbiota. These pathogens have transmission routes related to non-potable use such as inhalation through showering. In addition, many water softeners are attached to the entire water supply to a building, affecting all water systems. In a most recent study (Richard et al., 2021), showed that a whole-building water softener could have a detrimental effect on water quality including increasing water pH and trihalomethanes and decreasing disinfectant residual.

While most studies of opportunistic pathogens focus on hot water systems, in this study, 100%, 98.3%, 60% and 18.3% of the cold-water samples were positive for *Legionella* spp., *Mycobacterium* spp., *M. avium* and *Acanthamoeba* spp., respectively as measured by qPCR. A multistate survey of cold-water taps in the U.S. also indicated a high prevalence of *L. pneumophila* Serogroup 1 (Donohue et al., 2014) and nontuberculous mycobacteria (NTMs) including *M. avium*, *M. mucogenicum* and *M. gordonae* in potable cold water (Donohue et al., 2015). The colonization of these opportunistic pathogens in cold-water taps implies the presence of risk factors that favor their growth not just in hot-water systems, but also in cold-water systems.

The densities of *Legionella* and *Mycobacterium* had strong temporal variations in water systems of the building with higher concentrations during the summer break. This could be due to relatively low water usage when the building was not fully occupied by students and staff during summer months. The prolonged hydraulic retention time in plumbing due to reduced water usage could promote stagnation and the loss of disinfectant residual, therefore favoring the growth of pathogens. This is consistent with a study of water quality in a residential building over the course of one year which showed that reduced water usage increased water stagnation times, and, in turn, increased *Legionella* spp. and *Mycobacterium* spp. colonization in building plumbing (Ley et al., 2020). A recent literature review based on 22 studies also highlights a positive association between water stagnation and increased

colonization of *Legionella* in potable water systems (Nisar et al., 2020).

In this study, the densities of *Mycobacterium* spp. were generally higher than those of *Legionella* spp. in first-draw water samples, indicating their possible release from pipe biofilms. In a study of microbiome characterization of showerhead biofilms, *Mycobacterium* spp. were found to be the dominant members associated with biofilms (Feazel et al., 2009). The results of this study also showed that chloramine was less effective for controlling the growth of *Mycobacterium* spp. and reducing their occurrence at the tap although chloramine is a more promising community-level disinfectant to prevent colonization of *Legionella* in buildings (Lytle et al., 2021; Moore et al., 2006). Interestingly, *M. avium* was detected in 75% of water samples. This is in contrast to other studies that reported low detection rate of *M. avium* in drinking water samples (Covert et al., 1999; Donohue et al., 2015). *M. avium* is currently on the U.S. EPA candidate contaminant list, a list of drinking water contaminants that are known or anticipated to occur in water systems and are not currently regulated. The high prevalence of *M. avium* in potable water could pose potential health risk to children. It has been recognized that *M. avium* is the most common of the NTM that cause cervical lymphadenitis (an acute symptomatic enlargement of the cervical lymph nodes) among healthy children (Haverkamp et al., 2004; Lai et al., 1984; Thegerström et al., 2008; Wolinsky, 1995). Potable water could be a potential source of *M. avium* infection due to their high resistance to both chemical disinfectants and ultra violet (UV) irradiation (Whiley et al., 2012).

In this study, *Acanthamoeba* spp. were detected in water samples using qPCR although the detection rate was lower than the detection rate for other opportunistic pathogens. *Acanthamoeba* spp. are free-living amoebae commonly found in natural water environments and engineered water systems (Marciano-Cabral et al., 2010; Stockman et al., 2011). *Acanthamoeba* spp. can cause severe infections of the eye, skin and central nervous system, more common in immunocompromised individuals (Marciano-Cabral and Cabral, 2003). However, *Acanthamoeba* keratitis is an eye infection that typically occurs in healthy individuals particularly contact lens users and can result in permanent visual impairment. *Acanthamoeba* spp. have been shown to serve as hosts for pathogenic bacteria such as *Legionella* spp. and *Mycobacterium* spp. in drinking water systems (Thomas et al., 2006, 2008).

For selected samples, both culture and qPCR assays were concurrently used for the enumeration of *Legionella*. There were discrepancies between culture and qPCR enumeration of *Legionella* spp. in this study (25% of samples were positive using culturing method whereas 100% were positive using qPCR). This is in agreement with previous studies, which detected *Legionella* in water samples at a higher rate using qPCR compared to culture assay (Wullings and van der Kooij, 2006). Culture assay is currently considered to be the standard method for the detection and identification of *Legionella* and other opportunistic pathogens. However, the fastidious nature of the bacteria and the lengthy incubation periods (e.g., up to 10 days for *Legionella* and up to months for *Mycobacterium*) necessary for growth make routine environmental monitoring challenging. Compared to culturing methods, molecular techniques such as qPCR have the advantage of high specificity and sensitivity, high throughput, short turnaround time which allow for routine monitoring and rapid response, and the ability to detect viable but not cultivable cells. Although qPCR allows a good reliability of the quantification, this method can lead to an overestimation of bacteria because it allows for the detection of dead cells. The lack of correlations between culture and qPCR assays highlights the need for standardizing testing protocol for *Legionella* and other opportunistic pathogens in environmental samples that is sufficient for risk assessment and management.

One of the objectives of this study was to assess the correlations between opportunistic pathogen concentrations and physicochemical water parameters in the full-scale building plumbing. Interestingly, variations in gene copy numbers of opportunistic pathogen, particularly *Legionella* spp. did not correlate well to the physicochemical water parameters. This suggests that the measured physicochemical parameters of water had little impact on the densities of *Legionella* and *Mycobacterium* in this study. Moreover, the low variation observed for most physiochemical parameters in this study could explain the insignificant fluctuation of bacterial densities in relation with physicochemical properties changes. The association between *Mycobacterium* and TOC suggests that limiting essential nutrients such as organic carbon in water distribution systems could control pathogen regrowth in the plumbing. Future studies should rely on more frequent and prolonged sampling to more accurately assess the relationships between densities of opportunistic pathogens and physicochemical water parameters in the full-scale plumbing.

5. Conclusions

Collectively, this study highlights how microbial drinking water quality varies seasonally and spatially throughout low flow plumbing in a school building and the possible unintended consequences associated with reduced water usage during school breaks. The results of this study can help inform building water quality sampling and plumbing design. Due to the lack of water quality monitoring data, the safety of school drinking water remains largely unknown. Proactive water testing at existing school buildings would determine the extent of water quality problems associated with plumbing and could direct action to remediate those problems.

While not all microbial growth is harmful, it is undesirable to introduce any uncontrolled microbial colonization in plumbing systems past the property line. Routine monitoring for water quality including potential opportunistic pathogens in school buildings should be performed as part of an effort to reduce risk of microbial proliferation and children's exposure due to water systems. It is suggested that water quality testing to be conducted in school buildings after a prolonged period of low water use during school breaks and periodically when school in session. Currently, there is no consensus on the methods for environmental monitoring of opportunistic pathogens in plumbing, particularly parallel detection of multiple pathogens. Moreover, the complexity of plumbing hinders the development of universal sampling plans that are representative of the actual colonization of opportunistic pathogens and risk of exposure (Wang et al., 2017). Unified monitoring approaches are urgently needed for informing proactive risk prevention and outbreak response.

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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.2022.113945.

References

Barna, Z., Kádár, M., Kálmán, E., Scheirich Szax, A., Vargha, M., 2016. Prevalence of Legionella in premise plumbing in Hungary. Water Res. 90, 71–78.

Bédard, E., Laferrière, C., Charron, D., Lalancette, C., Renaud, C., Desmarais, N., Déziel, E., Prévost, M., 2015. Post-outbreak investigation of Pseudomonas aeruginosa faucet contamination by quantitative polymerase chain reaction and environmental factors affecting positivity. Infect. Control Hosp. Epidemiol. 36, 1337–1343.

Beer, K.D., Gargano, J.W., Roberts, V.A., Hill, V.R., Garrison, L.E., Kutty, P.K., Hilborn, E. D., Wade, T.J., Fullerton, K.E., Yoder, J.S., 2015. J.M.M.a.m.w.r.. Surveillance for

Waterborne Disease Outbreaks Associated with Drinking Water—United States, vol. 64, p. 842, 2011–2012.
Benedict, K.M., Reses, H., Vigar, M., Roth, D.M., Roberts, V.A., Mattioli, M., Cooley, L.A., Hilborn, E.D., Wade, T.J., Fullerton, K.E., Yoder, J.S., Hill, V.R., 2017. Surveillance for waterborne disease outbreaks associated with drinking water - United States, 2013-2014. MMWR Morb. Mortal. Wkly. Rep. 66, 1216–1221.
Borella, P., Montagna, M.T., Stampi, S., Stancanelli, G., Romano-Spica, V., Triassi, M., Marchesi, I., Bargellini, A., Tato, D., Napoli, C., 2005. Legionella contamination in hot water of Italian hotels. Appl. Environ. Microbiol. 71, 5805–5813.
Bruijnesteijn van Coppenraet, E.S., Lindeboom, J.A., Prins, J.M., Peeters, M.F., Claas, E. C.J., Kuijper, E.J., 2004. Real-time PCR assay using fine-needle aspirates and tissue biopsy specimens for rapid diagnosis of mycobacterial lymphadenitis in children. J. Clin. Microbiol. 42, 2644–2650.
Buse, H.Y., Morris, B.J., Gomez-Alvarez, V., Szabo, J.G., Hall, J.S., 2020. Legionella diversity and spatiotemporal variation in the occurrence of opportunistic pathogens within a large building water system. Pathogens 9, 567.
Chern, E.C., King, D., Haugland, R., Pfaller, S., 2014. Evaluation of quantitative polymerase chain reaction assays targeting Mycobacterium avium, M. intracellulare, and M. avium subspecies paratuberculosis in drinking water biofilms. J. Water Health 13, 131–139.
Costa, D., Bousseau, A., Thevenot, S., Dufour, X., Laland, C., Burucoa, C., Castel, O., 2015. Nosocomial outbreak of Pseudomonas aeruginosa associated with a drinking water fountain. J. Hosp. Infect. 91, 271–274.
Covert, T.C., Rodgers, M.R., Reyes, A.L., Stelma Jr., G.N., 1999. Occurrence of nontuberculous mycobacteria in environmental samples. Appl. Environ. Microbiol. 65, 2492–2496.
Cradock, A.L., Jones, S.E., Merlo, C., 2019. Examining differences in the implementation of school water-quality practices and water-access policies by school demographic characteristics. Prev. Med. Rep. 14, 100823.
Donohue, M.J., Mistry, J.H., Donohue, J.M., O'Connell, K., King, D., Byran, J., Covert, T., Pfaller, S., 2015. Increased frequency of nontuberculous mycobacteria detection at potable water taps within the United States. Environ. Sci. Technol. 49, 6127–6133.
Donohue, M.J., O'Connell, K., Vesper, S.J., Mistry, J.H., King, D., Kostich, M., Pfaller, S., 2014. Widespread molecular detection of Legionella pneumophila serogroup 1 in cold water taps across the United States. Environ. Sci. Technol. 48, 3145–3152.
Edagawa, A., Kimura, A., Doi, H., Tanaka, H., Tomioka, K., Sakabe, K., Nakajima, C., Suzuki, Y., 2008. Detection of culturable and nonculturable Legionella species from hot water systems of public buildings in Japan. J. Appl. Microbiol. 105, 2104–2114.
Feazel, L.M., Baumgartner, L.K., Peterson, K.L., Frank, D.N., Harris, J.K., Pace, N.R., 2009. Opportunistic pathogens enriched in showerhead biofilms. Proc. Natl. Acad. Sci. Unit. States Am. 106, 16393–16399.
Flemming, H.-C., 1987. Microbial growth on ion exchangers. Water Res. 21, 745–756.
Han, X.Y., Ihegword, A., Evans, S.E., Zhang, J., Li, L., Cao, H., Tarrand, J.J., El-Kweifi, O., 2015. Microbiological and clinical studies of legionellosis in 33 patients with cancer. J. Clin. Microbiol. 53, 2180–2187.
Haverkamp, M.H., Arend, S.M., Lindeboom, J.A., Hartwig, N.G., van Dissel, J.T., 2004. Nontuberculous mycobacterial infection in children: a 2-year prospective surveillance study in The Netherlands. Clin. Infect. Dis. 39, 450–456.
Herwaldt, L.A., Gorman, G.W., McGrath, T., Toma, S., Brake, B., Hightower, A.W., Jones, J., Reingold, A.L., Boxer, P.A., Tang, P.W., 1984. A new Legionella species, Legionella feeleii species nova, causes Pontiac fever in an automobile plant. Ann. Intern. Med. 100, 333–338.
Hookey, J., Saunders, N., Fry, N., Birtles, R., Harrison, T., 1996. Phylogeny of Legionellaceae based on small-subunit ribosomal DNA sequences and proposal of Legionella lytica comb. nov. for Legionella-like amoebal pathogens. Int. J. Syst. Evol. Microbiol. 46, 526–531.
Huang, S., Hsu, B., Ma, P., Chien, K., 2009. Legionella prevalence in wastewater treatment plants of Taiwan. Water Sci. Technol. 60, 1303–1310.
Inkinen, J., Kaunisto, T., Pursiainen, A., Miettinen, I.T., Kusnetsov, J., Riihinen, K., Keinänen- Toivola, M.M., 2014. Drinking water quality and formation of biofilms in an office building during its first year of operation, a full scale study. Water Res. 49, 83–91.
Kline, S., Cameron, S., Streifel, A., Yakrus, M.A., Kairis, F., Peacock, K., Besser, J., Cooksey, R.C., 2004. An outbreak of bacteremias associated with Mycobacterium mucogenicum in a hospital water supply. Infect. Control Hosp. Epidemiol. 25, 1042–1049.
Kruse, E.-B., Wehner, A., Wisplinghoff, H., 2016. Prevalence and distribution of Legionella spp in potable water systems in Germany, risk factors associated with contamination, and effectiveness of thermal disinfection. Am. J. Infect. Control 44, 470–474.
LaBombardi, V.J., O'brien, A.M., Kislak, J.W., 2002. Pseudo-outbreak of Mycobacterium fortuitum due to contaminated ice machines. Am. J. Infect. Control 30, 184–186.
Lai, K.K., Stottmeier, K.D., Sherman, I.H., McCabe, W.R., 1984. Mycobacterial cervical lymphadenopathy: relation of etiologic agents to age. JAMA 251, 1286–1288.
Ley, C.J., Proctor, C.R., Singh, G., Ra, K., Noh, Y., Odimayomi, T., Salehi, M., Julien, R., Mitchell, J., Nejadhashemi, A.P., 2020. Drinking water microbiology in a water-efficient building: stagnation, seasonality, and physicochemical effects on opportunistic pathogen and total bacteria proliferation. Environ. Sci.: Water Res. Technol. 6, 2902–2913.
Lytle, D.A., Pfaller, S., Muhlen, C., Struewing, I., Triantafyllidou, S., White, C., Hayes, S., King, D., Lu, J., 2021. A comprehensive evaluation of monochloramine disinfection on water quality, Legionella and other important microorganisms in a hospital. Water Res. 189, 116656.
Marciano-Cabral, F., Cabral, G., 2003. Acanthamoeba spp. as agents of disease in humans. Clin. Microbiol. Rev. 16, 273–307.
Marciano-Cabral, F., Jamerson, M., Kaneshiro, E.S., 2010. Free-living amoebae, Legionella and Mycobacterium in tap water supplied by a municipal drinking water utility in the USA. J. Water Health 8, 71–82.
Moore, M.R., Pryor, M., Fields, B., Lucas, C., Phelan, M., Besser, R.E., 2006. Introduction of monochloramine into a municipal water system: impact on colonization of buildings by Legionella spp. Appl. Environ. Microbiol. 72, 378–383.
Mull, B.J., Narayanan, J., Hill, V.R., 2013. Improved method for the detection and quantification of Naegleria fowleri in water and sediment using immunomagnetic separation and real-time PCR. J. Parasitol. Res. 2013, 608368. Article ID 608367.
Nazarian, E.J., Bopp, D.J., Saylors, A., Limberger, R.J., Musser, K.A., 2008. Design and implementation of a protocol for the detection of Legionella in clinical and environmental samples. Diagn. Microbiol. Infect. Dis. 62, 125–132.
Neil, K., Berkelman, R., 2008. Increasing incidence of legionellosis in the United States, 1990– 2005: changing epidemiologic trends. Clin. Infect. Dis. 47, 591–599.
Nisar, M.A., Ross, K.E., Brown, M.H., Bentham, R., Whiley, H., 2020. Water stagnation and flow obstruction reduces the quality of potable water and increases the risk of legionelloses. Front. Environ. Sci. 8, 611611.
Parsons, S.A., 2000. The effect of domestic ion-exchange water softeners on the microbiological quality of drinking water. Water Res. 34, 2369–2375.
Patel, A.I., Hecht, C.E., Cradock, A., Edwards, M.A., Ritchie, L.D., 2020. Drinking water in the United States: implications of water safety, access, and consumption. Annu. Rev. Nutr. 40, 345–373.
Qvarnstrom, Y., Visvesvara, G.S., Sriram, R., da Silva, A.J., 2006. Multiplex real-time PCR assay for simultaneous detection of Acanthamoeba spp., Balamuthia mandrillaris, and Naegleria fowleri. J. Clin. Microbiol. 44, 3589–3595.
Ra, K., Odimayomi, T., Ley, C., Aw, T.G., Rose, J.B., Whelton, A.J., 2020. Finding building water quality challenges in a 7 year old green school: implications for building design, sampling, and remediation. Environ. Sci.: Water Res. Technol. 6, 2691–2703.
Rhoads, W.J., Pruden, A., Edwards, M.A., 2016. Survey of green building water systems reveals elevated water age and water quality concerns. Environ. Sci.: Water Res. Technol. 2, 164–173.
Richard, R., Hamilton, K.A., Westerhoff, P., Boyer, T.H., 2021. Physical, chemical, and microbiological water quality variation between city and building and within multistory building. ACS ES&T Water 1, 1369–1379.
Salinas, M.B., Fenoy, S., Magnet, A., Vaccaro, L., Gomes, T.D., Hurtado, C., Ollero, D., Valdivieso, E., Del Águila, C., Pozuelo, M.J., 2021. Are pathogenic Legionella non-pneumophila a common bacteria in Water Distribution Networks? Water Res. 196, 117013.
Stamm, J.M., Engelhard, W.E., Parsons, J.E., 1969. Microbiological study of water-softener resins. Appl. Microbiol. 18, 376–386.
Stockman, L.J., Wright, C.J., Visvesvara, G.S., Fields, B.S., Beach, M.J., 2011. Prevalence of Acanthamoeba spp. and other free-living amoebae in household water, Ohio, USA—1990–1992. Parasitol. Res. 108, 621–627.
Thegerström, J., Romanus, V., Friman, V., Brudin, L., Haemig, P.D., Olsen, B., 2008. Mycobacterium avium lymphadenopathy among children, Sweden. Emerg. Infect. Dis. 14, 661.
Thomas, V., Herrera-Rimann, K., Blanc, D.S., Greub, G., 2006. Biodiversity of amoebae and amoeba-resisting bacteria in a hospital water network. Appl. Environ. Microbiol. 72, 2428–2438.
Thomas, V., Loret, J.F., Jousset, M., Greub, G., 2008. Biodiversity of amoebae and amoebae- resisting bacteria in a drinking water treatment plant. Environ. Microbiol. 10, 2728–2745.
Vaccaro, L., Gomes, T.S., Izquierdo, F., Magnet, A., Llorens Berzosa, S., Ollero, D., Salso, S., Alhambra, A., Gómez, C., López Cano, M., 2021. Legionella feeleii: ubiquitous pathogen in the environment and causative agent of pneumonia. Front. Microbiol. 2074.
Wang, H., Bedard, E., Prevost, M., Camper, A.K., Hill, V.R., Pruden, A., 2017. Methodological approaches for monitoring opportunistic pathogens in premise plumbing: a review. Water Res. 117, 68–86.
Wang, H., Edwards, M.A., Falkinham, J.O., Pruden, A., 2012. Molecular survey of occurrence of Legionella spp., Mycobacterium spp., Pseudomonas aeruginosa and amoeba hosts in two chloraminated drinking water distribution systems. Appl. Environ. Microbiol. 78, 6285–6294.
Whiley, H., Keegan, A., Fallowfield, H., Bentham, R., 2014. Detection of Legionella, L. pneumophila and Mycobacterium avium complex (MAC) along potable water distribution pipelines. Int. J. Environ. Res. Publ. Health 11, 7393–7405.
Whiley, H., Keegan, A., Giglio, S., Bentham, R., 2012. Mycobacterium avium complex–the role of potable water in disease transmission. J. Appl. Microbiol. 113, 223–232.
Wolinsky, E., 1995. Mycobacterial lymphadenitis in children: a prospective study of 105 nontuberculous cases with long-term follow-up. Clin. Infect. Dis. 20, 954–963.
Wullings, B.A., van der Kooij, D., 2006. Occurrence and genetic diversity of uncultured Legionella spp. in drinking water treated at temperatures below 15 C. Appl. Environ. Microbiol. 72, 157–166.
Yoder, J.S., Straif-Bourgeois, S., Roy, S.L., Moore, T.A., Visvesvara, G.S., Ratard, R.C., Hill, V.R., Wilson, J.D., Linscott, A.J., Crager, R., 2012. Primary amebic meningoencephalitis deaths associated with sinus irrigation using contaminated tap water. Clin. Infect. Dis. 55, e79–e85.

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The modifying effect of trait anxiety on the association of fine particulate matter with heart rate variability variables

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ABSTRACT

Background: Previous studies have shown that exposures to ambient particulate matter with aerodynamic diameter <2.5 μm ($PM_{2.5}$) and stress are associated with adverse cardiovascular health effects.
Objectives: To investigate the potential modifying effect of trait anxiety on the association between short-term exposures to $PM_{2.5}$ and HRV variables.
Methods: A panel of 92 middle-aged and elderly adults in Tianjin and Shanghai were recruited for repeated follow-ups with measurements of 24-h personal exposures to air pollutants and Holter ECG monitoring. Heart rate variability (HRV) variables calculated over 5-minute segments during the 24 h, including low frequency power (LF), high frequency power (HF), standard deviation of normal-to-normal intervals (SDNN) and root mean square of successive differences (rMSSD), were included in the analysis. The Trait Anxiety Inventory was used to investigate the long-term general anxiety level of the participants. Generalized linear mixed-effects model was used to analyze the association between exposure factors and HRV variables, and potential effect modification by trait anxiety.
Results: Data on 87 participants were included in final analysis after exclusions. Higher exposure to $PM_{2.5}$ was associated with lower levels of LF, HF, SDNN and rMSSD, and the largest decreases in LF, HF, SDNN and rMSSD were found at 3-h moving average. Trait anxiety significantly modified the associations of $PM_{2.5}$ with LF, HF, SDNN and rMSSD, with stronger inverse associations found in high trait anxiety group than in low trait anxiety group. For an IQR (27.3 $\mu g/m^3$) increase in $PM_{2.5}$ at 3-h moving average, there were decreases of 3.50% (95% CI: −4.46%, −2.54%) and 3.50% (95% CI: −4.49%, −2.50%) in the high trait anxiety group, and decreases of 0.81% (95% CI: −1.22%, −0.40%) and 0.65% (95% CI: −1.07%, −0.23%) in the low trait anxiety group in HF and rMSSD, respectively (both p for interaction<0.01).
Conclusion: Our study suggests that trait anxiety could modify the association of short-term exposure to $PM_{2.5}$ with HRV variables, indicating that higher trait anxiety may increase the cardiac susceptibility to air pollution in the study participants.

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1. Introduction

Ambient particulate matter with aerodynamic diameter <2.5 μm ($PM_{2.5}$) refers to a variety of solid or liquid particles dispersed uniformly in the aerosol system and is an important component of air pollution. The main sources of particulate matter in the urban environment are human activities, including automobile exhausts, coal burning and industrial emissions (Zhu et al., 2018). There is increasing evidence that $PM_{2.5}$ exposure may be associated with adverse cardiovascular health effects. Previous studies have shown that short-term $PM_{2.5}$ exposure within a few days may increase the morbidity and mortality of cardiovascular diseases (Kunovac et al., 2020; Pope et al., 2015) and the hospital admissions for cardiovascular diseases (Ren et al., 2021). The development of cardiovascular disease is accompanied by a series of changes in subclinical indicators, including blood pressure and blood lipids (Xu et al., 2014). In addition, acute $PM_{2.5}$ exposure can affect the cardiac autonomic function in healthy individuals, which in turn leads to decreased levels of heart rate variability (HRV) indicators (Hu et al., 2020; Tobaldini et al., 2018).

In recent years, anxiety has become an important factor affecting population health, anxiety not only increases the risk of cardiovascular disease by increasing unhealthy behaviors such as smoking and alcohol consumption (Strine et al., 2005), but also increases the risk of major cardiac events in patients with coronary artery disease (Allgulander 2016; Celano et al., 2016). As an important component of anxiety, trait anxiety refers to the stable tendency to attend to, experience, and report negative emotions such as fears, worries, and anxiety across many situations (Gidron 2013). A previous study on trait anxiety found that staying in a state of prolonged anxiety could affect the level of stress response in the cardiovascular system (de Rooij et al., 2010). Higher trait anxiety has been associated with a delayed systolic blood pressure (SBP) response to stress (Wiggert et al., 2016) and increased coronary artery calcification in healthy individuals (Hernandez et al., 2014). Previous studies have shown that trait anxiety was significantly associated with decreased HRV, and individuals with higher trait anxiety had lower HRV levels (Levy 2014; Svendsen et al., 2016; Williams et al., 2017).

Effect modification refers to whether the strength of the association between an exposure factor and an outcome has different magnitudes depending on the existence of a third variable (Kamangar 2012). The potential effect of air pollution exposure on the occurrence of cardiovascular disease events has been found to be more pronounced in communities with higher stressor levels (Clougherty. et al., 2021), and the association between particulate matter exposure and blood pressure has also been found to be stronger in individuals with higher psychological stress (Hicken et al., 2014). In addition, adults with higher unemployment rates who also lived in high air pollution environment were found to have a higher risk for cardiovascular disease (Dragano et al., 2009). These findings suggest that stress may modify the relationship between air pollution and cardiovascular health, that was, simultaneous exposures to air pollution and high stress may lead to stronger adverse health effects from the joint actions of both health risk factors. Previous studies have shown that air pollution and stress may affect cardiovascular health through similar biological pathways mediated by stress hormones (Miller et al., 2016; Zorn et al., 2017), therefore, the role of stress should not be ignored while studying the potential effects of air pollution on cardiovascular health. However, no study has even reported the potential modifying effect of trait anxiety on the association between air pollution and cardiovascular health to date.

Evidence from previous studies regarding whether trait anxiety may modify the effects of short-term exposure to major air pollutants such as $PM_{2.5}$ on the cardiovascular health in human individuals is lacking, which is not conducive to the identification of susceptible population and the formulation of targeted prevention and control measures for the health hazards of air pollution. This panel study was thus designed to investigate the potential modifying effect of trait anxiety on the association between short-term exposures to $PM_{2.5}$ and cardiac autonomic function, as reflected by changes in HRV.

2. Methods

2.1. Study population

In this panel study, a group of middle-aged and elderly adults in Tianjin and Shanghai were recruited for three to six repeated follow-up visits with air pollution exposure and Holter electrocardiogram (ECG) measurements. Participants underwent three repeated follow-up visits during each study period (summer/autumn or winter/spring), or six repeated follow-up visits if they participated in both study periods (summer/autumn and winter/spring). The panel study was conducted in Tianjin (Aug 22, 2018 to Oct 24, 2018; Jan 7, 2019 to Apr 4, 2019) and Shanghai (Jul 24, 2019 to Sept 25, 2019; Dec 20, 2019 to Jan 14, 2020; May 24, 2020 to Jul 6, 2020). Due to the COVID-19 pandemic, participants only completed one follow-up visit in winter in Shanghai, and the remaining follow-up visits were completed in the following summer in 2020. A schematic diagram of the study design was provided in the supplementary material (Fig. S1). We screened individuals willing to participate in the project by placing recruitment advertisements and included those who met the inclusion/exclusion criteria in the study. No participants were related to each other. The inclusion/exclusion criteria for participant recruitment were as follows: inclusion criteria: (1) $35 \leq$ age < 70, (2) $18.5 \leq$ BMI < 30 kg/m^2, (3) no smoking history; exclusion criteria: suffering from respiratory, cardiovascular or other chronic diseases. There was an interval of at least 2 weeks between repeated follow-up visits for a given participant. The follow-up visits were conducted according to the following standard procedures: participants were measured for their 24-h personal exposures to air pollutants using professional equipment from 8:00–9:00 a.m. the first day until 8:00–9:00 a.m. the following day, and an ambulatory electrocardiogram device was also worn to collect real-time HRV data. Information regarding subject name, age, gender, height, weight, passive smoking status, alcohol intake, occupation, educational level and annual household income per capita was obtained from baseline questionnaires. Due to the impact of COVID-19 pandemic, study participants were required to wear disposable surgical masks during post-epidemic follow-ups. Each participant was issued a disposable surgical mask before the instrument wearing time, and was told not to use other types of mask except the issued one during the 24-h measurement until the next morning. No participant wore a non-disposable mask or replaced another mask of the same type in the study. The study protocol was approved by the Institutional Review Board of Peking University Health Science Center. All participants signed the written informed consent before the study began.

2.2. Exposure assessment

A real-time $PM_{2.5}$ counter was installed at the locations where the participants usually stayed to measure their 24-h continuous exposures to $PM_{2.5}$. The study participants also carried a backpack with personal exposure monitoring instruments inside to measure their 24-h continuous exposures to black carbon (BC) and meteorological variables. The real-time $PM_{2.5}$ counter was placed on a table near the study participants, and would be moved as well when they move to other places. At night, the $PM_{2.5}$ sampling system was placed on a table about 2 m away from the participant while the participant was sleeping. The table was about 1 m above the ground, close to the study participant's breathing zone. The backpack was carried by the study participants during the day and was put beside them when they slept at night. When they stayed at home but not sleeping, they were also required to wear the backpack, and if they needed to work or do other activities, the backpack was placed within 1 m of them. The real-time concentrations of $PM_{2.5}$ were measured using a real-time particulate counter (SidePak AM520 Aerosol

Monitor, TSI Incorporated, USA), and the instrument flow rate was set at 1.7 L/min. The real-time concentrations were measured using a portable BC monitor (MicroAeth AE51, Aethlabs, USA). Meanwhile, the real-time levels of temperature and relative humidity (RH) were recorded using a HOBO Pro v2 logger (Onset Corp., Pocasset, MA, USA). The recording intervals of the above instruments were all set at once per minute. Instruments were calibrated according to the manufacturer's specifications and passed a parallelism test (i.e., several instruments were placed in the same environment and sampled simultaneously and the results were compared for consistency) before the sampling began. The MicroAeth monitors would be replaced with a new sampling diaphragm filter for each measurement during the study.

2.3. Heart rate variability measurement

While carrying backpacks for recording exposure factors, all participants were asked to wear an electrographic Holter monitor (CBox-1012, Microcardio, China) to record their ambulatory ECG signals continuously for 24 h. ECG signals were recorded at the same time when the personal exposure monitoring instruments started working, and lasted until the instruments were retrieved the next morning (totally 24 h). Since the study participants were not convenient to exercise or do other sports when carrying the equipment, they only maintained normal life activities that did not evidently affect the recording results. ECG signals were exported and processed by specialized physicians using professional ambulatory ECG analysis software (Dynamic ECG workstation V1.4.3.0) to calculate HRV variables. The ECG signals were divided into 5-min segments, and the values of the HRV variables during the recording time were calculated from the ECG signals during these 5-min segments. Two frequency-domain parameters including low frequency power (LF) and high frequency power (HF) and two time-domain parameters including standard deviation of normal-to-normal intervals (SDNN) and root mean square of successive differences (rMSSD) were included in the analysis.

2.4. Trait anxiety information

The Chinese version of Trait Anxiety Inventory (TAI, 20 items) was used to investigate the general anxiety level, including long-term experience or feeling of fear, tension and anxiety of the participants. The TAI scale has been validated in Chinese (Shek 1993) and widely used in China (Han et al., 2020; Zhou et al., 2020). Participants were asked to report the frequency of their feelings on a Likert scale (1-almost never, 2- sometimes, 3- often, 4- almost always) for each item in the scale at their first follow-up visits in this study. A rating of 4 indicates the presence of high levels of anxiety for eleven TAI scale items (#2, 4, 5, 8, 9, 11, 12, 15, 17, 18, 20) (e.g., "When I think about my current affairs and interests, I get into a state of tension," "I feel like crying"), and a high rating for the remaining nine TAI scale items (e.g., "I feel pleasant," "I feel safe") indicates the absence of anxiety. The TAI scale items were scored with the same weighting as the numbers selected on the test form, and the scoring weights for the anxiety-absent items were reversed. Two participants with more than 2 missing TAI scale items were excluded, and the total TAI scores were calculated by multiplying the arithmetic mean of non-missing items by 20 (the total number of items) for the remaining participants (Fountoulakis et al., 2006; Knowles and Olatunji 2020).

2.5. Statistical analysis

Characteristics of the study participants were described as mean ±SD for continuous variables, and as number (proportion) for categorial variables. The average personal exposure levels of $PM_{2.5}$ during 1 h–5 h before the HRV variables measurements were calculated and matched with the HRV data. The average temperature and RH at same exposure time windows were also adjusted for in the models as confounding factors. A log10 conversion was performed to improve the normality of the HRV variables. Generalized linear mixed-effects models were used to analyze the association between $PM_{2.5}$ and the HRV variables. The mixed-effects models included age (continuous), gender (male or female), body mass index (BMI) (continuous), passive smoking (yes or no), alcohol drinking (yes or no), city (Tianjin or Shanghai), day of study (continuous) and its squared variable (Penttinen et al., 2001), day of week (Monday to Friday, categorial), hour of HRV measurement (categorial), occupation (worker, retired, others), educational level (lower than high school, high school, university or above), annual household income per capita (<10,000, 10,000–30,000, 30,000–50,000, 50,000–100,000, or >100,000 CNY), disposable surgical mask use (yes or no), temperature (continuous) and RH (continuous) as fixed-effect terms, and the random effects were estimated at the subject level. The regression coefficients were then converted into percentage changes with 95% confidence intervals (CI) to reflect the changes of the HRV variables associated with each IQR increase in the $PM_{2.5}$ concentration. The calculation formula was as follows: $(10^{\beta}\text{-}1) \times 100\%$ and $(10^{\beta \pm 1.96 \times SE}\text{-}1) \times 100\%$.

In order to estimate the modifying effect of trait anxiety on the association between short-term exposures to $PM_{2.5}$ and HRV variables, we performed subgroup analyses based on the summary score of the TAI scale using the same mixed-effects model. Because the participants included in this study were healthy individuals with a low range of TAI summary score, we classified participants into high trait anxiety group (higher than 75th percentile) and low trait anxiety group (less than or equal to 75th percentile) according to the summary score of the TAI scale (Dayan et al., 2002). The 75th percentile of the TAI summary score in this study was 39. Differences in the characteristics between the low and high trait anxiety groups were compared using two-sample t test for continsous variables and chi-squared test for categorical variables. In addition, a multiplicative interaction term for trait anxiety (dichotomized by 75th percentile) and $PM_{2.5}$ (continuous) was added in the mixed-effects model with the main effect terms of the TAI score and $PM_{2.5}$ to test the potential interaction between these two exposure factors. Furthermore, BC concentrations were adjusted in the models and stratified analyses according to before and during the pandemic were performed to evaluate the robustness of the results as sensitivity analyses. All statistical analyses were conducted using R software (version 3.6.1), and $p < 0.05$ (2-tailed) was set as the statistical significance level. The p for interaction <0.05 (2-sided) was considered to have statistically significant modification.

3. Results

3.1. Characteristics of the study participants and descriptive statistics of the exposure and health data

A total of 92 participants were enrolled in the study. After excluding 3 participants without ECG monitoring results and 2 participants without trait anxiety information, data from 87 participants were included in the current analysis (Fig. S2). Among them, 59 participants underwent three repeated follow-up visits and 28 participants underwent six repeated follow-up visits. Table 1 shows the characteristics of the involved 87 participants. The mean (SD) age of all participants was 51.9 (5.4) years, 26 (29.9%) of them were males, and the mean (SD) BMI was 24.2 (2.4) kg/m^2.

The personal exposure concentrations of $PM_{2.5}$ and meteorological variables over different exposure time windows are shown in Table 2. The 1-h means (SD) of $PM_{2.5}$, temperature and RH were 29.9 (44.8) μg/m^3, 24.6 (5.1) °C and 56.2 (16.9) %, respectively. Compared to other time windows, 5-h moving average (average of exposure levels during the 5 h before the recording time of HRV variables) had the highest mean (SD) of $PM_{2.5}$ concentrations at 30.4 (39.3) μg/m^3. The 5-min HRV levels of the study participants are shown in Table 3. The total number of 5-min HRV observations was 43,750, with 34,554 and 9,196 from the

Table 1
Characteristics of the study participants (N = 87).

Variables	Low trait anxiety	High trait anxiety	P value
	N = 66	N = 21	
Age (years) (mean ± SD)	53 ± 5	50 ± 6	0.005
Gender, Female (N, %)	43 (65.2)	18 (85.7)	0.073
BMI (kg/m^2) (mean ± SD)	24.0 ± 2.3	24.9 ± 2.6	0.168
Passive smoking (N, %)	29 (43.9)	10 (47.6)	0.768
Alcohol drinking (N, %)	16 (24.2)	6 (28.6)	0.691
Occupation (N, %)			0.087
Worker	20 (30.3)	2 (9.5)	
Retired	7 (10.6)	5 (23.8)	
Others	39 (59.1)	14 (66.7)	
Educational level (N, %)			0.720
Lower than high school	36 (54.5)	12 (57.1)	
High school	23 (34.8)	8 (38.1)	
University or above	7 (10.6)	1 (4.8)	
Annual household income per capita (CNY) (N, %)			0.018
<10,000	7 (10.6)	0 (0)	
10,000–30,000	22 (33.3)	4 (19.0)	
30,000–50,000	21 (31.8)	7 (33.3)	
50,000–100,000	8 (12.1)	9 (42.9)	
>100,000	8 (12.1)	1 (4.8)	
24-h average $PM_{2.5}$ (μg/m^3) (mean ± SD)	37.3 ± 34.3	28.6 ± 34.4	0.618

Abbreviations: BMI, body mass index; CNY, Chinese Yuan; SD, standard deviation.

Table 2
Descriptives of exposure factors during the study.

Exposure factor	Exposure time windows	Mean ± SD	Min	Median	Max	IQR
$PM_{2.5}$ (μg/m^3)	1-h	29.9 ± 44.8	0.1	16.4	670.7	27.2
	2-h	30.0 ± 42.8	0.1	16.7	546.1	27.2
	3-h	30.0 ± 41.1	0.1	17.2	507.5	27.3
	4-h	30.2 ± 40.2	0.1	17.4	496.2	27.6
	5-h	30.4 ± 39.3	0.1	17.7	439.8	27.7
Temperature (°C)	1-h	24.6 ± 5.1	9.9	25.9	37.7	7.6
	2-h	24.6 ± 5.1	10.0	25.9	37.6	7.6
	3-h	24.6 ± 5.1	10.0	25.9	37.4	7.6
	4-h	24.6 ± 5.1	10.1	25.9	36.9	7.6
	5-h	24.6 ± 5.1	10.1	26.0	36.4	7.5
RH (%)	1-h	56.2 ± 16.9	9.0	60.0	90.0	21.0
	2-h	56.1 ± 16.8	9.0	60.0	90.0	21.0
	3-h	56.1 ± 16.7	10.0	60.0	90.0	21.0
	4-h	56.1 ± 16.6	10.0	60.0	90.0	20.0
	5-h	56.1 ± 16.6	11.0	60.0	90.0	20.0

Abbreviations: IQR, interquartile range; SD, standard deviation; $PM_{2.5}$, particulate matter ≤2.5 μm in aerodynamic diameter; RH, relative humidity.

low and high trait anxiety groups, respectively. The HRV variables were non-normally distributed data in this study, and the medians (IQRs) of 5-min LF, HF, SDNN and rMSSD of all participants were 141.7 (86.0) ms^2, 150.4 (120.7) ms^2, 47.0 (32.0) ms and 25.0 (21.0) ms, respectively.

Table 3
Descriptives of 5-min heart rate variability variables by trait anxiety level of the study participants.

Variable	Trait anxiety level (Low, ≤75th percentile; High, >75th percentile)	Mean ± SD	Min	Median	Max	IQR
LF (ms^2)	Overall	157.6 ± 73.6	31.6	141.7	2167.5	86.0
	Low	158.4 ± 75.8	31.6	142.2	2167.5	88.2
	High	154.5 ± 64.6	35.1	140.7	581.6	77.4
HF (ms^2)	Overall	187.1 ± 116.6	28.0	150.4	1142.4	120.7
	Low	186.7 ± 116.5	28.0	150.1	1142.4	117.7
	High	188.9 ± 116.9	48.1	151.7	831.4	129.5
SDNN (ms)	Overall	54.0 ± 26.3	20.0	47.0	185.0	32.0
	Low	53.9 ± 26.5	20.0	47.0	185.0	33.0
	High	54.3 ± 25.5	20.0	48.0	181.0	32.0
rMSSD (ms)	Overall	30.1 ± 18.7	9.0	25.0	104.0	21.0
	Low	30.1 ± 18.8	9.0	25.0	104.0	21.0
	High	30.2 ± 18.6	9.0	25.0	104.0	23.0

Abbreviations: HF, high frequency power; IQR, interquartile range; LF, low frequency power; rMSSD, root mean square successive difference; SD, standard deviation; SDNN, standard deviation of normal-to-normal intervals.

3.2. Associations between $PM_{2.5}$ and heart rate variability variables

In generalized linear mixed-effects models, we found that higher exposure to $PM_{2.5}$ was significantly associated with lower LF, HF, SDNN and rMSSD. The largest decreases in LF, HF, SDNN and rMSSD were found at 3-h moving average. For an IQR (27.3 μg/m^3) increase in $PM_{2.5}$ at 3-h moving average, there was a 0.78% (95% CI: −1.07%, −0.47%) decrease in LF, a 1.17% (95% CI: −1.55%, −0.80%) decrease in HF, a 0.97% (95% CI: −1.31%, −0.63%) decrease in SDNN, and a 1.05% (95% CI: −1.44%, −0.66%) decrease in rMSSD (Fig. 1). The significance of the results did not change after additionally adjusting for BC concentrations (Fig. S3).

3.3. Estimated modifying effect of trait anxiety

Fig. 2 shows that trait anxiety could significantly modify the association between $PM_{2.5}$ and LF (at 1-h moving average to 5-h moving average), HF (at 1-h moving average to 5-h moving average), SDNN (at 1-h moving average to 4-h moving average) or rMSSD (at 1-h moving average to 5-h moving average). For an IQR (27.3 μg/m^3) increase in $PM_{2.5}$ at 3-h moving average, there was a 2.22% (95% CI: −2.95%, −1.49%) decrease in the high trait anxiety group and a 0.56% (95% CI: −0.89%, −0.22%) decrease in the low trait anxiety group in LF (p for interaction <0.01), a 3.50% (95% CI: -4.46%, -2.54%) decrease in the high trait anxiety group and a 0.81% (95% CI: -1.22%, -0.40%) decrease in the low trait anxiety group in HF (p for interaction<0.01), a 2.06% (95% CI: -2.93%, -1.18%) decrease in the high trait anxiety group and a 0.76% (95% CI: -1.13%, -0.39%) decrease in the low trait anxiety group in SDNN (p for interaction<0.01), and a 3.50% (95% CI: -4.49%, -2.50%) decrease in the high trait anxiety group and a 0.65% (95% CI: -1.07%, -0.23%) decrease in the low trait anxiety group in rMSSD (p for interaction<0.01). The significance of the results did not change after

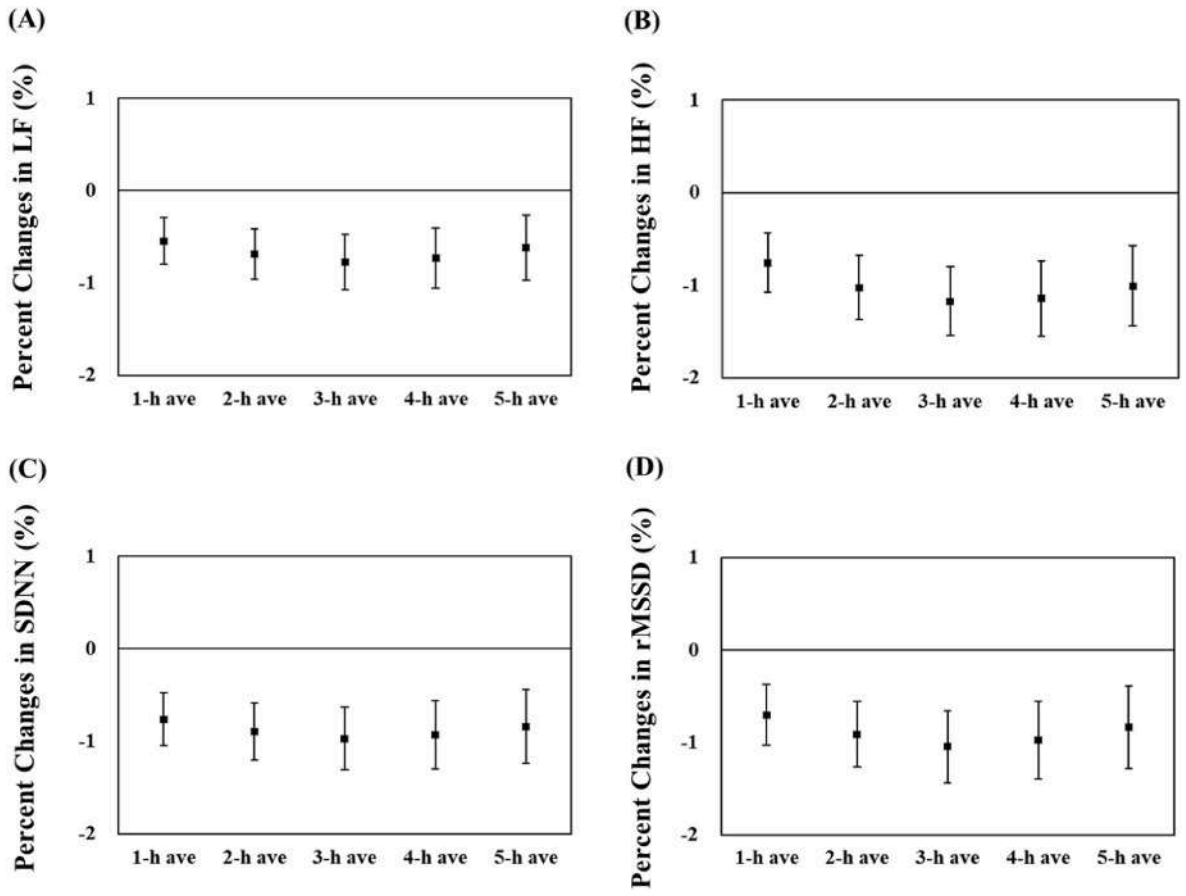

Fig. 1. The associations between $PM_{2.5}$ and 5-min heart rate variability variables at different exposure time windows in generalized linear mixed-effects models. (A) LF, (B) HF, (C) SDNN, (D) rMSSD. Models were adjusted for age, gender, BMI, passive smoking, alcohol drinking, city, time trend, day of week, hour, occupation, educational level, annual household income per capita, disposable surgical mask use, temperature and RH. 1-h ave to 5-h ave denote 1-h to 5-h moving averages of $PM_{2.5}$ concentrations.

additionally adjusting for BC concentrations (Fig. S4).

A stratified analysis was conducted according to before and during the pandemic and the results were presented in Fig. S5-Fig. S8 in the supplementary material. The results of the before the pandemic period were similar to the overall results. The HRV variables decreased with higher pollutant exposures (Fig. S5) and showed more apparent reductions in the high trait anxiety group (Fig. S6). The results of the during the pandemic period showed that only LF increased with pollutant exposures, while the results of the remaining variables were insignificant (Fig. S7). The results of analysis stratified by trait anxiety during the pandemic period showed that the differences between the high and low trait anxiety groups were similar to the before the pandemic period and overall results, with the high trait anxiety group showing more inverse associations compared with the low trait anxiety group (Fig. S8). These results suggest that the potential modifying effect of trait anxiety on the association of $PM_{2.5}$ with heart rate variability variables was stable.

4. Discussion

Our study examined the potential modifying effect of trait anxiety on the associations between short-term exposure to $PM_{2.5}$ and cardiac autonomic function, as reflected by changes in HRV variables, in a panel of middle-aged and elderly adults. The results suggest that trait anxiety could significantly modify the associations of $PM_{2.5}$ with LF, HF, SDNN and rMSSD. The associations of $PM_{2.5}$ with HRV variables in the high trait anxiety group were generally more apparent than those in the low trait anxiety group. To our knowledge, this is the first study to explore the modifying effect of trait anxiety on the associations between short-term exposures to $PM_{2.5}$ and cardiovascular health variables in human participants.

A recent report by the Health Effects Institute showed that associations between the air pollutants and cardiovascular disease events were attenuated when adjusting for social stressors, suggesting that psychosocial stress may play a mediating role in the relationship between the air pollutants and cardiovascular diseases (Clougherty. et al., 2021). However, few studies have been conducted to investigate the role of psychological stress on modifying the association between air pollution and cardiovascular health indicators. One study in China showed that psychological stress significantly modified the association between short-term ozone (O_3) exposure and systolic blood pressure (SBP), with individuals with low perceived stress showing a decrease in SBP whereas individuals with high perceived stress showing an increase in SBP, (Chen et al., 2021). Another study in the United States showed that the association between short-term $PM_{2.5}$ exposure and SBP was stronger in participants with higher psychosocial stress. For each 10 $\mu g/m^3$ increase in $PM_{2.5}$ exposure, participants with higher psychosocial stress in Southwest neighborhood showed an increase of 9.05 mmHg (95% CI: 3.29, 14.81) in SBP, much higher than the increase of 2.94 mmHg (95% CI: −0.85, 6.72) with lower psychosocial stress (Hicken et al., 2014), suggesting that psychosocial stress may increase the sensitivity of the cardiovascular system to air pollution. The results of our study shows that trait anxiety may have a modifying effect on the association between $PM_{2.5}$ and HRV variables. The higher trait anxiety group had decreased parasympathetic nervous activity (reflected by reductions in HF and rMSSD) and decreased sympathetic nervous activity (reflected

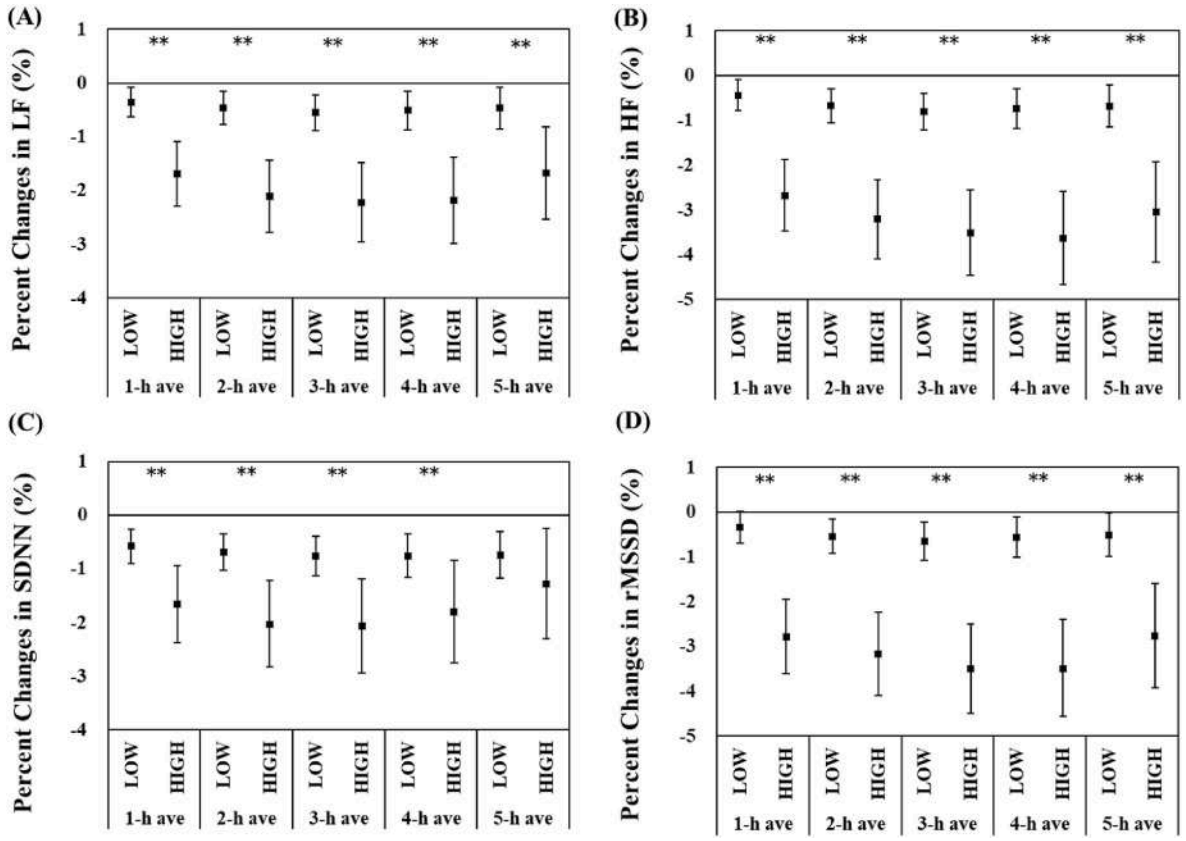

Fig. 2. Modification of trait anxiety on the associations between $PM_{2.5}$ and 5-min heart rate variability variables at different exposure time windows in generalized linear mixed-effects models. (A) LF, (B) HF, (C) SDNN, (D) rMSSD. Stratified analyses were conducted for the high trait anxiety group (>75th percentile) and low trait anxiety group (≤75th percentile) divided according to trait anxiety score. Models were adjusted for age, gender, BMI, passive smoking, alcohol drinking, city, time trend, day of week, hour, occupation, educational level, annual household income per capita, disposable surgical mask use, temperature and RH. 1-h ave to 5-h ave denote 1-h to 5-h moving averages of $PM_{2.5}$ concentrations. ** interaction $p < 0.05$.

by reductions in LF) under short-term $PM_{2.5}$ exposure than the lower trait anxiety group. HRV variables reflect the heart's ability to adapt to the environment. When the HRV decreases, the body is less capable to adapt to the environment and may suggest serious health damage (McCraty and Shaffer 2015). The SDNN is believed to reflect the overall variability, and a decrease in SDNN represents an overall decrease in HRV level (Weippert et al., 2013). LF is believed to reflect the sympathetic tone and HF is believed to reflect parasympathetic tone that both influence the heart beat (Cygankiewicz and Zareba 2013). The rMSSD value reflects the ability to resist stress and the body's fatigue level (Djaoui et al., 2017; Plews et al., 2013), and a decrease in rMSSD indicates reduced parasympathetic activity (Järvelin-Pasanen et al., 2018). The results suggest that participants with higher trait anxiety are less capable to adapt to air pollution and need more attention.

Since the participants recruited in this study were healthy individuals, and their trait anxiety scores, which represent the prolonged anxiety levels, were relatively low, and the differences in the absolute values of HRV variables between the subgroups with different trait anxiety levels may not be apparent. However, a mixed-effects model for analyzing repeated-measure data was used in this study and the subject was treated as a random effect term in the model, the results were estimated as the percentage change in HRV variables per unit increase in air pollution exposure levels, which tended to be more apparent in the high trait anxiety group. The results of the modifying effect analysis also showed that the estimated effects of air pollution on HRV variables were significantly different among different subgroups, and therefore it could support that higher trait anxiety may increase susceptibility of the cardiovascular health upon air pollution exposure. The possible reason is that trait anxiety and air pollution may have the same mechanism to jointly affect cardiovascular health. Similar to the mechanism by which trait anxiety stimulates the sympathetic nervous system, activates the hypothalamus-pituitary-adrenal axis and causes the body's stress response (Holwerda et al., 2018; Labad et al., 2020), air pollution may also affect the sympathetic nervous system, increase the body's inflammatory factor levels and activate the hypothalamus-pituitary-adrenal-axis (Dar et al., 2019; Martínez-Lazcano et al., 2013; Niu et al., 2018), which leads to increased levels of glucocorticoids (Spiga et al., 2014). Glucocorticoids acting on myocardial glucocorticoid receptors may not only lead to abnormal left ventricular function (Oakley et al., 2019), but also cause arrhythmias and altered HRV (Cruz-Topete et al., 2019; Ling et al., 2018). Elevated levels of glucocorticoids may further affect levels of vasoreactivators by affecting vascular smooth muscle glucocorticoid receptors, resulting in elevated angiotensin II and endothelin 1 (Kozhevnikova et al., 2007; Xue et al., 2014), which may subsequently cause vascular wall constriction and affect blood pressure (Gallardo-Ortíz et al., 2015). In addition, short-term exposure to air pollution may impair vascular function (Cengel and Sahinarslan 2006), cause damage such as impaired immune response (Girod and Brotman 2004), and further increase blood pressure (Harrison et al., 2011), which may have a synergistic effect in addition to the influence of trait anxiety on cardiovascular health (Ushakov et al., 2016).

In addition, we found similar effects of air pollution and chronic anxiety on the autonomic nervous system which were reflected by reductions in HF and rMSSD that indicate decreased parasympathetic activity. Air pollutants can stimulate defensive sensory nerves within the cardiopulmonary system, thereby causing autonomic dysfunction (Taylor-Clark 2020). Similar to our study, a previous study has shown

that air pollutants may affect cardiac autoregulation within a few hours after exposure, with decreases in rMSSD reflecting parasympathetic activity associated with $PM_{2.5}$ exposures (Weichenthal et al., 2014). It has also been shown that decreased parasympathetic activity occurred with increased anxiety levels (Michopoulos et al., 2017), and the mechanism may be that a situation of high anxiety leads to higher norepinephrine levels, which in turn affects parasympathetic function (Nahman-Averbuch et al., 2016). This may be the reason why indicators reflecting parasympathetic activity such as HF and rMSSD decreased more significantly with increasing concentrations of $PM_{2.5}$ exposure in the high trait anxiety group compared to the low trait anxiety group in this study. As some of the influencing factors of trait anxiety, such as sleep quality, may directly affect the cardiovascular system (Kwok et al., 2018; Norbury and Evans 2019), the mechanism by which trait anxiety and air pollution affect cardiovascular health together needs to be further explored.

Our study had several strengths. First, this study made the first attempt to investigate the modifying effect of trait anxiety on the association of $PM_{2.5}$ with cardiovascular health variables in human participants, which may provide reference for subsequent studies. Second, previous studies have only investigated the potential modifying effects of stress-related factors on the potential effects of air pollution on cardiovascular disease events and blood pressure, whereas our study further investigated the joint impacts of air pollution and stress on cardiac autonomic function (reflected by changes in HRV variables), expanding the literature by showing strong effect modification of trait anxiety on the association between short-term exposure to $PM_{2.5}$ and HRV variables. Third, most of the existing studies investigating the potential effects of air pollution and stress on cardiovascular health used data from fixed-site monitoring data or model simulations in exposure assessment, and thus could not avoid the issue of exposure misclassification. In contrast, our study used personal exposure instruments to monitor the real-time exposure levels of the participants, and thus significantly improved the exposure assessment accuracy. Finally, most of the existing studies have been conducted in countries with relatively low air pollution levels, whereas our study is more representative of the population living in developing countries in the context of high air pollution levels.

Our study also has some limitations. First, this study only investigated the association between changes in air pollutant levels and cardiovascular health variables within a 24-h period. Although this allows for accurate observation of acute changes in cardiovascular health variables in association with air pollution exposure, the potential cumulative effects of longer-term exposure to air pollutants (e.g., up to several months or 1 year) may be ignored. Second, this study focused on the effect of trait anxiety on the association between short-term $PM_{2.5}$ exposure and HRV variables in middle-aged and older participants, and therefore the generalizability of the findings may be limited when extrapolating to younger individuals. Third, although time trends were controlled for in the analysis, some other unmeasured factors that influencing air pollution changes and HRV during the study period may still affect the results. Fourth, this study only investigated the modifying effect but not mediating effect of trait anxiety on the association between short-term air pollution exposure and cardiovascular health variables. However, the TAI scale was used to capture the general anxiety status of the participants over long durations, and therefore it was unlikely that the measured stress could mediate the potential effects of personal exposures measured within a few hours. Therefore, compared with the potential mediating role played by the state anxiety that capturing short-term anxiety, the TAI scale that capturing prolonged anxiety is more likely to elicit a modification effect on the observed associations. Finally, although other air pollutants such as nitrogen dioxides, sulfur dioxides and carbon monoxide may affect the results, this study did not collect real-time monitoring data of these air pollutants, and further research is encouraged to investigate the question of interest with regards to these air pollutants.

5. Conclusions

In summary, our study suggests that trait anxiety could modify the association of $PM_{2.5}$ with HRV variables in middle-aged and elderly adults, and many of the associations are stronger in the high trait anxiety group than in the low trait anxiety group. Therefore, trait anxiety may increase the cardiovascular system's susceptibility to air pollution in the study participants. These results may provide scientific evidence for the formulation of targeted prevention and control measures for the cardiovascular health hazards related to air pollution in the population in the future.

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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.2022.113933.

References

Allgulander, C., 2016. Anxiety as a risk factor in cardiovascular disease. Curr. Opin. Psychiatr. 29, 13–17.
Celano, C.M., Daunis, D.J., Lokko, H.N., Campbell, K.A., Huffman, J.C., 2016. Anxiety disorders and cardiovascular disease. Curr. Psychiatr. Rep. 18, 101.
Cengel, A., Sahinarslan, A., 2006. Nitric oxide and cardiovascular system. Anadolu Kardiyol. Derg. 6, 364–368. AKD = the Anatolian journal of cardiology.
Chen, Q., Li, H., Liu, Q., Wang, W., Deng, F., Sun, Z., et al., 2021. Does psychosocial stress modify the association of fine particulate matter and ozone with cardiovascular health indicators? Environ. Pollut. 277, 116726.
Clougherty, J.E., Humphrey, J.L., Kinnee, E.J., Robinson, L.F., McClure, L.A., Kubzansky, L.D., et al., 2021. Social Susceptibility to Multiple Air Pollutants in Cardiovascular Disease. Research Report 206. Health Effects Institute, Boston, MA.
Cruz-Topete, D., Oakley, R.H., Carroll, N.G., He, B., Myers, P.H., Xu, X., et al., 2019. Deletion of the cardiomyocyte glucocorticoid receptor leads to sexually dimorphic changes in cardiac gene expression and progression to heart failure. J. Am. Heart Assoc. 8, e011012.
Cygankiewicz, I., Zareba, W., 2013. Heart rate variability. Handb. Clin. Neurol. 117, 379–393.
Dar, T., Radfar, A., Abohashem, S., Pitman, R.K., Tawakol, A., Osborne, M.T., 2019. Psychosocial stress and cardiovascular disease. Curr. Treat. Options Cardiovasc. Med. 21, 23.
Dayan, J., Creveuil, C., Herlicoviez, M., Herbel, C., Baranger, E., Savoye, C., et al., 2002. Role of anxiety and depression in the onset of spontaneous preterm labor. Am. J. Epidemiol. 155, 293–301.
de Rooij, S.R., Schene, A.H., Phillips, D.I., Roseboom, T.J., 2010. Depression and anxiety: associations with biological and perceived stress reactivity to a psychological stress protocol in a middle-aged population. Psychoneuroendocrinology 35, 866–877.
Djaoui, L., Haddad, M., Chamari, K., Dellal, A., 2017. Monitoring training load and fatigue in soccer players with physiological markers. Physiol. Behav. 181, 86–94.
Dragano, N., Hoffmann, B., Moebus, S., Möhlenkamp, S., Stang, A., Verde, P.E., et al., 2009. Traffic exposure and subclinical cardiovascular disease: is the association modified by socioeconomic characteristics of individuals and neighbourhoods? Results from a multilevel study in an urban region. Occup. Environ. Med. 66, 628–635.
Fountoulakis, K.N., Papadopoulou, M., Kleanthous, S., Papadopoulou, A., Bizeli, V., Nimatoudis, I., et al., 2006. Reliability and psychometric properties of the Greek translation of the state-trait anxiety inventory form y: preliminary data. Ann. Gen. Psychiatr. 5, 2.
Gallardo-Ortíz, I.A., Rodríguez-Hernández, S.N., López-Guerrero, J.J., Del Valle-Mondragón, L., López-Sánchez, P., Touyz, R.M., et al., 2015. Role of α1d -adrenoceptors in vascular wall hypertrophy during angiotensin ii-induced hypertension. Autonom. Autacoid Pharmacol. 35, 17–31.
Gidron, Y., 2013. Trait anxiety. In: Gellman, M.D., Turner, J.R. (Eds.), Encyclopedia of Behavioral Medicine. Springer, New York, NY. New York, 1989-1989.
Girod, J.P., Brotman, D.J., 2004. Does altered glucocorticoid homeostasis increase cardiovascular risk? Cardiovasc. Res. 64, 217–226.
Han, Y., Fan, J., Wang, X., Xia, J., Liu, X., Zhou, H., et al., 2020. Factor structure and gender invariance of Chinese version state-trait anxiety inventory (form y) in university students. Front. Psychol. 11, 2228.
Harrison, D.G., Guzik, T.J., Lob, H.E., Madhur, M.S., Marvar, P.J., Thabet, S.R., et al., 2011. Inflammation, immunity, and hypertension. Hypertension 57, 132–140.

Hernandez, R., Allen, N.B., Liu, K., Stamler, J., Reid, K.J., Zee, P.C., et al., 2014. Association of depressive symptoms, trait anxiety, and perceived stress with subclinical atherosclerosis: results from the chicago healthy aging study (chas). Prev. Med. 61, 54–60.
Hicken, M.T., Dvonch, J.T., Schulz, A.J., Mentz, G., Max, P., 2014. Fine particulate matter air pollution and blood pressure: the modifying role of psychosocial stress. Environ. Res. 133, 195–203.
Holwerda, S.W., Luehrs, R.E., Gremaud, A.L., Wooldridge, N.A., Stroud, A.K., Fiedorowicz, J.G., et al., 2018. Relative burst amplitude of muscle sympathetic nerve activity is an indicator of altered sympathetic outflow in chronic anxiety. J. Neurophysiol. 120, 11–22.
Hu, J., Fan, H., Li, Y., Li, H., Tang, M., Wen, J., et al., 2020. Fine particulate matter constituents and heart rate variability: a panel study in shanghai, China. Sci. Total Environ. 747, 141199.
Järvelin-Pasanen, S., Sinikallio, S., Tarvainen, M.P., 2018. Heart rate variability and occupational stress-systematic review. Ind. Health 56, 500–511.
Kamangar, F., 2012. Effect modification in epidemiology and medicine. Arch. Iran. Med. 15, 575–582.
Knowles, K.A., Olatunji, B.O., 2020. Specificity of trait anxiety in anxiety and depression: meta-analysis of the state-trait anxiety inventory. Clin. Psychol. Rev. 82, 101928.
Kozhevnikova, L.M., Avdonin, P.P., Sukhanova, I.F., Avdonin, P.V., 2007. [the Role of Desensitization of Glucocorticoid Receptors in the Development of Vascular Resistance to Endogenous Vasoconstrictors in Traumatic Shock]. Vestnik Rossiiskoi akademii meditsinskikh nauk, pp. 3–8.
Kunovac, A., Hathaway, Q.A., Pinti, M.V., Taylor, A.D., Hollander, J.M., 2020. Cardiovascular adaptations to particle inhalation exposure: molecular mechanisms of the toxicology. Am. J. Physiol. Heart Circ. Physiol. 319, H282–h305.
Kwok, C.S., Kontopantelis, E., Kuligowski, G., Gray, M., Muhyaldeen, A., Gale, C.P., et al., 2018. Self-reported sleep duration and quality and cardiovascular disease and mortality: a dose-response meta-analysis. J. Am. Heart Assoc. 7, e008552.
Labad, J., Salvat-Pujol, N., Armario, A., Cabezas, Á., Arriba-Arnau, A., Nadal, R., et al., 2020. The role of sleep quality, trait anxiety and hypothalamic-pituitary-adrenal axis measures in cognitive abilities of healthy individuals. Int. J. Environ. Res. Publ. Health 17.
Levy, B., 2014. Illness severity, trait anxiety, cognitive impairment and heart rate variability in bipolar disorder. Psychiatr. Res. 220, 890–895.
Ling, N., Li, C.L., Wang, Z.Z., Zhang, H.N., Xu, H., An, X.J., 2018. Heart rate variability in children with myocarditis presenting with ventricular arrhythmias. Eur. Rev. Med. Pharmacol. Sci. 22, 1102–1105.
Martínez-Lazcano, J.C., González-Guevara, E., del Carmen Rubio, M., Franco-Pérez, J., Custodio, V., Hernández-Cerón, M., et al., 2013. The effects of ozone exposure and associated injury mechanisms on the central nervous system. Rev. Neurosci. 24, 337–352.
McCraty, R., Shaffer, F., 2015. Heart rate variability: new perspectives on physiological mechanisms, assessment of self-regulatory capacity, and health risk. Glob. Adv. Health Med. 4, 46–61.
Michopoulos, V., Powers, A., Gillespie, C.F., Ressler, K.J., Jovanovic, T., 2017. Inflammation in fear- and anxiety-based disorders: Ptsd, Gad, and beyond. Neuropsychopharmacology vol. 42, 254–270 official publication of the American College of Neuropsychopharmacology.
Miller, D.B., Ghio, A.J., Karoly, E.D., Bell, L.N., Snow, S.J., Madden, M.C., et al., 2016. Ozone exposure increases circulating stress hormones and lipid metabolites in humans. Am. J. Respir. Crit. Care Med. 193, 1382–1391.
Nahman-Averbuch, H., Sprecher, E., Jacob, G., Yarnitsky, D., 2016. The relationships between parasympathetic function and pain perception: the role of anxiety. Pain Pract. 16, 1064–1072 the official journal of World Institute of Pain.
Niu, Y., Chen, R., Xia, Y., Cai, J., Ying, Z., Lin, Z., et al., 2018. Fine particulate matter constituents and stress hormones in the hypothalamus-pituitary-adrenal axis. Environ. Int. 119, 186–192.
Norbury, R., Evans, S., 2019. Time to think: subjective sleep quality, trait anxiety and university start time. Psychiatr. Res. 271, 214–219.
Oakley, R.H., Cruz-Topete, D., He, B., Foley, J.F., Myers, P.H., Xu, X., et al., 2019. Cardiomyocyte glucocorticoid and mineralocorticoid receptors directly and antagonistically regulate heart disease in mice. Sci. Signal. 12.
Penttinen, P., Timonen, K.L., Tiittanen, P., Mirme, A., Ruuskanen, J., Pekkanen, J., 2001. Number concentration and size of particles in urban air: effects on spirometric lung function in adult asthmatic subjects. Environ. Health Perspect. 109, 319–323.
Plews, D.J., Laursen, P.B., Stanley, J., Kilding, A.E., Buchheit, M., 2013. Training adaptation and heart rate variability in elite endurance athletes: opening the door to effective monitoring. Sports Med. 43, 773–781.
Pope 3rd, C.A., Turner, M.C., Burnett, R.T., Jerrett, M., Gapstur, S.M., Diver, W.R., et al., 2015. Relationships between fine particulate air pollution, cardiometabolic disorders, and cardiovascular mortality. Circ. Res. 116, 108–115.
Ren, Z., Liu, X., Liu, T., Chen, D., Jiao, K., Wang, X., et al., 2021. Effect of ambient fine particulates (pm(2.5)) on hospital admissions for respiratory and cardiovascular diseases in wuhan, China. Respir. Res. 22, 128.
Shek, D.T., 1993. The Chinese version of the state-trait anxiety inventory: its relationship to different measures of psychological well-being. J. Clin. Psychol. 49, 349–358.
Spiga, F., Walker, J.J., Terry, J.R., Lightman, S.L., 2014. Hpa axis-rhythms. Compr. Physiol. 4, 1273–1298.
Strine, T.W., Chapman, D.P., Kobau, R., Balluz, L., 2005. Associations of self-reported anxiety symptoms with health-related quality of life and health behaviors. Soc. Psychiatr. Psychiatr. Epidemiol. 40, 432–438.
Svendsen, J.L., Osnes, B., Binder, P.E., Dundas, I., Visted, E., Nordby, H., et al., 2016. Trait self-compassion reflects emotional flexibility through an association with high vagally mediated heart rate variability. Mindfulness 7, 1103–1113.
Taylor-Clark, T.E., 2020. Air pollution-induced autonomic modulation. Physiology 35, 363–374.
Tobaldini, E., Bollati, V., Prado, M., Fiorelli, E.M., Pecis, M., Bissolotti, G., et al., 2018. Acute particulate matter affects cardiovascular autonomic modulation and ifn-γ methylation in healthy volunteers. Environ. Res. 161, 97–103.
Ushakov, A.V., Ivanchenko, V.S., Gagarina, A.A., 2016. Psychological stress in pathogenesis of essential hypertension. Curr. Hypertens. Rev. 12, 203–214.
Weichenthal, S., Hatzopoulou, M., Goldberg, M.S., 2014. Exposure to traffic-related air pollution during physical activity and acute changes in blood pressure, autonomic and micro-vascular function in women: a cross-over study. Part. Fibre Toxicol. 11, 70.
Weippert, M., Behrens, K., Rieger, A., Stoll, R., Kreuzfeld, S., 2013. Heart rate variability and blood pressure during dynamic and static exercise at similar heart rate levels. PLoS One 8, e83690.
Wiggert, N., Wilhelm, F.H., Nakajima, M., al'Absi, M., 2016. Chronic smoking, trait anxiety, and the physiological response to stress. Subst. Use Misuse 51, 1619–1628.
Williams, D.P., Feeling, N.R., Hill, L.K., Spangler, D.P., Koenig, J., Thayer, J.F., 2017. Resting heart rate variability, facets of rumination and trait anxiety: implications for the perseverative cognition hypothesis. Front. Hum. Neurosci. 11, 520.
Xu, Y., Zeng, F.F., He, L.P., Ling, W.H., Chen, W.Q., Chen, Y.M., 2014. Comparison of predictive value of cardiometabolic indices for subclinical atherosclerosis in Chinese adults. PLoS One 9, e93538.
Xue, Q., Patterson, A.J., Xiao, D., Zhang, L., 2014. Glucocorticoid modulates angiotensin ii receptor expression patterns and protects the heart from ischemia and reperfusion injury. PLoS One 9, e106827.
Zhou, Z., Wang, Y., Niu, Y., He, Z., Huang, M., Zhou, Y., et al., 2020. How we assess the perioperative anxiety of surgical patients with pulmonary nodules: the revision of state-trait anxiety inventory. J. Cardiothorac. Surg. 15, 324.
Zhu, Y., Huang, L., Li, J., Ying, Q., Zhang, H., Liu, X., et al., 2018. Sources of particulate matter in China: insights from source apportionment studies published in 1987-2017. Environ. Int. 115, 343–357.
Zorn, J.V., Schür, R.R., Boks, M.P., Kahn, R.S., Joëls, M., Vinkers, C.H., 2017. Cortisol stress reactivity across psychiatric disorders: a systematic review and meta-analysis. Psychoneuroendocrinology 77, 25–36.