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Approaches to mixture risk assessment of PFASs in the European population based on human hazard and biomonitoring data

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ABSTRACT

Per- and polyfluoroalkyl substances (PFASs) are a highly persistent, mobile, and bioaccumulative class of chemicals, of which emissions into the environment result in long-lasting contamination with high probability for causing adverse effects to human health and the environment. Within the European Biomonitoring Initiative HBM4EU, samples and data were collected in a harmonized way from human biomonitoring (HBM) studies in Europe to derive current exposure data across a geographic spread. We performed mixture risk assessments based on recent internal exposure data of PFASs in European teenagers generated in the HBM4EU Aligned Studies (dataset with N = 1957, sampling years 2014–2021). Mixture risk assessments were performed based on three hazard-based approaches: the Hazard Index (HI) approach, the sum value approach as used by the European Food Safety Authority (EFSA) and the Relative Potency Factor (RPF) approach. The HI approach resulted in the highest risk estimates, followed by the RPF approach and the sum value approach. The assessments indicate that PFAS exposure may result in a health risk in a considerable fraction of individuals in the HBM4EU teenager study sample, thereby confirming the conclusion drawn in the recent EFSA scientific opinion. This study underlines that HBM data are of added value in assessing the health risks of aggregate and cumulative exposure to PFASs, as such data are able to reflect exposure from different sources and via different routes.

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1. Introduction

1.1. Mixture exposure to PFASs in human biomonitoring

Per- and polyfluoroalkyl substances (PFASs) are a highly persistent, mobile, and bioaccumulative class of chemicals, of which emissions into the environment are expected to result in long-lasting contamination with high probability for causing adverse effects to human health and the environment (ECHA 2012a; ECHA 2012b; ECHA 2012c; ECHA 2012d; ECHA 2013a; ECHA 2013b; ECHA 2015; ECHA 2017a; ECHA 2017b; ECHA 2019a; ECHA 2019b). Several subclasses among the PFASs, such as perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl ether carboxylic acids (PFECAs) comprise substances that induce multi-organ toxicity (ATSDR 2021; EFSA 2020a). Due to their widespread use, persistency, bioaccumulation, and high bioavailability in the environment, humans are exposed to a variety of PFASs via, e.g., contaminated drinking water, food, consumer products, or house dust (Poothong et al., 2020; Colles et al., 2020).

Our current regulatory systems are not designed to adequately address combined exposure to multiple chemicals or aggregate exposure to the same chemical from multiple sources and pathways. Historically, risk assessment is performed for single chemicals and specific applications or uses within confined regulatory domains (Drakvik et al., 2020). For PFASs, as may be the case for many compounds, exposure is underestimated if combined exposure to multiple PFASs and aggregated exposure to the same PFAS from multiple sources and pathways is not included in risk assessment. In addition, considering that several PFASs result in the same adverse health effects, the risk assessment for individual compounds and single exposure sources may lead to an underestimation of the risk (EFSA 2019a). Therefore, mixture toxicity currently receives much attention, to bring forward the importance of assessing combined exposure to substances with similar toxicity profiles (European Commission 2020; Drakvik et al., 2020; EFSA 2019a). In a few exemplary cases, such as for dioxins and related PCBs in food and feed (EFSA 2018a), for phthalates in articles (ECHA 2017c) and food contact materials (EFSA 2019b), and pesticides in food (EFSA 2020b), the cumulative risk resulting from combined exposure to multiple substances has been assessed in a regulatory context.

Human biomonitoring (HBM) is a highly relevant tool to empirically observe aggregated exposure to PFASs in human blood or breast milk, particularly for the long half-life PFASs, and has an important role in screening for exposure to novel PFASs (Kaiser et al., 2021; Kang et al., 2020; Li et al., 2020a; Liu et al., 2019; Miaz et al., 2020). Commonly, studies report highly positive correlation coefficients between PFASs, illustrating that simultaneous exposure to multiple PFASs occurs at the individual level (EFSA 2020a; Kotlarz et al., 2020; Yu et al., 2020; Appendix A, Table A18-A27). This highlights the importance of focussing on combined exposure to multiple PFASs in risk assessment. The HBM4EU Aligned Studies collected samples and data in a harmonized way to derive current internal exposure data for the European population across a geographic spread (Gilles et al., 2021). Blood concentrations were measured in teenagers from nine studies (dataset with N = 1957, sampling period between 2014 and 2021) for 12 different PFASs (Gilles et al., 2021; Govarts et al., submitted; Gilles et al., 2022; Richterova et al., 2023). This dataset served as the basis of internal exposure data for the current paper.

1.2. Aim of this study

We present several ways of assessing the risk of mixtures of PFASs and address the challenges related to risk assessment of combined exposure to several similarly acting substances, when using exposure measurements in the blood plasma/serum of teenagers as primary input.

2. Methods

2.1. Methodologies for calculating mixture risk

2.1.1. The hazard index approach

The hazard index (HI) approach is a generally accepted tool for pragmatic mixture risk assessment that builds on the assumption of dose addition for calculating risk to chemical mixtures (Meek et al., 2011; Boberg et al., 2019). By using this approach, a hazard quotient is calculated for each single compound in the mixture based on the exposure level in the numerator (here human exposure levels, HBM data measured in the HBM4EU project) relative to the effect level (here defined as the effect level observed in human epidemiological studies) (Equation (1)). By summing the hazard quotients (HQs), the HI is calculated and a HI exceeding 1 indicates that a potential risk to human health may exist.

$$Hazard\ Index = \frac{Exp_1}{EL_1} + \frac{Exp_2}{EL_2} + \frac{Exp_3}{EL_3} + \ \ldots \ \frac{Exp_i}{EL_i} \qquad \text{(Eq. 1)}$$

Exp_i: exposure to compound *i*, expressed as ng/mL blood serum or plasma.

EL_i: the effect level of compound *i,* in ng/mL blood serum or plasma. In this study, we have used human internal exposures (presented as median or geometric mean plasma/serum concentration per study) statistically associated with either a given effect on immunotoxicity or on birth weight reductions.

Hazard Index = the sum of the hazard quotients of each chemical, which is the ratio of the human exposure to the substance relative to the effect level.

2.1.2. The sum value approach

EFSA derived a sum value approach for PFOA, PFNA, PFHxS and PFOS (hereafter called 'EFSA-4') and established a group tolerable weekly intake (TWI) of 4.4 ng/kg bw/week, corresponding to a serum concentration of 6.9 ng/mL in women of reproductive age, based on a serum concentration of 17.5 ng/mL in children of 1-year-old (EFSA 2020a). In their risk assessment, EFSA relied on the assumption that the EFSA-4 are equipotent for immunotoxic effects in humans and can be added without correction for potential differences in toxic potencies.

The cumulative risk, also defined as the risk characterization ratio (RCR), is estimated by summing the serum concentrations of the EFSA-4 in each individual and dividing this by the serum concentration that correspond to the TWI, i.e. the HBM guidance value (GV) (Equation (2)).

$$Cumulative\ risk = \frac{\sum Exp_i}{HBM\ GV_{PFASs}} \qquad \text{(Eq. 2)}$$

Exp_i = exposure to compound *i* per individual, whereby compound *i* is PFOA, PFNA, PFHxS or PFOS, in ng/mL blood serum or plasma.

HBM GV PFASs = the PFAS plasma level of 6.9 ng 'EFSA-4'/mL in women of reproductive age that corresponds to a level of 17.5 ng 'EFSA-4'/mL in children.

Cumulative risk = the combined risk from aggregate exposures to multiple PFASs.

2.1.3. The relative potency factor approach

The relative potency factor (RPF) approach for mixture risk assessment of PFAS builds on the assumption of dose addition, setting the potency of the index compound PFOA for liver toxicity in rat studies to 1, and expressing the toxicity of the other compounds relative to this as relative potency factors (Bil et al., 2021). For the purpose of evaluating mixtures in blood, RPFs were derived based on (modelled) serum concentrations in the male rat, thus reflecting internal relative potencies (Bil et al., 2022). Internal RPFs were available for nine PFASs (PFBA, PFHxA, PFOA, PFNA, PFDoDA, PFBS, PFHxS, PFOS and HFPO-DA), meaning that for some substances in the HBM4EU survey no internal RPF was available (PFHpS, PFPeA, PFHpA, PFDA, PFUnDA) due to absence of

suitable toxicity and/or toxicokinetic information available, whereas for some substances with an internal RPF (PFBA, HFPO-DA) no measurements were performed in the HBM4EU survey.

PFOA equivalent (PEQ) exposures for each PFAS were calculated by multiplying internal exposure of each individual participant (expressed as concentration in blood serum or plasma) by internal RPFs of the respective PFASs (Equation (3)). Subsequently, PEQ exposures were summed to obtain the sum PEQ for each individual. These may be expressed as percentiles per cohort or aggregated otherwise to reflect population exposure and can be used for risk assessment as if they represented exposure to PFOA solely. The cumulative risk is estimated by summing the PEQ exposure per individual and dividing this by the serum concentrations that correspond to the TWI (Equation (4)).

In combining the RPF approach with the HBM GVs of EFSA, it is assumed that not only the EFSA-4 cause immunotoxicity in humans but also the other PFASs for which RPFs are derived, and that the potency ranking of PFASs based on liver toxicity in animals can be extrapolated to the potency ranking of human toxicities such as immunotoxicity.

$$PFOA\ equivalent\ (PEQ) = Exp_i * RPF_i \quad \text{(Eq. 3)}$$

and

$$Cumulative\ risk = \frac{\sum PEQ_i}{HBM\ GV_{PFASs}} \quad \text{(Eq. 4)}$$

Exp_i = exposure to compound i per individual, whereby compound i is PFBA, PFHxA, PFOA, PFNA, PFDoDA, PFBS, PFHxS, PFOS or HFPO-DA, in ng/mL blood serum or plasma.

RPF_i = internal relative potency factor of compound i, whereby compound i is PFBA, PFHxA, PFOA, PFNA, PFDoDA, PFBS, PFHxS, PFOS or HFPO-DA.

PEQ_i = exposure to compound i per individual expressed in PFOA equivalents, whereby compound i is PFBA, PFHxA, PFOA, PFNA, PFDoDA, PFBS, PFHxS, PFOS or HFPO-DA.

HBM GV PFASs = the PFAS plasma level of 6.9 ng 'EFSA-4'/mL in women of reproductive age that corresponds to a level of 17.5 ng 'EFSA-4'/mL in children.

Cumulative risk = the combined risk from aggregate exposures to multiple PFASs.

2.2. Human biomonitoring data from the HBM4EU aligned studies

HBM4EU aimed to collect exposure data in European countries to sufficiently cover defined geographic regions, with preference given to data on PFAS exposure in teenagers of 12–19 years (Table 1). Serum, plasma or whole blood were the matrices of choice to measure exposure to PFASs (Vorkamp et al., 2021). The 12 PFASs that were included in the HBM4EU survey were PFBS, PFHxS, PFHpS, PFOS, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, and PFDoDA. General information on the number of individuals within each study, sex, age, sampling period, BMI, limit of detection (LOD) and limit of quantification (LOQ) is presented in Appendix A. More information on the data transformation to arrive at harmonized variables may be found in Gilles et al. (2021).

Exposure data are presented as percentiles of exposure per study (Table A6-A17; Appendix A). The 50th (P50) or 95th (P95) percentile values reflect an average or worst-case exposure scenario per study, respectively. These percentiles were used to calculate the HI for the mixture exposure to PFASs per study. When P50 or P95 was <LOD, between LOD-LOQ, or <LOQ, two scenarios were used to calculate the HI. In one scenario, the plasma/serum concentrations below LOQ were set at 0, and hence the HQ of these PFASs were not included in the HI. This scenario is used in the main paper.

In the other scenario, the summary statistics were imputed by LOD/2 (in case of <LOD), (LOD + LOQ)/2 (in case of between LOD and LOQ), or LOQ/2 (in case of <LOQ and LOD not available). This scenario is used in the Supplement (Appendix D). By using the latter approach, the cumulative risk may be driven by the number of substances for which the level is <LOD, between LOD-LOQ, or <LOQ. Moreover, because the LOD and LOQ values differ between studies, the degree by which this imputed value drives the cumulative risk may also differ among studies. The LOD and LOQ values for each study are provided in Appendix A.

In a deterministic approach, such as the HI approach described above, one relies on the assumption that the pairwise correlation among PFAS exposures is 1. However, pairwise correlations among PFAS vary (see Table A18-A27; Appendix A, for Spearman correlations between PFASs per cohort), and the magnitude of the correlations are strongly dependent on exposure source(s) (EFSA 2020a).

For the sum value approach and the RPF approach, we therefore used the mixture exposure at the individual level, using the raw exposure data of the PFAS mixtures for each person separately. This means that for the sum value approach, the serum or plasma concentration for PFOA, PFNA, PFHxS and PFOS were summed per individual, and the percentiles for the EFSA-4 per country were then used to interpret the risk. For the RPF approach, we multiplied the RPF with PFAS exposure concentrations to obtain PEQs per individual, and then presented the percentiles for the sum PEQs per country to interpret the risk.

For the sum value and RPF approach, two scenarios were used for the individuals that had measured PFAS values below LOD/LOQ. In one scenario, all values <LOQ were set at zero, after which the individual PFAS concentrations were summed to obtain sum exposure per individual. In the other scenario, the sum value was calculated in a way that individual PFAS measurements below the LOD/2 (in case of <LOD), LOD + LOQ/2 (in case of between LOD and LOQ), or LOQ/2 (in case of <LOQ) (Govarts et al. submitted). The first scenario is used in the main paper, and the outcome of the latter scenario is provided in the Supplement (Appendix E and F).

Please see Table 2 for an overview of the inclusion of PFASs in the different mixture risk assessments and the data used in these different methods.

Table 1
Human biomonitoring studies aligned within HBM4EU measuring PFASs in teenagers (Gilles et al., 2021).

Study name	Number of participants	Country	HBM4EU region
Riksmaten adolescents[a, c]	300	Sweden	North
Norwegian environmental biobank (NEB) II [b]	177	Norway	North
PCB cohort [a]	292	Slovakia	East
Exposure of children and adolescents to selected chemicals through their habitat environment (SLO CRP) [a]	94	Slovenia	South
Cross-Mediterranean Environment and Health Network (CROME) [a]	52	Greece	South
Biomonitorización en adolescents (BEA) [a]	300	Spain	South
Étude de santé sur l'environnement, la biosurveillance, l'activité physique et la nutrition (ESTEBAN) [a]	299	France	West
German Environmental Survey (GerES-sub) V [b,d]	300	Germany	West
Flemish Environment and Health Studies (FLEHS) IV [a]	143	Belgium	West

[a] Measured in blood serum.
[b] Measured in blood plasma.
[c] Data were obtained in a laboratory that did not participate in the HBM4EU Quality assurance/Quality control (QA/QC) program and consequently was not qualified within HBM4EU. Furthermore, data were initially reported as ng/g. To convert them to ng/mL, the assumption was made that 1 mL blood serum = 1 g blood serum.
[d] This is an unweighted subsample of the German Environmental Survey 2014–2017.

Table 2
Overview of the different mixture risk assessment approaches, the number of PFASs included in these assessments, and the different data that were used in these approaches.

	Hazard index	Sum value	Relative potency factor
Exposure assessment			
PFASs included	PFNA, PFDA, PFUnDA, PFDoDA, PFOS[a] PFOA, PFDA, PFHxS [b] PFOA, PFNA, PFHpS, PFOS [c] PFNA, PFDA, PFDoDA, PFUnDA[d]	PFOA, PFNA, PFHxS, PFOS	PFHxA,[e] PFOA, PFNA, PFDoDA, PFBS, PFHxS, PFOS
Type of data	Summary data, P50 and P95 per substance	Individual data, P50 mixture, P95 mixture	Individual data, P50 mixture, P95 mixture
Hazard assessment			
Type of effect studied in the mixture risk assessment	Birth weight reduction Immunotoxicity	Immunotoxicity	Immunotoxicity
Type of data used to account for differences in toxic potency	Effect levels (ELs) based on immunotoxicity and birth weight reductions in epidemiological studies (Table 3)	None	Internal relative potency factors (RPFs) obtained from rodent liver toxicity data (Appendix C) to convert PFAS serum concentrations to PFOA Equivalents (PEQs)
HBM GV used	None	EFSA sum value for teenagers and their (future) children	EFSA sum value for teenagers and their (future) children

[a] Using the ELs based on Kielsen et al. (2016).
[b] Using the Els based on Grandjean et al. (2012).
[c] Using the ELs based on Meng et al. (2018).
[d] Using the ELs based on Wang et al. (2016).
[e] The Norwegian Environmental Biobank (NEB) II did not contain measurements on PFHxA.

3. Results

3.1. Hazard assessment

3.1.1. Selection of effect levels for critical endpoints in the hazard index approach

EFSA (2020a) considered adverse endpoints on immunotoxicity as the most critical risk factor for exposure to PFASs. Furthermore, it identified decreases in birth weight as potential critical end-point (as well as increase in serum cholesterol and high serum levels of ALT), but noted that the TWI based on immune effects would be protective for the other potential critical end-points. We included impaired vaccination responses as the measure of immunotoxicity as well as decreased birth weight as the critical health effects in the hazard index approach.

All human epidemiological studies mentioned in EFSA (2020a) on immunotoxicity and birth outcomes were reviewed (except the critical study of Abraham et al. (2020)). Studies in which no or only few PFASs showed statistically significant (inverse) associations to immune effects or birth weight were excluded. A total of four studies were selected for further calculations. In these studies, single or multiple linear regression analysis was used to reveal associations between exposure to single PFASs and developmental- or immunological effects. We included the geometric mean or median PFAS exposure as effect level (i.e. point of departure, POD) when the regression coefficients (beta values and 95% confidence intervals) for these PFASs showed a statistically significant association with the exposure. The beta value reflects the magnitude of the effect (including its 95% confidence intervals) that is associated with a doubling of PFAS exposure. Some studies also complemented linear regression with quartile methods, but due to lack of statistical significance in pairwise comparison between quartiles of many of these associations and because not all studies used quartile methods, these outcomes were not used as effect level. A more detailed overview of these studies, including a summary of their conduct and outcomes, is provided in Appendix B. The corresponding PODs can be found in Table 3 and Appendix B.

3.1.2. TWI derivation for the sum value approach as discussed in EFSA (2020a)

Abraham et al. (2020) examined the relation between plasma PFAS concentrations in 1-year-old infants (N = 101) and antibody response against diphtheria, tetanus and Haemophilus influenza type b. EFSA employed benchmark dose (BMD) modelling for exposure to the sum of four PFASs, using a benchmark response of 10% decrease ($BMDL_{10}$) in antibody titres. The tolerable weekly 'EFSA-4' PFASs intake of mothers (of 4.4 ng/kg bw/week), in their life up to pregnancy, was then estimated. The extrapolation of the $BMDL_{10}$ to the TWI consisted of several intermediate steps, of which one was a modelled serum level of 6.9 ng 'EFSA-4' PFASs/mL in mothers at the age of 35 (EFSA 2020a). This value should protect their children from reaching a body burden of 17.5 ng 'EFSA-4' PFASs/mL via breastfeeding.

Thus, the TWI should prevent mothers from reaching PFAS levels in breastmilk at the age of 35 that would lead to a serum/plasma level in their infants that is associated with an impaired immune response. Consequently, high PFAS exposure of breastfed children is considered in derivation of the TWI. Therefore, EFSA (2020a) specifically mentioned that the intake of infants should not be compared to the TWI value. For teenagers we decided to use both a HBM GV value of 17.5 and 6.9 ng/mL for interpreting the risk based on internal exposures. That is, the HBM GV of 6.9 ng/mL should have been lower to be protective for future children of this specific female teenage subgroup, due to further build-up of PFASs in the body up to the age of 35, and therefore slightly underestimates the risk. For exceedance of the HBM GV of 17.5 ng/mL, we assume that teenagers are equally sensitive to immunosuppression by PFASs, however this is an assumption that warrants further study.

3.1.3. Selection of hazard data for RPF derivation

A database for 16 PFASs was previously created based on liver hypertrophy, absolute liver weight increase, and relative to body weight (bw) liver weight increase in the male rat upon oral (gavage) subchronic exposure (42–90 days) (Bil et al., 2021). This database was used for derivation of internal RPFs of nine PFASs, whereby internal, kinetically modelled, time-weighted average serum concentrations in the male rat were expressed against relative (to bw) liver weight increase to obtain RPFs (Bil et al., 2022). The internal RPFs for PFASs are presented in Table 4. The hazard database of the selection of these nine PFASs is to be found in Appendix C. The establishment of toxicokinetic models for many PFASs was hampered by the absence of toxicokinetic information for parametrization, and thus resulted in a lower number of internal RPFs compared to external RPFs.

The RPF approach for PFASs builds on the assumption that the potency ranking based on internal doses giving rise to liver toxicity in the male rat can be applied to humans. Moreover, we assume that the liver RPFs obtained in the male rat also apply to other endpoints, such as immunotoxicity and that all PFASs for which liver RPFs are available will cause an immunotoxic response.

Table 3
Associations between PFASs exposure and either immunotoxic effects or reduced birth weight in new-borns. The beta value represents the magnitude of the effect caused by a doubling of PFAS exposure. Statistically significant associations, of which the median or geometric mean were used as point of departures/effect levels for the hazard quotient calculations, are indicated in bold.

Study	Study population	N	Sample matrix	Sampling period	Median/geometric mean exposure and range in ng/mL[a]		Association between PFASs and health effects (beta ± 95% confidence interval)[b], per doubling of exposure		
Meng et al. (2018)	Mother-child pairs	3535 (PFOS and PFOA)	Blood plasma of mothers	First trimester, 1996–2002	PFOS	**30.1** (22.9–39.0)	Birth weight in boys + girls (gr)	PFOS	**−45.2** (−76.8, −13.6)
					PFOA	**4.6** (3.3–6.0)		PFOA	**−35.6** (−66.3, −5.0)
					PFHxS	1.0 (0.7–1.3)		PFHxS	1.2 (−28.3, 30.7)
		2120 (other PFASs)			PFNA	**0.5** (0.4–0.6)		PFNA	**−36.3** (−70.6, −2.0)
					PFHpS	**0.4** (0.3–0.5)		PFHpS	**−38.9** (−72.6, −5.1)
					PFDA	0.2 (0.1–0.2)		PFDA	−9.0 (−43.2, 25.2)
Wang et al. (2016)	Mother-child pairs	106[c]	Blood serum of mothers	Third trimester, 2000–2001	PFOA	1.98 (1.69–2.32)	Birth weight in girls (gr)	PFOA	−80 (−180, 10)
					PFNA	**1.44** (1.19–1.74)		PFNA	**−80** (−160, 0)
					PFDA	**0.37** (0.32–0.42)		PFDA	**−140** (−260, −20)
					PFUnDA	**2.89** (2.12–3.94)		PFUnDA	**−60** (−110, −10)
					PFDoDA	**0.30** (0.25–0.35)		PFDoDA	**−120** (−210, −20)
Grandjean et al. (2012)	Children followed from birth to year 7	440	Blood serum of mothers and children	At age of 5 (children), 2002–2005	PFOS	16.7 (13.5–21.1)	Percentage change in specific antibody response to tetanus vaccine at 7 yrs of age, 2 yrs post-vaccination	PFOS	−23.8 (−44.3, 4.2)
					PFOA	**4.06** (3.33–4.96)		PFOA	**−35.8** (−51.9, −14.2)
					PFHxS	**0.63** (0.45–0.88)		PFHxS	**−19.7** (−31.6, −5.7)
					PFNA	1.00 (0.76–1.24)		PFNA	−17.4 (−34.1, 3.6)
					PFDA	**0.28** (0.21–0.38)		PFDA	**−22.3** (−35.8, −5.8)
Kielsen et al. (2016)	Adults	12	Blood serum	Adults (average age 37 years), NA	PFHxS	0.37 (0.27–0.70)	Percentage change in specific antibody response to diphtheria vaccine 4–10 days post-vaccination	PFHxS	−13.31 (−25.07, 0.29)
					PFHpA	0.12 (0.094–0.14)		PFHpA	6.52 (−28.04, 57.7)
					PFOS	**9.52** (5.38–14.3)		PFOS	**−11.90** (−21.92, −0.33)
					PFOA	1.69 (1.30–2.79)		PFOA	−8.22 (−20.85, 6.44)
					PFNA	**0.66** (0.46–0.80)		PFNA	**−17.90** (−27.99, −6.39)

(*continued on next page*)

Table 3 (*continued*)

Study	Study population	N	Sample matrix	Sampling period	Median/geometric mean exposure and range in ng/mL[a]		Association between PFASs and health effects (beta ± 95% confidence interval)[b], per doubling of exposure	
					PFDA	**0.30** (0.20–0.32)	PFDA	**−18.18** (−29.52, −5.00)
					PFUnDA	**0.21** (0.18–0.27)	PFUnDA	**−12.11** (−22.06, −0.90)
					PFDoDA	**0.039** (0.035–0.048)	PFDoDA	**−15.64** (−28.14, −0.98)

[a] For Meng et al. (2018), median exposure and interquartile range (Q1-Q3) are presented. For Wang et al. (2016), geometric mean and 95% confidence interval are presented. For Grandjean et al. (2012), geometric mean and interquartile range (Q1-Q3 are presented. For Kielsen et al. (2016), median and interquartile range (Q1-Q3) are presented.

[b] For Meng et al. (2018), birth weight was adjusted for infant sex, infant birth year, gestational week of blood draw, maternal age, parity, socio-occupational status, pre-pregnancy body mass index (BMI), smoking and alcohol intake during pregnancy. For Wang et al. (2016), birth weight in girls was adjusted for family annual income, maternal age at delivery, maternal education, maternal previous live children, and maternal pre-pregnancy BMI. Grandjean et al. (2012), antibody response to tetanus vaccination was adjusted for age, sex, and booster type. Kielsen et al. (2016) antibody response was adjusted for sex and age. P-value in all studies was 0.05.

[c] A total of 223 mother-child couples were included in the study, but only statistically significant effects were seen for girls.

Table 4
Internal relative potencies for PFASs for liver toxicity in rodents compared to PFOA that was selected as the reference compound (Bil et al., 2022).

Compound	Internal RPF	Measured in HBM4EU
PFBS	0.2	Yes
PFHxS	0.6	Yes
PFOS	3	Yes
PFBA	2	No
PFHxA	10	Yes
PFOA	1	Yes
PFNA	5	Yes
PFDoDA	10	Yes
HFPO-DA	9	No

3.2. PFAS mixture risk assessments

3.2.1. Calculation of the cumulative risk based on the hazard index approach

Table 5 reports the final HIs calculated for teenagers based on the median exposure (P50). The outcome of this exercise illustrates that for immune effects, the HI is exceeded by taking the average exposure in all study populations except the Slovakian, German, and Spanish study populations, whereas for the Flanders study population the results are equivocal. For decreased birth weight, the HI is exceeded in the French study population, but not in that of Germany, Slovakia, Slovenia, Spain and Flanders. The results are equivocal for the Norwegian, Greek and Swedish study populations. Note that in Table 5, the LOD or LOQ was set at zero in cases where the substance could not be quantified in human blood.

For a more conservative illustration of exposure, the P95 may be used. For individuals with a relatively high exposure to PFASs, there is a risk for a compromised immune response in all cohorts from the various European study populations based on the results of Kielsen et al. (2016) and Grandjean et al. (2012). Moreover, a risk for decreased birth weight caused by PFAS exposure is indicated in all study populations based on the studies by Meng et al. (2018) and Wang et al. (2016) (Table 6).

Calculations that include setting values at half the LOD or LOQ are presented in Appendix D (Table D1 and Table D2). Especially for the studies with a high LOD and/or LOQ, the risk was significantly higher when taking this approach. This indicates that for these study populations, estimation of the risk is highly uncertain since it is mainly driven by the LOD and/or LOQ value.

3.2.2. Calculation of the cumulative risk based on the sum value approach of EFSA (2020a)

In Table 7 (and Appendix E, Table E2), the percentiles of exposure to the EFSA-4 are provided. Exposure to the EFSA-4 resulted in exceedance of the HBM GV in 1.3–24% of individuals per study population when the value of 6.9 ng/mL was used, and when non-detects for the separate compounds were treated as null exposure when calculating the sum. Risk Characterization Ratios (RCRs) were higher than one only in the highly exposed individuals (P95 scenario).

0–1.7% of the study populations exceeded the value of 17.5 ng/mL. The P50 and P95 did not exceed this HBM GV in any of the study populations, and consequently all RCRs remained below one (Table 7). When values below LOD/LOQ were attributed a value of half LOD/LOQ, there was only a marginal difference (Appendix E, Table E1). This is explained by the fact that only a low number of exposure values of the EFSA-4 are below LOD/LOQ (Appendix A).

Another important observation to note is that PFOS exposure is highest among all PFAS congeners, whereby exposure to this substance alone already resulted in exceedance of the HBM GV of 6.9 ng/mL at the higher percentiles of some of the study populations (Appendix A, Table A9).

3.2.3. Calculation of the cumulative risk based on internal RPFs

In Table 8 (and Appendix F, Table F2), the percentiles of exposure to either six (as PFHxA was not analysed in the Norwegian study) or seven PFASs is provided, expressed as PEQs.

Exposure to the sum PEQ resulted in exceedance of the HBM GV of 6.9 ng/mL in 41–96% of the study populations, when non-detects were treated as nulls. 1.7–23% of the study population exceeded the HBM GV of 17.5 ng/mL. RCRs were higher than one for the highly exposed individuals (P95) in all study populations, and below one for the median exposed individuals (P50). Some compounds contributed more to the cumulative risk because they had a high internal RPF (e.g. PFOS, PFNA).

In the scenario where values below LOD/LOQ were attributed a value of half LOD/LOQ, these attributed values were multiplied with their respective RPF. For substances with low detection frequency and for which the LOD/LOQ values were relatively high (e.g. for PFBS, PFHxA, PFDoDA in the French, Flemish and German study), these values contributed significantly to the risk estimate. Interpretation of the risk based on studies with such high LOD/LOQ is uncertain.

3.2.4. Comparison between approaches

PFAS exposure may pose a health risk in the teenagers' HBM4EU study population where risk estimates exceed an RCR/HI of one (Figs. 1 and 2). The HI approach resulted in the highest risk estimates based on

Table 5
PFAS mixture risk assessment based on the Hazard Index (HI) approach. HIs for PFASs were calculated for nine European teenage populations based on P50 values, where non-detects are treated as zeros. Hazard quotients are summed up to calculate the HI. A HI >1 indicates a potential risk.

	EL (ng/mL)		Norway	Sweden	Slovakia	Slovenia	Greece	Spain	Belgium	France	Germany
Immunotoxicity											
Kielsen et al. (2016)											
Adults											
PFOS	9.52	HQ	0.29	0.28	0.14	0.17	0.22	0.14	0.23	0.21	0.27
PFNA	0.66		0.67	0.57	0.26	0.38	0.63	0.43	0.48	0.82	ND
PFDA	0.3		0.43	0.49	0.17	0.47	0.57	ND	ND	0.72	ND
PFUnDA	0.21		0.41	ND	ND	0.29	0.17	ND	ND	0.52	ND
PFDoDA	0.039		ND	ND	ND	ND	ND	ND	ND	0.00	ND
		HI	**1.81**	**1.34**	**0.57**	**1.30**	**1.58**	**0.57**	**0.72**	**2.27**	**0.27**
Grandjean et al. (2012)											
Children											
PFOA	4.06	HQ	0.32	0.28	0.17	0.21	0.22	0.16	0.27	0.36	0.31
PFHxS	0.63		0.75	0.62	0.46	0.37	0.44	ND	0.78	1.09	0.62
PFDA	0.28		0.46	0.53	0.18	0.50	0.61	ND	ND	0.77	ND
		HI	**1.53**	**1.43**	**0.81**	**1.08**	**1.27**	**0.16**	**1.05**	**2.22**	**0.93**
Birth weight reduction											
Meng et al. (2018)											
Mothers											
PFOA	4.6	HQ	0.28	0.25	0.15	0.19	0.19	0.14	0.24	0.32	0.27
PFOS	30.1		0.09	0.09	0.05	0.05	0.07	0.04	0.07	0.07	0.09
PFNA	0.5		0.89	0.75	0.34	0.50	0.83	0.56	0.64	1.08	ND
PFHpS	0.4		ND	NA	0.08	0.08	0.13	ND	ND	ND	NA
		HI	**1.26**	**1.09**	**0.61**	**0.82**	**1.22**	**0.75**	**0.95**	**1.47**	**0.36**
Wang et al. (2016)											
Mothers											
PFNA	1.44	HQ	0.31	0.26	0.12	0.17	0.29	0.20	0.22	0.38	ND
PFDA	0.37		0.35	0.40	0.14	0.38	0.46	ND	ND	0.59	ND
PFDoDA	2.89		ND	ND	ND	ND	ND	ND	ND	ND	ND
PFUnDA	0.3		0.29	ND	ND	0.20	0.12	ND	ND	0.36	ND
		HI	**0.95**	**0.66**	**0.25**	**0.75**	**0.86**	**0.20**	**0.22**	**1.33**	**0**

EL = effect level.
HI = hazard index.
HQ = hazard quotient.
NA = not available.
ND = not detected (values below LOD or LOQ were set at zero).

P95 exposure, up to an HI of 6.2 for immune effects seen in the French study population. This is followed by the RPF approach, for which the highest RCR was 4.3 in the Swedish study population based on P95 exposures. By using the sum value approach, the highest RCR based on P95 exposure was 1.8, observed for the Swedish study population.

4. Discussion

The current work explores the use of HBM data in risk assessment, and the outcomes of each risk assessment should be interpreted in light of the uncertainties of the approaches considered. In order to perform these mixture risk assessment exercises, we have used approaches that have their advantages as well as some built-in assumptions and limitations, as we explain below.

4.1. Internal exposure data

In the mixture risk assessments, we have included recent PFASs exposure data from 2014 or later from teenagers in the European population. The HBM4EU Aligned Studies collected samples and data in a harmonized way and analysed them in laboratories that were qualified in the HBM4EU QA/QC program, but also built further on existing capacity. For this reason, there is still some heterogeneity in the data, e.g. the age of the study participants and sampling years and geographical representativity for Europe (Appendix A, Table A1). Furthermore, most studies quantified PFASs in serum, but two studies quantified PFASs in plasma (NEB II and GerES V). Consequently, differences due to this different exposure matrix cannot be excluded, although it is anticipated that the concentrations quantified in serum or plasma only differ slightly (Poothong et al., 2017). Lastly, not all studies measured total PFASs levels, being the sum of branched and linear forms, but only considered the linear form (i.e. the ESTEBAN and FLEHS IV studies). It may be that serum/plasma levels may have been higher by including both branched and linear forms.

In the HBM4EU Aligned Studies, short as well as long chain PFASs were measured in blood samples, but the detection frequencies of substances such as PFBS, PFPeA, and PFHxA were overall low. Apart from the high LOD/LOQ in some studies that could explain this, it may also have to do with the exposure matrix in which the substances were measured. In general, for short-chain PFASs, the half-life in humans is quantified in the range of days to months compared to years for the long-chain PFASs (EFSA, 2020a). For this reason, urine may also be a relevant matrix to consider to assess the exposure, complementary to blood samples. In a recent HBM study among the US population, paired urine and blood samples were obtained, which showed that urine may also be a relevant exposure matrix for PFASs with a short elimination half-live

Table 6
PFAS mixture risk assessment based on the Hazard Index (HI) approach. HIs for PFASs were calculated for nine European teenage populations based on P95 values, whereby non-detects are set at zero. Hazard quotients are summed up to calculate the hazard index. A HI >1 indicates a potential risk.

	EL (ng/mL)		Norway	Sweden	Slovakia	Slovenia	Greece	Spain	Belgium	France	Germany
Immunotoxicity											
Kielsen et al. (2016)											
Adults											
PFOS	9.52	HQ	0.74	0.86	0.65	0.61	0.55	0.32	0.77	0.65	0.62
PFNA	0.66		1.52	1.30	0.70	0.77	1.27	0.91	1.12	2.09	1.11
PFDA	0.3		1.01	1.43	0.57	0.94	1.33	0.92	1.67	1.69	1.24
PFUnDA	0.21		1.28	1.67	0.38	0.59	0.81	1.12	1.14	1.37	ND
PFDoDA	0.039		ND	ND	ND	0.77	0.63	ND	ND	ND	ND
		HI	**4.55**	**5.27**	**2.30**	**3.68**	**4.58**	**3.28**	**4.70**	**5.80**	**2.96**
Grandjean et al. (2012)											
Children											
PFOA	4.06	HQ	0.51	0.58	0.34	0.36	0.54	0.25	0.44	0.65	0.77
PFHxS	0.63		1.89	1.75	1.58	0.67	1.41	1.21	2.22	3.69	1.56
PFDA	0.28		1.08	1.54	0.61	1.00	1.42	0.99	1.79	1.81	1.32
		HI	**3.48**	**3.87**	**2.53**	**2.03**	**3.37**	**2.46**	**4.45**	**6.15**	**3.65**
Birth weight reduction											
Meng et al. (2018)											
Mothers											
PFOA	4.6	HQ	0.45	0.51	0.30	0.31	0.48	0.22	0.39	0.57	0.68
PFOS	30.1		0.23	0.27	0.20	0.19	0.17	0.10	0.24	0.20	0.20
PFNA	0.5		2.01	1.72	0.93	1.02	1.68	1.20	1.48	2.76	1.46
PFHpS	0.4		0.25	NA	0.49	0.28	0.36	0.63	ND	ND	NA
		HI	**2.95**	**2.51**	**1.92**	**1.81**	**2.69**	**2.16**	**2.11**	**3.54**	**2.34**
Wang et al. (2016)											
Mothers											
PFNA	1.44	HQ	0.70	0.60	0.32	0.35	0.58	0.42	0.51	0.96	0.51
PFDA	0.37		0.82	1.16	0.46	0.76	1.08	0.75	1.35	1.37	1.00
PFDoDA	2.89		ND	ND	ND	0.01	0.01	ND	ND	ND	ND
PFUnDA	0.3		0.89	1.17	0.27	0.41	0.57	0.79	0.80	0.96	ND
		HI	**2.41**	**2.93**	**1.05**	**1.53**	**2.23**	**1.95**	**2.67**	**3.29**	**1.51**

EL = effect level.
HI = hazard index.
HQ = hazard quotient.
NA = not available.
ND = not detected (values below LOD or LOQ were set at zero).

Table 7
PFAS mixture risk assessment based on the sum value approach. Percentiles of the distribution for the sum of EFSA-4 (PFOA, PFNA, PFOS, PFHxS) calculated for nine European teenage populations, where non-detects were set at zero. Risk Characterization Ratios (RCR) were calculated for each population (for median and high exposures, respectively) as well as the percentage of participants exceeding the HBM GV.

	Participants	Percentiles of the sum EFSA-4 in serum or plasma (ng/mL)		Participants exceeding the HBM GV of 6.9 ng/mL		RCR based on the P50 and the HBM GV of 6.9 ng/mL	RCR based on the P95 and the HBM GV of 6.9 ng/mL	Participants exceeding the HBM GV of 17.5 ng/mL		RCR based on the P50 and the HBM GV of 17.5 ng/mL	RCR based on the P95 and the HBM GV of 17.5 ng/mL
	N	P50	P95	N	%	RCR P50	RCR P95	N	%	RCR P50	RCR P95
Norway	177	5.15	11.0	31	17.5	0.75	1.59	0	0	0.29	0.63
Sweden	300	4.82	12.4	69	23.0	0.70	1.80	5	1.7	0.28	0.71
Slovakia	292	2.59	9.24	22	7.5	0.38	1.34	4	1.4	0.15	0.53
Slovenia	94	2.99	8.15	7	7.5	0.43	1.18	0	0	0.17	0.47
Greece	52	3.83	8.91	7	13.5	0.56	1.29	0	0	0.22	0.51
Spain	299	2.38	5.18	4	1.3	0.34	0.75	1	0.3	0.14	0.30
Belgium	300	4.18	10.2	51	17.0	0.61	1.48	1	0.3	0.24	0.58
France	143	4.91	11.3	34	23.8	0.71	1.64	2	1.4	0.28	0.65
Germany	300	4.35	9.78	49	16.3	0.63	1.42	3	1	0.25	0.56

(Calafat et al., 2019). The CONTAM panel recommended to conduct additional human biomonitoring studies on paired samples (blood--urine), to identify the relevant matrices for biomonitoring of various PFASs (EFSA 2020a). This would be a useful follow-up in additional HBM studies on PFASs under the PARC initiative.

Organofluorine mass balance analysis measures the total extractable

Table 8
PFAS mixture risk assessment based on the Relative Potency Factor (RPF) approach. Percentiles of the distribution for the sum of six or seven PFASs calculated for nine European teenage populations, where non-detects were set at zero. The exposure levels were corrected relative to the potency of PFOA, and all values were expressed as ng PFOA equivalents (PEQ)/mL serum or plasma. Risk Characterization Ratios (RCR) were calculated for each population (for median and high exposures, respectively), and the percentage of participants exceeding the HBM GV for the sum of four PFASs.

	Substances in sum PEQ	Participants	Percentiles of the sum of PFASs in serum or plasma expressed as PEQ ng/mL		Participants exceeding the HBM GV of 6.9 ng/mL		RCR based on the P50 and the HBM GV of 6.9 ng/mL	RCR based on the P95 and the HBM GV of 6.9 ng/mL	Participants exceeding the HBM GV of 17.5 ng/mL		RCR based on the P50 and the HBM GV of 17.5 ng/mL	RCR based on the P95 and the HBM GV of 17.5 ng/mL
	#	N	P50	P95	N	%	RCR P50	RCR P95	N	%	RCR P50	RCR P95
Norway	6[b]	177	12.4	28.5	169	95.5	1.80	4.13	32	18	0.71	1.63
Sweden	7[a]	300	11.8	29.9	266	88.7	1.71	4.33	68	23	0.67	1.71
Slovenia	7[a]	94	7.24	21.9	51	54.3	1.05	3.17	7	7.5	0.41	1.25
Slovakia	7[a]	292	6.88	26.2	145	49.7	1.00	3.80	27	9.3	0.39	1.50
Greece	7[a]	52	11.1	25.8	49	94.2	1.61	3.74	9	17	0.63	1.47
Spain	7[a]	299	6.26	13.7	123	41.1	0.91	1.99	5	1.7	0.36	0.78
Belgium	7[a]	300	9.86	27.6	230	76.7	1.43	4.00	48	16	0.56	1.58
France	7[a]	143	10.7	27.4	130	90.9	1.55	3.97	25	17	0.61	1.57
Germany	7[a]	300	9.63	23.9	232	77.3	1.40	3.46	30	10	0.55	1.37

[a] PFBS, PFHxS, PFOS, PFHxA, PFOA, PFNA, PFDoDA.
[b] PFBS, PFHxS, PFOS, PFOA, PFNA, PFDoDA.

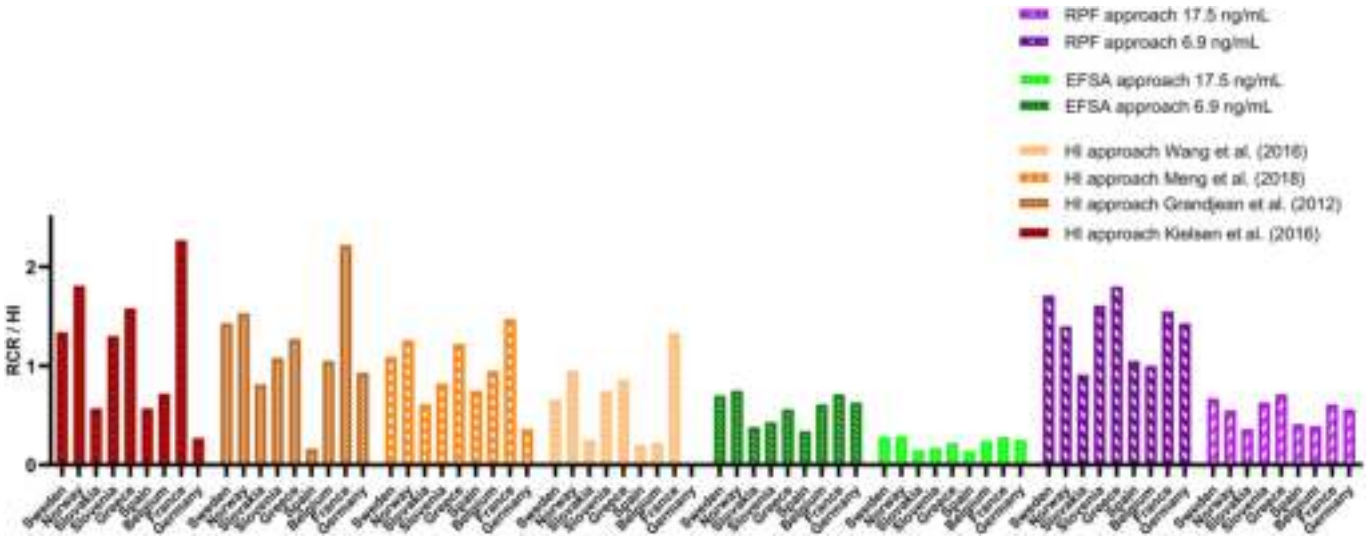

Fig. 1. Risk characterization ratio (RCR)/hazard index (HI) for simultaneous exposure to multiple PFASs based on P50 sum exposure in nine European teenage populations. Exposure values below limit of quantification (LOQ) were treated as nulls. Information on the number of individuals within the human biomonitoring study, sex, age, sampling period, LOD and LOQ is presented in Appendix A.

organofluorine (EOF) in the blood, and provides an indication of what fraction of the EOF can be attributed to particular PFASs, when performed together with a targeted PFAS analysis (Aro et al., 2021). Several studies have shown that the percentage of EOF accounted for by the sum of identified legacy PFASs in the human blood decreased in recent years, meaning that the fraction of unknown PFASs in human blood increased during this period (Aro et al., 2021; Miaz et al., 2020; Yeung and Mabury 2015). This stipulates the importance of organofluorine mass balance analysis and non-targeted- and suspect screening to gain insight into the exact PFAS composition in human blood, thereby enabling the identification of new and emerging PFASs. Currently the total PFASs mixture exposure is likely underestimated by focussing mainly on known PFCAs and PFSAs.

4.2. Hazard data

In both the HI approach and the sum value approach, we fully relied on human epidemiological data in risk assessment to estimate mixture risk, which is a novelty in risk assessment. However, the human epidemiological data we have used in the HI approach have limitations that one should be aware of. A clear distinction between cross-sectional studies, prospective studies, and case-control studies has not been made in the hazard assessment. Furthermore, the models used in these epidemiological studies were based on single or multiple linear regression analysis, and did not consider model averaging to address model uncertainty. Another inherent issue to the use of hazard data obtained from human epidemiological studies is the lack of a background response. This means that it is generally unknown how the response observed relates to the response if very low or no exposure occurs. This problem may be overcome by the use of an interpercentile range, whereby the data are divided over a sufficient number of ventiles/deciles/quartiles so that the lower percentiles(s) may serve as a background response (Li et al., 2020b).

The PODs we took for the HI approach are the average PFAS serum

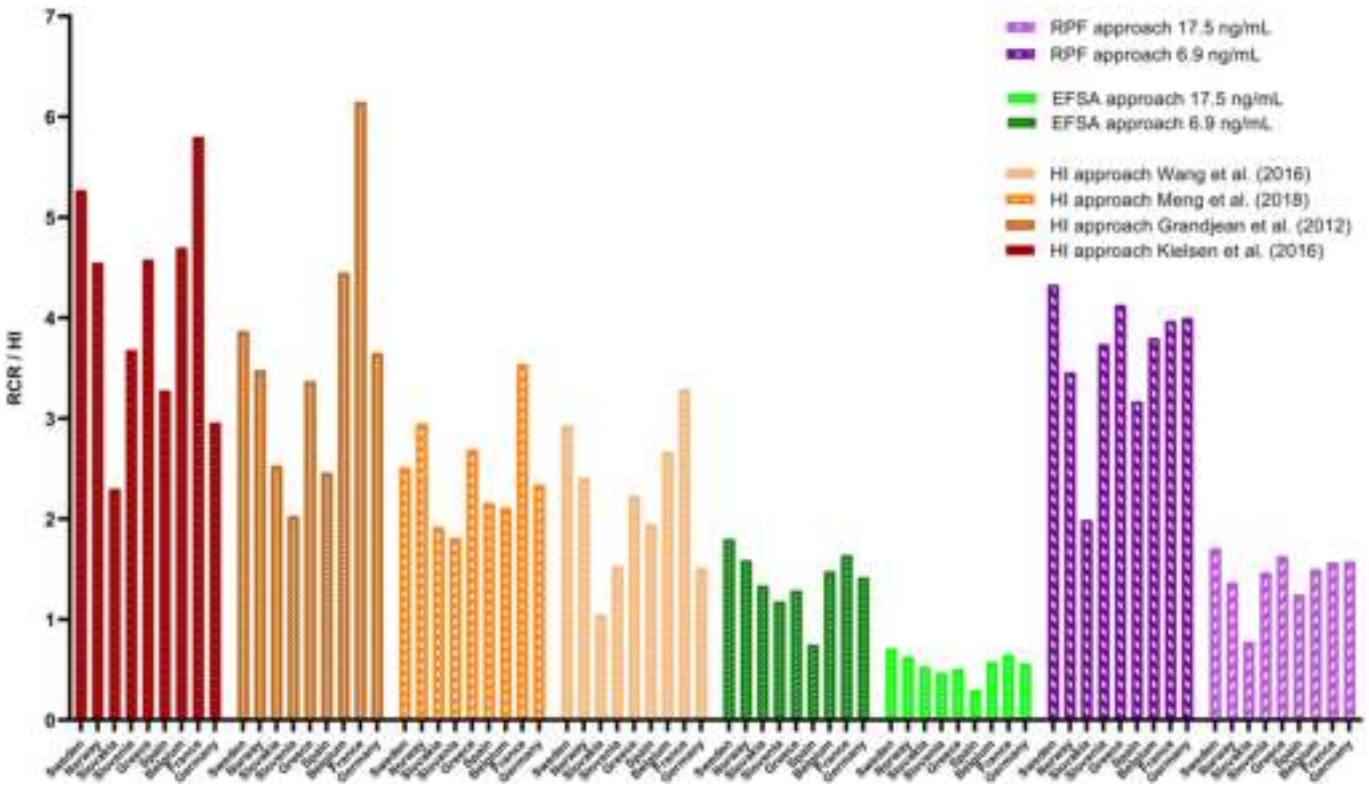

Fig. 2. Risk characterization ratio (RCR)/hazard index (HI) for simultaneous exposure to multiple PFASs based on P95 sum exposure in nine European teenage populations. Exposure values below limit of quantification (LOQ) were treated as nulls. Information on the number of individuals within the human biomonitoring study, sex, age, sampling period, LOD and LOQ is presented in Appendix A.

levels in a given study statistically significantly associated with the health outcome, which depends on the population studied and the exposure to PFASs in these populations. Furthermore, the serum concentration of one PFAS may be highly correlated with the serum concentration of another PFASs (as indicated in Appendix D) and therefore, it is impossible to determine which PFAS(s) drive(s) the association. If one of the components would be completely responsible for the adverse effect observed in the study, the others would not be expected to have any impact. A specific consequence of this is that PFASs with low concentrations (e.g. PFNA or PFHxS) may be statistically correlated with the health outcome in the study using a single pollutant model, but this may (at least partly) be due to confounding with the higher concentration PFASs (e.g. PFOS) with which it is correlated. In such cases, the POD of the low concentration PFASs would be overprotective. Discussion on the issues of using epidemiological data for risk assessment took place in 2018, when EFSA presented its scientific opinion on PFOS and PFOA (EFSA 2018b; EFSA 2018c). EFSA later performed BMD modelling for the sum of four PFASs in the recent opinion, to derive a $BMDL_{10}$ assuming equipotency of the four PFASs and absence of other PFASs/-substances or stressors which could also (partly) contribute to the effect.

As a default assumption, EFSA assumed in their risk assessment equipotency of PFOA, PFNA, PFOS and PFHxS (the 'EFSA-4') at POD level (immune effect in the child). In human epidemiological studies it is difficult to determine relative potencies and EFSA noted that the stronger association for PFOA compared to PFOS as indicated by the human epidemiological data, conflicted with the higher potency that is seen for PFOS compared to PFOA in various animal studies. Studying relative potencies in epidemiological studies is difficult in practice, as the serum concentration of one PFAS may be highly correlated with the serum concentration of other PFASs. Therefore, data from experimental animal studies can be used to explore if such an assumption on equipotency is likely, even if this approach suffers from the general issue of species differences. Our exercise on the derivation of internal relative potencies leads to question the assumption of equipotency between PFOA, PFOS, PFHxS and PFNA, because differences in potency between PFAS were observed at the serum level.

Concerning the limitations of the RPF approach, it is assumed that the PFASs for which an RPF is available exert the same toxicodynamic features as PFOA during similar exposure durations. Nevertheless, this extrapolation step needs some critical reflection. For other data-rich substances included in the assessment, such as PFOS, it would be valuable to evaluate whether the use of the RPF under- or overestimates the risk when substance-specific exposure and hazard data are used. Furthermore, it must be noted that the derivation of the internal RPFs is based on a limited dataset, namely liver effects in the male rat. The relevance of rodent data for use in risk assessment of PFASs has been questioned due to uncertainties in species concordance for certain endpoints, such as lipid perturbations and liver carcinogenicity, and differences in toxicokinetics and bioaccumulation potential among species (Corton et al., 2018; Pizzurro et al., 2019; Fenton et al., 2021). Such apparent discrepancies led to the conclusion that more information is needed regarding the mode(s) of action and adverse outcome pathways for PFAS toxicity, PFAS toxicokinetics in humans and experimental animals, and dose-response relationships among sexes, species and life-stages, whereas for many effects concordance between human epidemiological findings and experimental animal data exists (Fenton et al., 2021).

By using the RPFs in combination with the EFSA TWI, it is (provisionally) assumed that (1) other PFASs apart from 'EFSA-4' exert an adverse effect on the immune system and (2) the potency ranking of PFAS for liver toxicity in the rodent is the same as the potency ranking of PFAS for immunotoxicity in the human. A thorough comparison of PFASs relative potencies in relation to other effects, sex, life-stages, and species, and in particular for critical effect such as immune effects and mammary gland development, would be of added value.

4.2.1. Risk characterization

With regard to the different approaches:

- The mixture risk assessment using the HI approach demonstrated PFAS exposure may result in a health risk in the HBM4EU study population, when considering both P50 and P95 values. In this assessment, more than four PFASs were included, which is an advantage over the sum value approach. The basis of this mixture risk assessment was human data only, which is an advantage compared to the RPF methodology. However, due to the positive correlation between different PFASs in epidemiological studies this approach is likely to result in overestimation of risk. Finally, the HI approach used as such is more conservative to the other two approaches caused by the fact that combined exposure has not been assessed at the individual level but only at the population level.
- The sum value methodology is the most straight forward PFAS mixture risk assessment performed here, as the HBM GV of the 'EFSA-4' is directly compared to the recent European mixture exposure of the same compounds. This approach led to the conclusion that exposure to the sum of the EFSA-4 resulted in exceedance of the HBM GVs only when looking at P95 exposure. Since the P50 and P95 values were based on summing exposure to the EFSA-4 per individual, the influence of >1 correlations is avoided. This approach relies on human data, therefore not involving issues regarding interspecies differences. However, only four substances were included in the assessment, whereas exposure to more PFASs is apparent in the HBM studies. Furthermore, this approach assumed equipotency of PFASs at the internal level, which may not be the case.
- The RPF method showed that exposure to six or seven PFASs, expressed as the sum PEQ, resulted in exceedance of the HBM GVs when considering P50 and P95 values. In this assessment, more than four PFASs were included, which is an advantage over the sum value approach. Furthermore, since the P50 and P95 values were based on summing PEQ per individual, the influence of >1 correlations was avoided. Moreover, differential potencies of the different PFASs is taken into account in the mixture risk assessment. However, this includes an uncertainty related to the extrapolation of the RPFs based on liver toxicity in experimental animals to immunotoxicity in humans. Ideally, this approach should include a serum concentration on which the toxicological point of departure is based, expressed in PEQs (i.e. an 'RPF adjusted' HBM GV). However, due to practical reasons it is currently not feasible to adjust the POD underlying the EFSA TWI from EFSA-4 sum exposure to PEQs. This is recommended to take into account later on, should the EFSA TWI be revised in the future.

4.3. Strengths of this study

In the current paper, we performed mixture risk assessments with HBM data as primary input. HBM data are very valuable to calculate the cumulative risk resulting from exposure to PFASs from multiple sources. Since exposure to PFASs stem from a wide variety of sources across different routes, exposure is underestimated if combined exposure to multiple PFASs and aggregated exposure to the same PFAS from multiple sources and pathways is not included in risk assessment. Hence, these mixture risk assessments targeted both combined exposure to multiple chemicals and aggregate exposure to the same chemicals from multiple sources and pathways.

Another strength of our study is the use of individual exposure data. Within HBM4EU, individual data could be shared via a single collaboration agreement, which restricted exchange of data to the information required to answer the research question defined (Gilles et al., 2021). Via this collaboration agreement, we were able to use individual exposure data to calculate summed exposure to PFASs on an individual level, rather than relying on summary statistics (e.g. P50, P95). In this way, sum exposure to multiple PFASs was not overestimated.

Lastly, EFSA considered exposure from dietary intake for specific age groups and expressed this against the TWI of 4.4 ng/kg bw/week. Based on these calculations, EFSA concluded on a risk for both adolescents and adults based on mean and high dietary intake. Overall, the results in the current mixture risk assessments are in line with the risk characterization in the EFSA opinion, which strengthens the overall conclusion that adverse health effects may arise in the European population due to PFAS mixture exposure.

5. Conclusion and perspectives

The mixture risk assessments show that combined exposure to PFASs is too high and may result in a human health risk in a substantial fraction of the HBM4EU study population, thereby confirming the conclusion drawn in the recent EFSA scientific opinion. Long-term exceedance of the HBM GV is undesirable and a reason to reduce human exposure to a level below this threshold. Thus, it is important to map exposure sources to PFASs and target them with effective policy measures so that human exposure to PFASs in general will be reduced.

For future research, the following recommendations aid to refine and improve mixture risk assessment of PFASs using human biomonitoring data:

- In general, we observed that risk assessments, based on HBM data obtained from studies that relied on relatively high LOD and LOQ, are very uncertain. We therefore stress the need for improving the analytical methods for human biomonitoring and for bringing down the LOD and LOQ of the PFAS analyses;
- We recommend to perform human biomonitoring studies that observe paired blood-urine samples to have a better insight into exposure to short-chain PFASs;
- We recommend to perform organofluorine mass balance analysis and non-targeted- and suspect screening to gain insight into the exact PFAS mixture composition in human blood, thereby enabling the identification of new and emerging PFASs;
- We recommend to further explore linkages between PFAS mixture exposure and health effects that would allow point of departure setting for PFAS mixture risk assessment, taking into account the background response of the health effects studied;
- We recommend to derive RPFs for PFASs that currently have none available, and to validate relative potencies of PFASs in relation to other effects, sex, life-stages, and species, and in particular for critical effects such as immune effects;
- We recommend to develop human-based *in vitro/ex vivo* models for immunotoxicity and developmental toxicity that can mimic the human responses. We advise, complementary to this, to investigate mode(s) of action and adverse outcome pathways for PFASs to overcome data gaps and to shed light on species concordance.

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Declaration of competing interest

The authors have no conflicts of interest to declare.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.114071.

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Corrigendum to "Harmonization of acronyms for volatile organic compound metabolites using a standardized naming system" [Int. J. Hygiene Environ. Health 235 (2021) 113749]

Denise S. Tevis [a], Sharon R. Flores [a], Brandon M. Kenwood [a], Deepak Bhandari [a,*], Peyton Jacob 3rd [b], Jia Liu [b], Pawel K. Lorkiewicz [c], Daniel J. Conklin [c], Stephen S. Hecht [d], Maciej L. Goniewicz [e], Benjamin C. Blount [a], Víctor R. De Jesús [a]

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The authors regret an error in Table 4 on page 113749. The chemical name for VOCM acronym 4HBeMA is misnamed as N-Acetyl-S-(4-hydroxy-3-buten-1-yl)-L-cysteine. The correct name is N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine.

The authors would like to apologise for any inconvenience caused.

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Corrigendum to "Impact of heat waves and cold spells on cause specific mortality in the city of São Paulo, Brazil" [Int. J. Hyg. Environ. Health 239 (2022), 113861]

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The authors regret that in Fig. 3 title text "* The confidence interval is not represented in the graph for better visualization of the other RRs. Confidence intervals are presented in Supplementary Table 1." is incorrectly shown as Supplementary Table 1. It should be corrected to "* The confidence interval is not represented in the graph for better visualization of the other RRs. Confidence intervals are presented in **Supplementary Table 2**"

Figs. 2 and 4 legends indicate the heat wave models as HW_92P_2d, HW_92P_3d, HW_92P_4d, HW_97P_2d, HW_97P_3d, and HW_97P_4d. It should be updated to: **HW_92.5P_2d, HW_92.5P_3d, HW_92.5P_4d, HW_97.5P_2d, HW_97.5P_3d, and HW_97.5P_4d.**

Corrected figures (legends) as follows:

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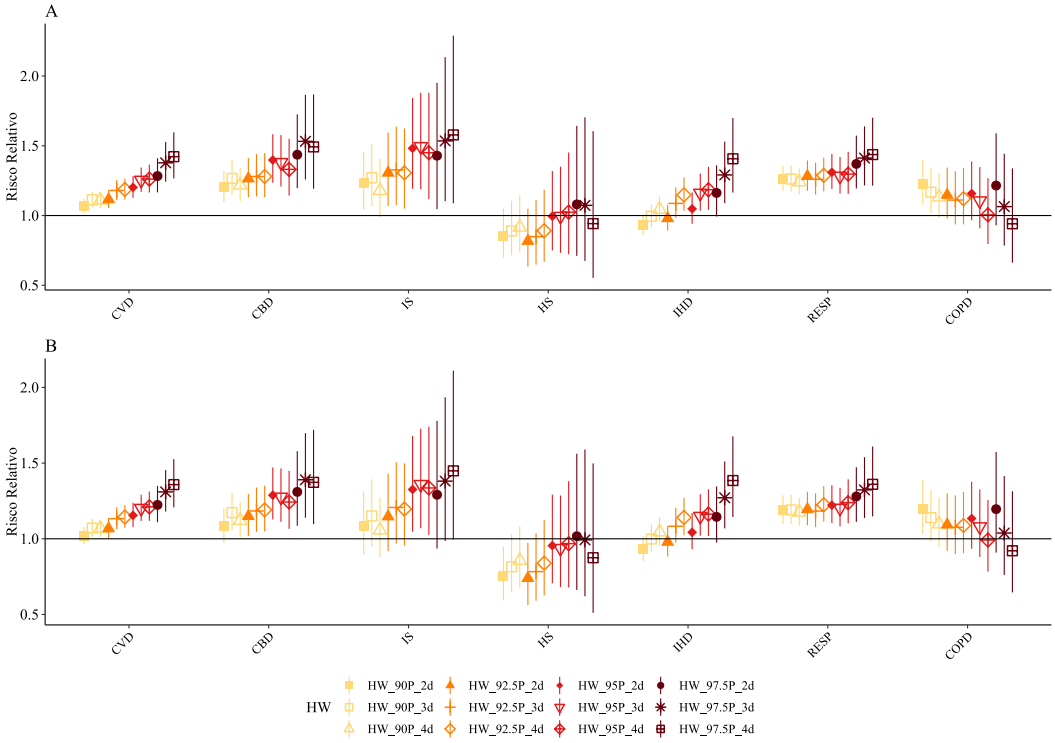

Fig. 2. Effects of heat waves under 12 definitions on mortality due to cardiovascular disease (CVD), cerebrovascular disease (CBD), ischemic stroke (IS), hemorrhagic stroke (HS), ischemic heart disease (IHD), respiratory disease (RESP), and chronic obstructive pulmonary disease (COPD) in people aged ≥65 years over lag 0–10 days in São Paulo. (A) Overall effects of heat waves without controlling for daily mean temperature (B) Added effects of heat waves after controlling for daily mean temperature.

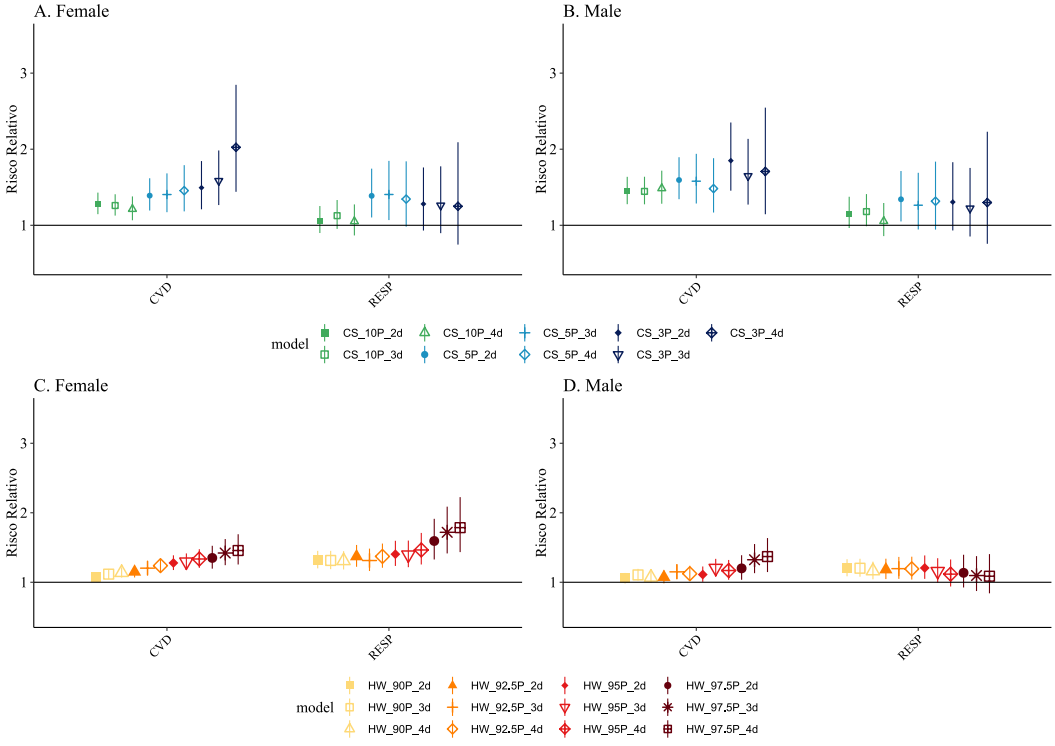

Fig. 4. Effects of cold spells (A and B) and heat waves (C and D) on cardiovascular diseases (CVD) and respiratory diseases (RESP) mortality stratified by sex (women and men).

The results were accurate in the original paper and were not affected by these corrections. The authors would like to apologise for any inconvenience caused.

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Corrigendum to "Substitutes mimic the exposure behaviour of REACH regulated phthalates – A review of the German HBM system on the example of plasticizers" [Int. J. Hyg. Environ. Health 236 (2021) 113780]

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The authors regret that some statements in chapter 3 "Human biomonitoring of phthalates and substitute plasticizers" are wrong due to missing text in the previously published version. Below is the corrected statement with changes in bold font.

For DPHP, **effects on the thyroid gland and pituitary gland, both observed in a subchronic toxicity study in rats, are considered critical (BfR, 2011; Bhat et al., 2014; German HBM Commission, 2015). DPHP is currently being under assessment for endocrine disruption (ECHA, 2021b). DEHTP is not considered toxic for reproduction and no warning for potential endocrine disrupting properties were identified under the regulatory management option analysis (ECHA, 2021a).** EFSA and also the German HBM Commission based their guidance values on the combined chronic and carcinogenicity dietary study in rats by Deyo (2008), in which effects on the retina were considered critical (Apel et al., 2017; Deyo, 2008; EFSA, 2008).

The authors would like to apologise for any inconvenience caused.

Reference

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Corrigendum

Corrigendum to "Transportation noise and incidence of hypertension" [Int. J. Hyg. Environ. Health 221 (2018) 1133–1141]

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The authors regret to inform about the following corrections to the publication "Transportation noise and incidence of hypertension". In our publication, we reported on an association between aircraft noise and incidence of hypertension in a population from Stockholm, Sweden, with a HR of 1.16 (95% CI 1.08–1.24) per 10 dB L_{den} 5 years preceding the event. However, after discovering a coding error leading to an underestimation of the person-time among exposed subjects, the association was no longer statistically significant (HR 1.03, 95% CI 0.94–1.12). On the other hand, in analyses using exposure at baseline, an increased risk of hypertension in relation to aircraft noise exposure was shown, with a HR 1.11 of (95% CI 1.03–1.19) per 10 dB L_{den} (see the updated version of 'Table 2'). In this table the analyses using exposure at baseline replace those based on the 10-year exposure in the original table which contained few cases. Furthermore, analyses based on exposure at baseline indicated a particularly high risk of hypertension among persons exposed to both aircraft and road traffic noise ≥45 dB L_{den}, with a HR of 1.28 (95% CI 1.06–1.55). Thus, although the coding error changed most of the detailed results, the main conclusions of the paper are still valid. A revised version of the manuscript is available upon request from the first author. The authors would like to apologise for any inconvenience caused.

Revised Table 2
Hazard ratio for hypertension in relation to noise exposure from road, railway or aircraft traffic at 1 and 5 years preceding diagnosis of hypertension and at baseline.

	1 year preceding the event				5 years preceding the event				At baseline			
	Person years	Cases	HR*	95% CI	Person years	Cases	HR*	95% CI	Person years	Cases	HR*	95% CI
Road traffic noise, dB, L_{den}												
<45	27,259	910	1.00	ref.	28,318	945	1.00	ref.	28,786	970	1.00	ref.
45–49	6,819	244	1.03	(0.90; 1.19)	6,699	244	1.05	(0.91; 1.21)	6,346	247	1.14	(0.99; 1.31)
50–54	4,293	171	1.13	(0.96; 1.33)	3,795	152	1.13	(0.95; 1.34)	3,639	131	1.04	(0.86; 1.24)
≥55	1,726	69	1.07	(0.84; 1.37)	1,353	53	1.03	(0.78; 1.36)	1,246	39	0.87	(0.63; 1.20)
per 10 dB	40,096	1,394	1.02	(0.95; 1.10)	40,165	1,394	1.03	(0.96; 1.12)	40,017	1,387	0.99	(0.91; 1.07)
Railway noise, dB, L_{den}												
<45	36,699	1,273	1.00	ref.	37,058	1,286	1.00	ref.	36,817	1,283	1.00	ref.
45–49	1,337	58	1.13	(0.87; 1.47)	1,269	58	1.22	(0.94; 1.59)	1,221	47	1.06	(0.79; 1.42)
50–54	943	35	1.01	(0.72; 1.41)	895	27	0.84	(0.57; 1.23)	934	28	0.85	(0.58; 1.24)
≥55	1,118	28	0.67	(0.46; 0.97)	944	23	0.65	(0.43; 0.99)	1,046	29	0.77	(0.53; 1.11)
per 10 dB	40,096	1,394	0.95	(0.86; 1.04)	40,165	1,394	0.94	(0.85; 1.04)	40,017	1,387	0.94	(0.85; 1.04)
Aircraft noise, dB, L_{den}												
<45	32,511	1,131	1.00	ref.	32,447	1,134	1.00	ref.	31,029	1,045	1.00	ref.
45–49	4,066	176	1.23	(1.05; 1.44)	3,312	147	1.28	(1.08; 1.52)	3,514	115	0.96	(0.79; 1.17)
50–54	2,654	63	0.82	(0.63; 1.06)	3,469	87	0.80	(0.64; 1.00)	4,308	180	1.25	(1.07; 1.47)
≥55	831	23	0.93	(0.61; 1.41)	896	25	0.92	(0.62; 1.37)	1,151	47	1.25	(0.93; 1.68)
per 10 dB	40,062	1,393	1.02	(0.93; 1.11)	40,124	1,393	1.03	(0.94; 1.12)	40,001	1,387	1.11	(1.03; 1.19)

*Hazard ratios (HR) and 95% confidence intervals (95% CI) adjusted for sex, educational level, physical activity during leisure time, psychological distress and diabetes heredity at recruitment.

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Corrigendum to Water and health seminar and special issue highlight ideas that will change the field. [International Journal of Hygiene and Environmental Health 226C (2020) 113529]

The authors regret that the printed version of the above article contained a number of errors. The correct and final version follows. The authors would like to apologise for any inconvenience caused.

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Domestic water security in the Arctic: A scoping review

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ABSTRACT

Introduction: More than 50 million people living in the Arctic nations remain without access to safely managed drinking water services. Remote northern communities, where large numbers of Indigenous peoples live, are disproportionally affected. Recent research has documented water and health-related problems among Indigenous communities, including poor water quality and insufficient quantities of water.
Objective: The objective of this scoping review is to examine the extent of available water security evidence as well as identify research gaps and intervention priorities to improve access to domestic water in the Arctic and Subarctic regions of the eight Arctic nations (Canada, the Kingdom of Denmark (Greenland), Finland, Iceland, Norway, Sweden, Russia, and the United States (Alaska)).
Methods: An extensive literature review was conducted to retrieve relevant documentation. Arctic & Antarctic Regions, Compendex, Geobase, Georef, MEDLINE and Web of Science databases were searched to identify records for inclusion. The initial searches yielded a total of 1356 records. Two independent reviewers systematically screened identified records using selection criteria. Descriptive analyses were used to summarize evidence of included studies.
Results: A total of 55 studies, mostly conducted in Canada and the United States, were included and classified by four predetermined major dimensions: 1) Water accessibility and availability; 2) Water quality assessment; 3) Water supply and health; 4) Preferences and risk perceptions.
Conclusions: This scoping review used a global approach to provide researchers and stakeholders with a summary of the evidence available regarding water security and domestic access in the Arctic. Culturally appropriate health-based interventions are necessary to ensure inclusive water services and achieve the Sustainable Development Goals (SDG) targets for universal access to water.

1. Introduction

Access to drinking water has been recognized as a fundamental human right by the United Nations General Assembly since 2010 (UN Committee on Economic Social and Cultural Rights, 2010). The WHO/UNICEF Joint Monitoring Programme for Water Supply, Sanitation and Hygiene (JMP) reported that, in 2020, 26% of the world population did not have access to safely managed drinking water services, that is located on premises, available when needed, and free from contamination (WHO/UNICEF, 2021). Whilst efforts to meet Agenda 2030 have been focussed on the world's most disadvantaged regions including sub-Saharan Africa, Eastern and South-Eastern Asia, and Central and South Asia, nearly 50 million people living in Europe and North America remain without access to safely managed drinking water services (WHO/UNICEF, 2021).

In the Arctic nations, i.e., Canada, Kingdom of Denmark, Finland, Iceland, Norway, Russian Federation, Sweden and the United States, nearly universal access to safely managed drinking water has been reported by JMP in 2020 (99% in Canada; >99% in the Kingdom of Denmark, Finland, Iceland, Norway and the United States, and 76% in the Russian Federation). In light of uncertainties in national statistics, it has been recognized that disaggregated estimate may not reflect the situation of small populations such as Indigenous groups (WHO/UNICEF, 2021). Despite the importance of targeting marginalized members of society, limited population data remains available, including the status of water, sanitation and hygiene (WASH) services (Hennessy and Bressler, 2016), in the Arctic region (i.e., above the Arctic circle), where approximately four million people live, around 10% of whom are

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Indigenous peoples (Stepien et al., 2014).

The presence of permafrost below many Arctic communities complicates the use of underground distribution networks, thus alternatives to piped water supply networks-such as trucked water to cistern or potable water dispensing units (PWDU) are commonly used in these areas. The use of these alternative distribution systems in remote communities, however, has been suggested to pose significant challenges to drinking water supply and distribution from the catchment area to the household, including water treatment, distribution and delivery, in-home storage, availability of accessible water, and difficulties in maintaining water quality standards from the point of entry to the point of use (Bradford et al., 2018; Farenhorst et al., 2017; Martin et al., 2007). For example, in Canada, many remote Indigenous communities have long been under drinking water advisories which have been shown to influence household perceptions and practices in accessing drinking water (Lam et al., 2017; Syme and Williams, 1993). Despite their disproportionate impact, water challenges faced by remote northern communities were found to be poorly discussed in national news articles in Canada (Lam et al., 2017). Further to the operationalization of water supply, households living in remote communities face additional water-related problems including poor in-home water quality, insufficient quantities of water (Daley et al., 2014) and lack of trust or rejection of the water delivered due to organoleptic properties (de França Doria, 2009). Results from a systematic review on climate, water and health in the Arctic, show that this area remains understudied. Similarly, an increase in water-borne illnesses and diseases was highlighted as an important concern associated with climate change in the Scandinavian countries (Harper et al., 2020; Semenza et al., 2012). Overall issues associated with drinking water services are not well documented in the Arctic (Bradford et al., 2016; Hennessy and Bressler, 2016), however, such information is essential for identifying appropriate interventions and developing sustainable solutions to ensure access to water for all and meet United Nations Sustainable Development Goal 6. This clearly highlights the needs to further investigate access to water and document the gap that may exist between Indigenous and non-Indigenous peoples.

Evidence-based knowledge is crucial to identify and develop culturally appropriate health-based interventions to improve water services, and this should be done through effective methods and appropriate data collection mechanisms (Bailie et al., 2004). With this in mind, the objectives of this scoping review are to appraise available evidence as well as identify knowledge gaps in the literature in view of addressing the following research questions: 1) What are the challenges affecting water supply services in the Arctic; 2) What considerations should be made when implementing interventions to improve access to domestic water in remote Indigenous communities.

2. Methods

This scoping review was conducted following the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) method (Tricco et al., 2018). The search strategy procedure was revised by the research team, with additional feedback sought from external collaborators and librarians. The protocol used for this study is available on request from the corresponding author.

2.1. Eligibility criteria

Studies focusing on drinking water supply services, access to water for drinking or domestic purposes or reporting related challenges were sought for inclusion in this review. Peer-reviewed articles and grey literature conducted in the Arctic nations (i.e., Canada, the Kingdom of Denmark (Greenland), Finland, Iceland, Norway, Sweden, the Russian Federation and the United States (Alaska)) were considered eligible for this review. No initial restriction on publication language was established, however, English was consistently used to yield searches.

2.2. Information sources

A comprehensive literature search of peer-review publications was conducted using bibliographic databases, including Arctic & Antarctic Regions, Compendex, Geobase, Georef, MEDLINE and Web of Science. The Arctic Science and Technology Information System (ASTIS) and High North Research Documents databases were also searched for peer-review publications and grey literature. Additional publications were identified using various search portal including Google Scholar, OpenGrey, Arctic Health Publications Database and Arctic Portal Library. Documentation from government agencies, non-profit organization, research institutes, and other organizations were considered.

2.3. Search strategy

The search strategy for bibliographic databases included three different sets of criteria related to access to water in remote communities in the Arctic: water, peoples and regions (Table 1). The search strategy combined free and standardized subject terms or controlled vocabulary search terms, including index terms or MeSh. The search strategy adapted for each database requirements and the approach used to identify grey literature are documented in Annexe 1.

2.4. Study selection and inclusion

The selection process was designed following the PRISMA chart flow and guideline (Page et al., 2021). The literature search was performed, and records were initially retrieved by Cassivi A on February 11, 2021. All records identified were uploaded using EndNote X9 reference management software and duplicates were removed before screening. Titles and abstracts were screened by two independent reviewers (Cassivi A., Covey A.) to determine which studies were potentially eligible and relevant to the aim of this review; in case of any disagreement a third reviewer (Guilherme S.) was consulted to reach consensus. The full texts of all studies were retrieved and downloaded, and interlibrary loans (ILL) were requested for records that were not part of Université Laval library's collection. All records selected and retrieved were screened for inclusion; records were initially screened by the first reviewer and those that were excluded were double screened by a second reviewer to avoid

Table 1
General search strategy used for identification of studies.

Set 1 – Water	
#1	((drink* OR domestic OR "in-home" OR potable OR piped OR safe OR (security OR insecurity) OR service* OR source* OR suppl* OR system* OR tap) NEAR/1 water)
Set 2 – Peoples [a]	
#2	(Aboriginal OR Aleut OR Alutiiq OR Athabaska OR Eskimo OR "First nation*" OR "Gwich'in" OR Indigenous OR Inupiaq OR Inuit* OR Metis OR Native* OR Saami OR Sami OR Yupik OR Tlingit)
#3	((Arctic OR Northern OR Remote) AND (communit* OR hamlet* OR people* OR settlement* OR village*))
#4	#2 OR #3
Set 3 – Regions	
#5	(Arctic OR circumpolar OR (Canada OR "Northwest Territories" OR Nunangat OR Nunavik OR Nunavut OR Nunatsiavut OR Inuvialuit OR Yukon) OR (Denmark OR "Faroe Islands" OR Greenland) OR (Finland OR Kainuu OR "Northern Ostrobothnia" OR Sapmi OR Lapland) OR Iceland OR (Norway OR Nordland OR "Troms and Finnmark" OR Svalbard OR "Jan Mayen") OR (Sweden OR Västerbotten OR Norrbotten) OR (Russia OR Murmansk OR Nenets OR "Yamal Nenets" OR "Chukotka Autonomous Okrugs" OR Arkhangelsk OR "Komi Republic" OR "Krasnoyarsk Territory" OR (Sakha OR Yakutia) OR Siberia) OR Alaska)
Final Search	
#6	#1 AND #4 AND #5

[a] The historic terms to describe Indigenous peoples (e.g., "*Eskimo*", "tribe") were included in the search strategy as once commonly used. The authors do not endorse the usage of these terms and will refer to the standard endonyms as defined in the Canadian Encyclopedia.

discrepancies. Again, in case of disagreement, the third reviewer was consulted for consensus.

The detailed inclusion and exclusion criteria are listed in Table 2. Original research articles including empirical evidence that focus on access to water for drinking and domestic purposes, i.e., accessibility, availability, quantity, quality, affordability, as well as related outcomes, e.g., hygiene and/or public health were considered for inclusion. The articles were required to refer to or have been conducted in the Arctic region; studies conducted in the Subarctic region were also considered eligible to transcend boundaries and respect to territorial claims. The population of interest included human populations (individuals, households and communities); articles referring to habitat-related, animal species or aquatic environment, as well as studies focusing on industrial, institutional or commercial sectors were excluded. Studies addressing water-related issues from a governance, law or policy perspective (e.g., regulations, guidelines, action plans and frameworks), as well as engagement (e.g., Indigenous knowledge) were considered outside the scope of this study and therefore also excluded. The studies focusing on engineering processes of water systems (e.g., design and/or construction of water treatment plant and/or distribution systems) or that related to the cross-discipline field of geology and hydrology (e.g., freshwater availability, watershed processes, water resources, groundwater processes) were also excluded from this review. The cited references of studies selected for inclusion in the review were also screened to ensure all relevant articles and documentations were included.

2.5. Data summary and synthesis

Data from the included sources of evidence was compiled by the first author using a structured form. Extracted data included general information, study design, information pertaining to the water supply and services, population and social determinants of health, as well as specific information on water security challenges as applicable. Studies were initially classified by the general research area from which specific information was drawn: 1) Water accessibility and availability; 2) Water quality assessment; 3) Water supply and health; 4) Preferences and risk perceptions. Conclusions, including strategies identified and suggestions for future research, were also extracted for this scoping review. Data was summarized and research trends and gaps were identified by analysing single record and comparing sets of records.

Table 2
Full-text screening inclusion and exclusion criteria.

	Inclusion	Exclusion
Type of publication	- Original articles including empirical evidence	- Letters, review articles, methods, newspaper or other special articles (e.g., editorials, commentaries)
Year of publication	- From 2000 onwards	- Before 2000
Geographic locations	- Arctic nations	- not in the Arctic nations and/or outside the Arctic or Subartic regions
Population of interest	- Individuals, household or communities	- habitat-related, animal species or aquatic environment, - industrial, institutional or commercial sectors.
Research area	- drinking water supply services and provision	- politics, governance and policy, including engagement and indigenous knowledge
	- access to drinking and/or domestic water, i.e., accessibility, affordability, availability, quality and quantity.	- engineering processes of water systems (e.g., design and/or construction of water treatment plant and/or distribution systems)
	- challenges that relate to access to water (e.g., behaviours, perceptions, practices, hygiene, health).	- geology and hydrology (e.g., watershed, water resources, groundwater)
	- other research that relates to access to drinking water	- other research area that is not related to access to water

3. Results

3.1. Selection of sources of evidence

The initial search yielded 1356 records and 974 records were screened for title and abstract eligibility after duplicates were removed. Of the records eligible 226 records were selected for full text screening, among which 6 records could not be retrieved. Overall, 216 records were assessed for eligibility, of which 55 records respected the inclusion criteria and were included in the synthesis (Fig. 1).

3.2. Results of individual sources of evidence

Studies retrieved were conducted in the Arctic and Subarctic regions. Most studies were conducted in Canada (67%) and the United States (24%), the other studies retrieved were conducted in Denmark, Russia or Scandinavia. Studies conducted in Canada, Denmark and the United States, all referred to Indigenous populations, namely Inuit, First Nations and Alaska Natives communities. The main research focuses of studies included: 1) Water accessibility and availability (31%); 2) Water quality assessments (20%); 3) Water supply and health (33%); 4) Preferences and risk perceptions (16%). General characteristics of studies, including study design, objectives and water supply systems are described in Table 3.

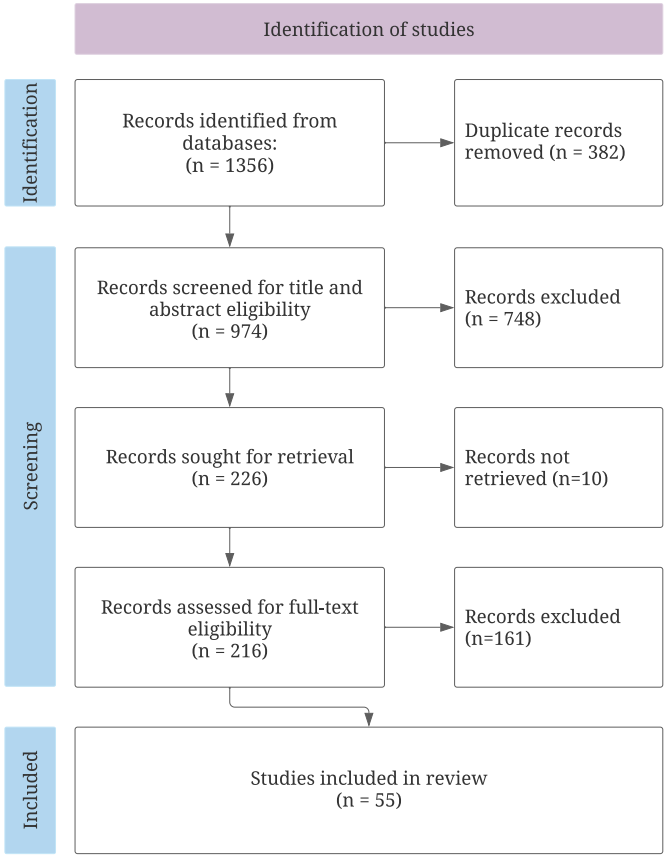

Fig. 1. Methods flow chart for study selection.

Table 3
Description of included studies (n = 55).

AUTHORS	YEAR	REGION	COUNTRY	INDIGENOUS PEOPLES	RESEARCH FOCUS	STUDY DESIGN	OBJECTIVES	WATER SERVICES
Anctil, M	2008	Arctic	Canada	Inuit	Health	Cross sectional	to monitor population health and its determinants	Truck-to-cistern (60%) Natural water sources (34%) Bottled water (6%)
Awume, O and al.	2020	Subarctic	Canada	First Nations	Preferences and risk perceptions	Cross sectional	to offer voice to other interpretations of water security based on semi-structured interviews with Indigenous participants representing varied backgrounds and communities	Piped supply Truck-to-cistern
Bermedo-Carrasco et al.	2018	Subarctic	Canada	First Nations	Preferences and risk perceptions	Community-based cross sectional	to describe the proportion of individuals who drink tap water and are satisfied with their tap water quality and then to identify factors associated with the satisfaction of water quality and reporting drinking tap water	Piped supply Truck-to-cistern Private wells
Bernier et al.	2009	Subarctic	Canada	First Nations	Water quality	Case study	to evaluate the microbial quality of raw source water used for drinking water purposes	Piped water Water collected from raw sources on the land
Bradford et al.	2018	Subarctic	Canada	First Nations	Water quality	Case study	to describe the truck-to-cistern water supply system and the risks associated with the supply chain	Piped supply Truck-to-cistern Private wells
Bradford et al.	2017	Subarctic	Canada	First Nations	Preferences and risk perceptions	Community-based participatory research	to address three main questions: 1) What values and perspectives do youth have about the water in their community? 2) What knowledge about water and health do youth have? 3) Does the adapted photovoice method work as a tool to share perspectives on water and health, and thereby contribute to cultural resilience?	Tap water
Brown et al.	2016	Subarctic	Canada	First Nations	Preferences and risk perceptions	Ecological study	to explore relationships between overall drinking water systems risk and community well-being, with a focus on socioeconomic factors.	Tap water (not specified)
Daley et al.	2018	Arctic	Canada	Inuit	Water quality	Exploratory and proactive research	to characterize the chemical and microbial drinking water quality from source to tap in three hamlets	Truck-to-cistern Mixed truck-to-cistern and piped
Daley et al.	2014	Arctic	Canada	Inuit	Water accessibility and availability	Exploratory case study	to conceptualize the pathways by which municipal water systems and services may be impacting health at the household and family levels, with consideration given to the underlying social and environmental determinants shaping health in the region	Truck-to-cistern
Daley et al.	2015	Arctic	Canada	Inuit	Preferences and risk perceptions	Qualitative case study	to identify and understand residents' perceptions of the functionality of current water and wastewater sanitation systems in a vulnerable context	Truck-to-cistern
Dudarev et al.	2013	Arctic	Russia	Unknown	Health	Ecological study	to assess the levels of food and waterborne diseases in selected regions of Russian Arctic, Siberia and the Far East and to compare disease levels among regions and with national levels Russia	Centralized water supply (not specified)
Dupont et al.	2014	Subarctic	Canada	First Nations	Preferences and risk perceptions health	Cross-sectional	to investigate whether there are differences in beliefs about health risks from tap water and bottled water purchases of residents in First Nations communities and a geographically diverse sample of non-First Nations Canadians across Canada; and to determine whether observed differences in health concerns translate into health risk avoidance expenditures and responses	Piped supply
Eichelberger, L	2018	Arctic	USA	Yupik	Water accessibility and availability	Ethnographic cross-sectional study	to examine household water insecurity in a remote village	Potable water dispensing unit
Eichelberger, LP	2010	Arctic	USA	Eskimo	Water accessibility and availability	Ethnographic case study	to describe the relationship between escalating energy costs, water scarcity and water insecurity based on ethnographic case studies	Piped water Water collected at the washeteria
Elwan et al.	2016	Arctic	USA	Alaska natives	Preferences and risk perceptions	Cross-sectional	to assess the frequency of sugar-sweetened beverage (SSB), water and milk consumption; to ascertain the attitudes towards consumption of water, milk and SSB of residents and to assess rural access to water, milk and SSB	Piped supply Water collected from community-based water points
Farenhorst et al.	2017	Subarctic	Canada	First Nations	Water quality	Cross-sectional	to quantify bacteria in drinking water sources of selected homes in a fly-in community	Piped supply Truck-to-cistern Standpipe (buckets)

(*continued on next page*)

Table 3 (*continued*)

AUTHORS	YEAR	REGION	COUNTRY	INDIGENOUS PEOPLES	RESEARCH FOCUS	STUDY DESIGN	OBJECTIVES	WATER SERVICES
Ford et al.	2019	Subarctic	Canada	First Nations	Preferences and risk perceptions	Community-based case study	to examine the impact of risk perception on human health risk associated with exposure to arsenic in unregulated well water using Bayesian methods and to determine factors influencing the presence of household water treatment and if current tap water safety can be predicted by risk perception and historical knowledge of water quality	Individual wells
Gessner, BD	2008	Arctic	USA	Alaska natives	Health	Ecological study	to test the hypothesis that the presence of modern water services in a community predicts respiratory tract infection risk	Piped water supply (69%)
Goldhar et al.	2013	Subarctic	Canada	Inuit	Water accessibility and availability	Case study	to understand how water security in remote communities is grounded in the perspectives of residents and the attributes of community drinking water systems	Piped water supply Store-bought water Water collected from the land
Goldhar, C, Bell, T and Wolf, J	2014	Subarctic	Canada	Inuit	Preferences and risk perceptions	Cross sectional	to identify the way in which residents of Rigolet are vulnerable to freshwater changes; the strategies and support residents are drawing on to adapt.	Piped water supply Store-bought water; Water collected from the land
Gora et al.	2020	Arctic	Canada	Inuit	Water quality	Comprehensive year-long survey	to examine how source water quality, treatment, distribution and building specific conditions influenced the general and microbiological quality of point of use drinking water in a decentralized drinking water system	Truck-to-cistern Store-bought water (reverse osmosis systems available at local store) Water collected from raw sources on the land
Gora et al.	2020	Arctic	Canada	Inuit	Water quality	Comprehensive year-long survey	to better characterize water quality risks related to metals and organics	Potable water dispensing unit
Gorbanev et al.	2020	Arctic	Russia	unknown	Water quality	Ecological study	to analyze drinking water quality from centralized water supply systems in Russian Arctic and to suggest measures to improve provision of Russian Arctic population with high quality drinking water.	Centralized water supply (not specified)
Hanrahan et al.	2014	Subarctic	Canada	Inuit	Water accessibility and availability	Case study	to understand the multiple dimensions and effects of long-term water insecurity (water-use patterns) in remote communities in Canada and to identify coping strategies	Potable water dispensing unit
Hanrahan et al.	2016	Subarctic	Canada	Inuit	Water accessibility and availability	Community-based case study	to fully describe water insecurity	Potable water dispensing unit
Hanrahan, M and N, M	2019	Subarctic	Canada	Inuit	Water accessibility and availability	Community-based case study	to better understand how water acquisition shapes emotional geographies and is shaped by these geographies	Potable water dispensing unit Shallow community wells Water collected from raw sources on the land
Harper et al.	2015	Arctic/ Subarctic	Canada	Inuit	Health	Retrospective cross sectional	to estimate the burden of community-level self-reported acute gastrointestinal illness in Inuit communities	Tap water Bottled water Water collected from raw sources on the land
Harper et al.	2011	Arctic	Canada	Inuit	Health	Ecological study	to describe seasonality of and explore associations between weather, water quality and occurrence of infectious gastrointestinal illnesses	Piped supply
Hennessy et al.	2008	Arctic	USA	Alaska natives	Health	Ecological study	to investigate the relationship between the presence of in-home piped water and wastewater services and hospitalization rates for respiratory tract, skin and gastrointestinal tract infections in rural Alaska	Piped supply Water collected
Jin et al.	2003	Subarctic	Canada	First Nations	Health	Ecological study	to determine if the incidence of hepatitis A among Aboriginal British Columbia is higher than in the total BC population, and to test hypotheses that it is associated with poverty and crowded, unsanitary living conditions	Not specified
Kuusi et al.	2005	Arctic	Finland	Unknown	Health	Retrospective cross sectional	to determine the cause, vehicle and environmental sources as well as the extent of a large outbreak of gastroenteritis	Piped supply
Ladefoged et al.	2011	Arctic	Denmark	Inuit	Health	Case-control study	to elucidate which social determinants and lifestyle factors might be associated with tuberculosis	Tap water (81% yes; 19% no) (not specified)

(*continued on next page*)

Table 3 (*continued*)

AUTHORS	YEAR	REGION	COUNTRY	INDIGENOUS PEOPLES	RESEARCH FOCUS	STUDY DESIGN	OBJECTIVES	WATER SERVICES
Lucier et al.	2020	Subarctic	Canada	First Nations	Preferences and risk perceptions	Community based participatory study	to unpack and explore the boil water advisories from the perspective of community members and provide considerations for current and future boil water advisory management	Tap water (not specified) Water collected from sources on the land Store-bought water
Marino et al.	2009	Arctic	USA	Yupik and Inupiaq	Preferences and risk perceptions	Case study	to examine how local social and environmental distinctions affect the acceptance of new technology (i.e., centralized systems) as primary source of drinking water	Centralized water supply Water collected from sources on the land
Martin et al.	2007	Arctic	Canada	Inuit	Water quality	Cross-sectional	to evaluate drinking habits that may increase the risk of disease for Nunavik residents in a climate change context	Piped water supply Store-bought water Water collected from the land
Masina et al.	2019	Arctic	Canada	Inuit	Water quality	Community based participatory study	to examine *Giardia* and *Cryptosporidium* in untreated surface water used for drinking	Mixed truck-to-cistern and piped
Mattos et al.	2020	Arctic	USA	Alaska natives	Water quality	Cohort study	to collect seasonal data on household water use, microbial quality from different water sources and waste management practices in remote communities	Water collected at the water treatment plant, or water sources on the land.
Melby et al.	2000	Subarctic	Scandinavia	unknown	Health	Cross sectional	to report on an outbreak of gastroenteritis in a subarctic community where clinical information, culture results and serum samples were available from a number of patients	Tap water (not specified)
Mercer, N and Hanrahan, M	2017	Subarctic	Canada	Inuit	Water accessibility and availability	Pilot cohort study	to assess changes in drinking/general purpose water consumption; perceived changes in health outcomes; and contributions to household economies as a result of a pilot project	Potable water dispensing unit
Messier et al.	2012	Arctic	Canada	Inuit	Health	Cross sectional	to measure antibodies against 8 pathogens in human sera, to evaluate the exposure to these different microorganisms in relation to sociodemographic and environmental criteria and to determine the risks for acquiring zoonotic infections relates to Inuit lifestyle and food habits	Truck-to-cistern
Mi et al.	2019	Subarctic	Canada	First Nations	Water quality	Cohort study	to examine water quality parameters in a broader range of First Nations households and communities	Piped supply Individual wells Truck-to-cistern
Miernyk et al.	2018	Arctic	USA	Alaska natives and non-natives	Health	Cross sectional	to determine the prevalence of *H. pylori* infection by both urea breath test and anti *H. pylori* IgC among Alaskans living in 4 regions of the state and to identify factors associated with infection	Piped water Truck-to-cistern Well water Store-bought water Water collected from the land
Moriarity et al.	2021	Subarctic	Canada	First Nation	Preferences and risk perceptions	Cross sectional	to examine whether the level of concern about environmental pollution is associated with self-reported behaviour, that is, water consumption and time-on-the-land.	Tap water (not specified) Store-bought water Water collected from the land
Mosites et al.	2020	Arctic	USA	Unkown	Health	Cross sectional secondary data analysis	to assess whether a community water service is associated with the frequency of sugar-sweetened beverages consumption, obesity or perceived health status in Alaska	Piped water Well water Closed haul system Water collected from the land
Ogorman, M and Penner, S	2018	Arctic/ Subarctic	Canada	First Nations	Health	Secondary data analysis and case study	to document the association between water and sanitation infrastructure and health indicators in Canada for First Nations, Métis and Inuit individuals living on and off-reserve in Canada	Piped supply Truck-to-cistern
Reisman et al.	2014	Arctic	USA	Alaska Natives	Health	Observational cross-sectional survey	to determine significant socioeconomic and demographic risk factors for pneumococcal colonization to better understand the dynamics of pneumococcal colonization in the Alaska Natives population	Tap water (not specified) Water collected from the land
Ritter et al.	2014	Arctic	USA	Alaska Natives	Preferences and risk perceptions	Cross sectional	to identify and analyze participant-reported motives for drinking untreated water and describe the interconnections among them	Trucks-to-cistern Water collected from the land
Sarkar et al.	2015	Subarctic	Canada	Inuit	Water accessibility and availability	Cross sectional	to explore the water insecurity, coping strategies and associated health risks in a small and isolated sub-arctic indigenous community in Canada	Potable water dispensing unit Water collected from the land

(*continued on next page*)

Table 3 (*continued*)

AUTHORS	YEAR	REGION	COUNTRY	INDIGENOUS PEOPLES	RESEARCH FOCUS	STUDY DESIGN	OBJECTIVES	WATER SERVICES
Spence, N and Walters, D	2012	Subarctic	Canada	First Nations	Preferences and risk perceptions	Ecological study	to identify the determinants of drinking water risk perception in the home among First Nations on reserve in Canada	Tap water (not specified) Store-bought water
Spicer et al.	2020	Arctic	Canada	First Nations	Preferences and risk perceptions	Cross sectional	to determine how risk perception and other social-economic variables influence individual drinking water consumption patterns and to explore how political jurisdiction and associated infrastructure and regulations influence individual drinking water consumption patterns	Piped supply Trucks-to-cistern
Thomas et al.	2016	Arctic	USA	Alaska natives	Health	Cohort study	to assess rates of acute respiratory, skin and gastrointestinal infections before and after installation of complete plumbing and hygiene education in these communities	Piped supply
Waldner, CL. Et al.	2017	Subarctic	Canada	First Nations	Preferences and risk perceptions	Community-based cross-sectional	to describe factors associated with self-reported health effects from tap water in 8 First Nations reserve communities in Saskatchewan	Piped supply Truck-to-cistern
Wenger et al.	2010	Arctic	USA	Alaska natives	Health	Ecological study	to evaluate invasive pneumococcal disease surveillance data from Alaska to further characterize the impact of vaccine introduction, and to evaluate potential associations between socioeconomic indicators, water supply and invasive pneumococcal disease in Alaskan children	Tap water Closed haul system Water collected
Wright et al.	2018	Subarctic	Canada	Inuit	Health	Cross sectional	to describe drinking water collection and storage practices, identify potential risk factors associated with water contamination between source and point of use, and examine possible associations between drinking water contamination and self-reported acute gastrointestinal illness	Piped supply Potable water dispensing unit Untreated brook water
Wright et al.	2018	Subarctic	Canada	Inuit	Preferences and risk perceptions	Cross sectional	to characterize drinking water perceptions and consumption patterns, examine use of water sources and changes	Piped supply Potable water dispensing unit Untreated brook wate Bottled water

3.3. Synthesis of results

3.3.1. Water accessibility and availability

Various types of water services were reported in the selected studies, including piped supply, truck-to-cistern, and off premises sources of water (e.g., potable water dispensing units) (Table 3). Most studies also included mentioned or discussed the use of alternative untreated water sources. Water supply services and/or the use of alternative water sources influence the quantities of water made available to consumers, which inherently relates to overall access to water, sanitation and hygiene.

Many communities in Canada and Alaska were found to be served by truck-to-cistern systems. In a truck-to-cistern systems in Nunavut, where the average resident uses approximately 110 L/day, more than half of the households interviewed reported experiencing water shortages at least once per month (Daley et al., 2014). Large households were most likely to report shortages of water, i.e., non-standard numbers of people living in households with standardized size tanks, and this issue was consistently reported in other studies (Bradford et al., 2018; Daley et al., 2014).

Some communities in the Arctic and Subarctic regions were also supplied with either potable water dispensing units (PWDU), i.e., public taps, exclusively, or a combination of PWDU and tap water. In Labrador, Wright et al. (2018c) found that the PWDU was the most popular source of drinking water, compared to tap water and natural water sources, and this was explained by a lack of trust and dislike of tap water as well as a reflection of Inuit culture and lifestyle. The location of the PWDU (over a mile from most houses), with decreased accessibility during winters and the high cost of water (2$ per litre), were, however, raised as important drawbacks for its use by community members (Hanrahan et al., 2014a, 2016). In Labrador, the average per capita water use was estimated to be less than one third of the Canadian national average (Hanrahan, 2017; Sarkar et al., 2015). In Alaska, where some communities exclusively rely on water from the community centralized watering point, the average per capita water consumption was reported as less than 20 L per day per capita (Eichelberger, 2010, 2018; Mattos et al., 2020).

Primary determinants of households fetching water from various alternative water sources were identified throughout the literature as having a vehicle (Eichelberger, 2010, 2018; Goldhar et al., 2013) and as relying on able-bodied male kin. In Alaska and northern Canada, fetching water is recognized as a gendered male activity and the quantities of water available and consumed by a household is positively correlated to both the presence and total number of men in that household (Eichelberger, 2010; Hanrahan, 2017). The absence of close males combined with the physically demanding nature of water collection and the associated dangers increases stress associated to water insecurity for elders/women who tend to limit water consumption, store water or purchase water retrieval services (Eichelberger, 2018; Hanrahan and Mercer, 2019).

Overall access to water influences the quantity of water allocated to hygiene. Various coping strategies were reported in cases where a limited quantity of water was available, including sharing bath water and recycling water for handwashing, laundry and washing out waste containers and/or walls (Hanrahan and Mercer, 2019; Mattos et al., 2020; Sarkar et al., 2015). Results from an intervention study show that psychological stress related to water insecurity was found to decrease after a water harvesting system was implemented (Mercer and Hanrahan, 2017). Results from a pilot project show an average increase of water availability from 215 to 251 gallons per household weekly. Water harvesting allowed households to increase their use of water for general purposes, e.g., personal and domestic hygiene, but did not increase the quantity of water used for drinking water (Mercer and Hanrahan, 2017).

3.4. Water quality assessment

Drinking water quality is an important component of water accessibility. Multiple studies reported in this scoping review aimed to examine water quality parameters and better evaluate water quality from source water (RW) to the point of use (POU) (Table 3). Most water quality studies focused on microbiological contamination issues. A diversity of microorganisms can generally be found in natural water sources, and source water may therefore contain strains of multiple pathogenic or non-pathogenic microorganisms. In a truck-to-cistern supply, various methods are used to treat source water, e.g., pretreatment (such as filtration), primary and secondary disinfection at the water treatment plant or chlorination of pumped water directly into the truck. Multiple studies showed increased changes in water quality or occurrences of microbial contamination during transport in truck-to-cistern systems, concurring with a decrease in secondary disinfectant availability (e.g., residual chlorine). In Saskatchewan and Manitoba, Canada, no total coliforms or *Escherichia coli* (*E. coli*) were found at the water treatment plants, however, total coliforms were found in increasing numbers through the water supply systems, i.e., water trucks, cisterns and household tap/standpipe buckets (Bradford et al., 2018; Farenhorst et al., 2017), which indicates changes in water quality during distribution. In Nunavik, total coliforms exceeding 10 total coliforms per 100 ml were found in one third of the residential water cisterns sampled (Martin et al., 2007). Results from a similar study conducted in Nunavut shows no coliform bacteria in the biofilm or water samples from water cisterns, however, at the genus level, Legionella was found to be more abundant in storage tanks compared to the water truck (Gora et al., 2020a). Results from a case study conducted in Saskatchewan showed an increased risk of microbial contamination in summer (coinciding with warmer, windier, and dustier environmental conditions) alongside an increased water demand and usage (therefore more frequent cistern refills) (Bradford et al., 2018).

In Canada, overall results show that the free and total residual chlorine concentrations were equal to or slightly higher in samples collected from the trucks compared to those collected from household taps (Bradford et al., 2018; Harper et al., 2011). Multiple studies found free chlorine residual below the limit at the POU (Daley et al., 2018; Farenhorst et al., 2017; Gora et al., 2020a; Mi et al., 2019). Tap water was generally warmer than in the truck (Daley et al., 2018), but temperature variations were not associated with residual chlorine variability in the water distribution systems (Bradford et al., 2018). Goldhar et al. (2013) suggested that additional chlorine would be needed in systems with high levels of organic matter, but additional chlorination could favour generation of additional disinfection by-products, such as trihalomethanes (THMs).

In Nunavut, Daley et al. (2018) found lead concentrations that exceeded guidelines for Canadian drinking water quality in many community buildings. In similar settings, Gora et al. (2020b) found that lead and copper was released from premise plumbing, which was identified as less problematic in more recently built buildings (Gora et al., 2020b). They suggested that the presence of organic matter, iron and manganese from the source water could influence the presence of lead through the premise plumbing of buildings in the community. This is also of important concern on Russian Arctic territory where 42.9% of surface and 16.4% of underground sources of centralized drinking water supply did not meet sanitary standards and rules for iron, manganese, sulfate, nickel, ammonia and nitrates (Gorbanev et al., 2020).

The use of untreated natural sources for drinking water purposes is common in many Arctic communities; this has been documented in a small number of studies, mostly in Indigenous communities. In Iqaluit, 30% of these untreated natural water samples tested positive for *E. coli*, 20% tested positive for *Giardia* and 1.8% of tested positive for *Cryptosporidium*, however, no association was found between *E. coli* and parasites (Masina et al., 2019). Results from a cross-sectional study conducted in Nunavik show large quantities of total coliforms, few *E. coli* or enterococci and no *Giardia* or *Cryptosporidium* parasites in these untreated natural sources (Martin et al., 2007).

Results from a case study conducted in the Cree community of Mistissini, where all households collect untreated water, shows high levels of contamination in containers used to store water. Overall, 71% of samples had total coliform counts above the acceptable drinking water guidelines, and *E. coli* and enterococci were detected in 9.5% and 19% of samples, respectively (Bernier et al., 2009). Similar results were found in Nunavik where 80% and 20% of the containers used to collect water contained more than 10 total coliforms and exceeded 1 *E. coli* per 100 ml, respectively (Martin et al., 2007). In Alaska, Mattos et al. (2020) detected total coliforms in 30% of stored household water samples from both treated and untreated sources, and 2% of the samples tested positive for *E. coli*. Although no correlation was found between the microbiological quality of water and the time and temperature of storage (Bernier et al., 2009), doubts regarding the effectiveness and frequency of the cleaning of containers in some communities were highlighted by Martin et al. (2007) and Mi et al. (2019), given the importance of cleaning in reducing contamination. It is recognized that water collection increases vulnerability to post-collection contamination from the point of water collection to the point of use. Likewise, the use of dippers and transfer devices were significantly associated with increased odds of total coliform presence in stored water (Wright et al., 2018c).

3.5. Water supply and health

Multiple studies included in this review focused on the health outcomes related to a lack of access to water, sanitation, and hygiene in addition to waterborne illness including gastrointestinal and respiratory infections (Table 3). In a cross-sectional study conducted in Alaska, access to piped or delivered drinking water was associated to a lower prevalence of Heliobacter pylori bacteria (*H. pylori*) (Miernyk et al., 2018). It was suggested that the Alaska Natives ethnicity, lower level of education, and larger family sizes or more crowded houses were also risk factors for prevalence of *H. pylori*. Authors highlighted the fact that lack of water availability would influence the lack of water for hygiene, which would likely facilitate community transmission (Miernyk et al., 2018). Similar findings were reported in Inuit communities where gastrointestinal symptoms were generally associated with access to tap water, and thus increased quantity of water available for hygiene purposes (Martin et al., 2007; Wright et al., 2018a). Consumption of water from alternative sources including natural sources on the land was significantly associated with an increase rate of acute and infectious gastrointestinal illness rate in the Inuit communities of Iqaluit and Rigolet, where incidence rate was measured as 2.9 and 3.9 case/-person/year respectively (Harper et al., 2011, 2015; Wright et al., 2018d). Additionally, water supply problems (e.g. no treatment system, untreated water or operational problems) were found to be positively correlated to the incidence of Hepatitis A (Hep A), and the incidence rate among First Nations communities was twice that of the general population of British Columbia. Children and young adults, as well as members of above-average-sized households were identified as at an increased risk of contracting Hep A (Jin and Martin, 2003). Similar results were found in Russia, where poor drinking water quality in the water supply was identified as the main cause of mass outbreaks of gastro illnesses, including Hep A (Dudarev et al., 2013). Two studies conducted in Scandinavia during an outbreak of Hep A also found conflicting associations between boiling water and illness. In Finland, 18.7% of illness was reported during the outbreak and the risk of illness was found to increase with daily consumption of boiled tap water (Kuusi et al., 2005). In Norway, an outbreak was shown to affect 15% of the study respondents, with no illness reported among those who boiled tap water during the outbreak (Melby et al., 2000).

Results from studies conducted in remote northern communities consistently show that the quality of water services can be associated with respiratory infections. In Alaska, lower respiratory tract infection

incidence rates among children less than 2 years old were strongly related to water services (Gessner, 2008). An extensive study evaluating disease surveillance data shows that better water services were also associated with a reduced rate of invasive pneumococcal disease rate in children aged less than 5 years old (Wenger et al., 2010). An additional study found that lack of in-home running water was associated with increased colonization prevalence of pneumococcus in children aged less than 10 years, which was also associated to Native [Indigenous] ethnicity, higher number of household members as well as having 3 or more children at home (Reisman et al., 2014). Similarly, in a case-control study conducted in Greenland, having no access to water or flushing toilets were identified as risk factors for tuberculosis (TB) (Ladefoged et al., 2011). Living in a small settlement was also identified as a risk factor but TB patients were not subject to higher degree of domestic crowding (Ladefoged et al., 2011), however this could reflect effect measure or confounding. Finally, results from an intervention study shows that the installation of piped water supply significantly increased water quantity available for consumers, decreasing clinic visits for respiratory infections by 16% (Thomas et al., 2016).

Other studies found associations between water supply services and protozoan parasites and zoonotic infections in Nunavik. Better water supply services and the use of water filtration within homes was associated with a reduced prevalence infection by toxoplasmosis gondii protozoa (*T. gondii*). Interestingly, the more frequently the reservoir was cleaned, the greater the proportion of individuals who were infected with *T. gondii* (Anctil, 2008). Similarly, Messier et al. (2012) reported a relationship between Echinococcus granulosus (sensu lato) seropositivity and the cleaning of the domestic water tank among the Inuit population exposed to a wide range of microorganisms responsible for zoonotic infections.

Finally, Mosites et al. (2020b) found that adults in rural Alaska without access to in-home water were more likely to consume sugar-sweetened beverages compared to participants with access to tap water, but no association was found between water supply problems and higher incidence of obesity (Mosites et al., 2020b). Overall, households collecting water from alternative sources were more likely to report a waterborne illness but access to running water was not significantly associated with superior self-reported health (Ogorman and Penner, 2018). As such, other studies that did not focus specifically on health outcomes revealed general concerns with regards to the safety of treated water and health problems, including lack of access for hygiene and cooking (Ritter et al., 2014; Waldner et al., 2017; Wright et al., 2018b).

3.6. Preferences and risk perceptions

Retrieving water from natural sources on the land, e.g., surface water, ice, rivers and springs, is an ancestral and ongoing practice in many communities. According to the scoping review, a general preference for untreated natural sources was noted in various geographical locations and populations, including among Alaskan natives (Marino et al., 2009; Ritter et al., 2014) as well as Inuit and First Nations communities in Canada (Awume et al., 2020; Bradford et al., 2017; Goldhar et al., 2014; Martin et al., 2007; Spicer et al., 2020; Wright et al., 2018a). Different factors influencing household perceptions and preferences, including traditional views, health, and socioeconomic conditions, were further identified.

Traditional views on caring for water and knowledge, coupled with the belief that water is alive (Goldhar et al., 2013) implies going beyond its physical, chemical or biological properties. The shared value of water, among other things, as opposed to the principle of privately owned, is at the core of the relationship between the spiritual and physical world and life for Indigenous Peoples (Bradford et al., 2017; Hanrahan et al., 2016). Deeply held cultural significance and ancestral practices, among other things, lead to the use of traditional natural sources on the land where fresh, clean and holy water can be found (Eichelberger, 2018; Martin et al., 2007). The preference for untreated [naturally filtered] water for drinking purposes is adverted as a major determinant for using it as an alternative to treated [chlorinated] water (Ritter et al., 2014). Studies conducted in First Nations communities in Canada found that individuals who were concerned about environmental issues, such as pollution, affecting water were more likely to be unsatisfied with tap water and prefer drinking water from natural sources (Bermedo-Carrasco et al., 2018; Moriarity et al., 2021).

Health and safety concerns regarding the use of chlorination were commonly reported as playing a crucial part in preventing individuals from drinking treated water, and this was more prevailing in Indigenous communities (Dupont et al., 2014; Eichelberger, 2018; Harper et al., 2015; Marino et al., 2009; Spicer et al., 2020). Ritter et al. (2014) reported that the use of chlorine to treat water in municipal treatment plants is considered a western practice conflicted with the widely held local preference for natural processes. Likewise, Marino et al. (2009) identified, through semi-structured interviews, that the action of serving coffee or flavoured beverages (e.g., Kool-Aid) made with treated chlorinated water to elderly people was believed to be disrespectful by many locals.

In addition to safety concerns, the use of chlorine is also reported to alter the taste of the water, playing a significant role in individual consumption preferences (Elwan et al., 2016; Spicer et al., 2020). Overall, multiple studies highlighted the widespread dissatisfaction, concerns, and overall lack of trust with water supply, especially in trucks-to-cistern system where stagnation and heating also occur (Martin et al., 2007; Ritter et al., 2014; Spicer et al., 2020). The organoleptic qualities of the water, including color, clarity, smell, and taste, are often reported as rejecting factors, and [abnormal] quality of water is commonly associated with illness (Eichelberger, 2018; Goldhar et al., 2013; Kuusi et al., 2005; Ritter et al., 2014; Waldner et al., 2017). For example, a significant association between drinking water risk perception and exceedance of an aesthetic objective or guideline was found in a first nation community in Saskatchewan, Canada (Ford et al., 2019). In similar settings, Bermedo-Carrasco et al. (2018) found that individuals who did not experience tap water odour were more likely to be satisfied with the quality of tap water and therefore to drink it. Goldhar et al. (2014) found that consumption of tap or bottled water represents a perceived decrease in water quality for the residents of a community in Labrador (Goldhar et al., 2014). Various concerns relating to the maintenance and cleanliness of water infrastructure were also highlighted (Waldner et al., 2017). In some truck-to-cistern settings, cisterns were reported to be insufficiently secured to protect them from outside contamination (e.g., people throwing garbage inside, insets/animals entering), which further discouraged water consumption (Ogorman and Penner, 2018; Spicer et al., 2020). Similar findings were also reported in a community supplied with a PWDU where households reported frequent breakdowns and closure periods as well as mistrust in the site because of animal activity in and around the raw (source) water intake (Hanrahan et al., 2014a).

Various socio-economic characteristics, including age, gender, education level and income, were identified as important determinants for households' perceptions regarding water. Adults, between 18 and 54 years of age, were more likely than elderly people to be concerned about the safety of tap water (e.g., presence of chlorine, pathogens in water) (Wright et al., 2018a). Adults were also more likely to report that tap water made them or someone else sick or produce other negative health effects (Dupont et al., 2014; Wright et al., 2018c), and tend to spend more on bottled water (Dupont et al., 2014). Using disaggregated data, Waldner et al. (2017) found that the association between self-reported health effects from tap water diminishes with an increased age, i.e., between 18 and 34 years, 35 and 54 years, and older than 55 years. Bermedo-Carrasco et al. (2018) found that adults aged 55 years and older, who were not concerned about environmental issues affecting water, had a higher probability of both consumption of, and satisfaction with, tap water, compared to young adults. Elderly people, however, were found to be more likely to use natural, traditional water sources

(Anctil, 2008; Ritter et al., 2014) and more likely to consume bottled water (Spicer et al., 2020). Finally, Spence and Walters (2012) found no clear evidence of an association between age and perception of risk for drinking water on First Nations reserves in Canada.

Compared to male respondents, females were more likely to have concerns about the presence of chemicals and chlorine and to have a greater perception of risk for tap water (Spence and Walters, 2012). Dupont et al. (2014) found that women were more likely to believe that someone had become sick from drinking water in their households, however, the study did not find any association between gender and health concerns regarding tap water specifically. Additionally, women more frequently reported that natural sources of water were unsafe to drink and more regularly consumed bottled water at home compared to males (Dupont et al., 2014; Spicer et al., 2020; Wright et al., 2018a). Results from a community based participatory research on reserve in Canada shows that men were more likely to adhere to boil water advisories and to correctly identify recommended practices (Lucier et al., 2020).

Spence and Walters (2012) found that a higher level of education was associated with a greater risk perception for drinking water among First Nations on reserve in Canada. Similar results were found in Nunavik Inuit communities, where individuals with secondary school education reported drinking less natural water and more bottled water than those with lower level of education (Anctil, 2008). Likewise, individuals of these communities with lower levels of education were more prone to *H. pylori* and zoonotic infection (Messier et al., 2012; Miernyk et al., 2018). In rural Alaska, lower formal education and lower income was also associated to an increase in sugar-sweetened beverage consumption as an alternative to drinking water, also increasing risk of obesity (Mosites et al., 2020b).

The use of bottled water varies in communities throughout the Arctic, but the high costs and sporadically availability are consensual (Goldhar et al., 2013; Hanrahan et al., 2014b). Multiple studies reported that the cost of purchasing potable water, which can be significant, limits accessibility as some members of communities are unable to afford this (Goldhar et al., 2014; Hanrahan et al., 2014a; Ritter et al., 2014; Sarkar et al., 2015). In some communities, responsibility for the costs of connecting their homes to the main water line falls to the residents, which may further constrain to access in low-income settings (Goldhar et al., 2013). In addition, employment and increased income were associated with a higher rate of bottled water consumption at home (Spicer et al., 2020; Wright et al., 2018b). According to the study by Ford et al. (2019), acceptance of water treatment, including filtration of the water consumed in the home, was more frequent among the respondents if personal or household income was higher: households were more likely to use domestic water treatment devices [type of device not specified] if the household annual income was greater than 50,000$. The level of education was not a significant predictor for the use of water treatment at the point of use (Ford et al., 2019). Finally, Sarkar et al. (2015) reported that the use of free but unmonitored and untreated water often remained the best option from a household's financial perspective.

4. Discussion

Inadequate access to safely managed drinking water, including poor water quality and insufficient in-home water quantity, pose significant threats to human health, particularly in settings where conventional piped water supply is not provided. In the Arctic nations, lack of access to water is associated with poverty, malnutrition, poor living conditions and poor hygiene, and environmental contamination (Daley et al., 2015; Gracey and King, 2009; Hennessy and Bressler, 2016; Hotez, 2010; Mosites et al., 2020a). The types of available water supply systems, e.g., piped water, trucks-to-cistern and/or PWDU, will influence overall access for households. Households served with tap water, though piped water or truck-to-cisterns, will have access to a greater quantity of water for domestic purposes compared to households without this service. However, availability of domestic tap water doesn't indicate that people will use it for drinking purposes. There is an important distinction between provision of water for domestic use and drinking purposes, and both should be differentiated when assessing and evaluating water supply.

It is important to consider how to provide a safe and reliable water supply which is also acceptable for the population through the unconventional systems used in remote settings. Various factors, including cultural characteristics, will influence household perceptions and preferences in accessing water. Deeply held cultural significance and ancestral practices regarding managing water should be considered for sustainable outcomes in remote Indigenous communities. A similar study conducted in remote Indigenous communities in Australia highlights the importance of working towards developing technology that is fit for purpose, people and place (Hall et al., 2022). Water retrieval from alternative, untreated natural water sources on the land (e.g., lakes, streams, rivers, rainwater, icebergs, snow) is a common practice among Indigenous communities in the Arctic and Subarctic regions. Interventions aiming to improve access to water should aim to ensure the provision of sufficient quantities of water for domestic hygiene as well as high quality of water for consumption, whichever source of water is used.

Results from Wright et al. (2018c) show that the population of Rigolet in Canada was favourable to the implementation of a PWDU in a community where no water supply system was available. However the motivations behind household reliance on alternative sources in settings where water is already provided through tap water are equivocal. The linkages between risk perceptions and practices, and therefore whether the implementation of culturally acceptable water supply, including water treatment methods, would reduce the use of untreated water sources on the land, remain unclear. The use of chlorine and other chemicals to treat water and subsequent reluctance to drink treated water, as opposed to "freshwater" from the land, is an important matter of discussion, which has also been raised in other contexts (Doria, 2006; Ritter et al., 2014). The safety of the water consumed is the upmost priority to prevent health issues, and its acceptability is key. The guidelines and regulations for the quality of drinking water should consider and specifically apply to the type of water supply.

A global approach to water security, respecting traditional views towards freshwater, should be considered, particularly in the context of Indigenous consumers, and working towards balancing the risks associated to water provision for domestic and drinking purposes will be necessary in upcoming decision-making. In times of climate and global changes and environmental degradation, monitoring should look at exposure to various sources of contaminants or chemicals in water sources that are used as intakes for municipal water supply and alternatively as direct sources of water (de França Doria, 2009). Alternative water sources that are used by the population should be adequately protected from outside contamination, they must be monitored, and easily accessible to allow water collection and prevent injuries (Curtis, 1986; Hanrahan and Mercer, 2019; Sarkar et al., 2015). The use of water tanks and containers for indoor storage, i.e., stagnant water, further increases vulnerability to bacterial regrowth and recontamination in water, and this calls for the promotion of good hygiene practices (Cassivi et al., 2020; Harris et al., 2013; Wright et al., 2004). The current condition, maintenance and cleanliness of water infrastructure, including pipelines, cistern trucks and water tanks, as well as the risks associated with water collection using individual containers, including post-collection contamination, require further attention.

The initial aim of this scoping review was to examine the extent of available water security evidence as well as identify research gaps and intervention priorities to improve access to domestic water in the Arctic and Subarctic regions of the eight Arctic nations, and to compare levels of service in Indigenous and non-Indigenous communities. However, results show that existing research mostly focuses on water-related

issues in the northernmost regions of Canada and the United States, where Indigenous communities are overrepresented. Evidence from the literature suggests that Indigenous communities in the Arctic are more affected by drinking water advisories and most likely disproportionally affected by lower water services compared to southern regions where coverage of access is nearly universal. Very few studies have focused on water provision and services in non-indigenous communities in the Arctic nations. Although this may suggest that such populations, mostly located in the subarctic and southern regions, are less affected by a lack of water services, more research would be necessary to better assess water coverage in the northernmost regions of the world. For example, little information is available on water provision in remote communities in Scandinavia, where universal coverage is presumably available, and in the Russian Federation, where the lowest safely managed drinking water service coverage is reported. It is noted that the search strategy for this review article was designed in English which might have limited access to non-English articles, such as those conducted in Scandinavian and Russian languages.

5. Conclusions

This scoping review highlights challenges that relate to, and result from, water security and insecurity in the Arctic, particularly in the context of Indigenous communities. Lack of access to water, including insufficient quantities, intermittent services, and poor in-home quality, is a threat to population health and well-being. This summary of evidence supports the importance of traditional views and individual preferences on household risk perceptions and practices in accessing water in Indigenous communities. It argues the importance of using evidence-based knowledge to provide inclusive and culturally adapted water services, including alternative natural [freshwater] drinking water sources. Future research should focus on optimizing overall operation of domestic water supply systems in northern communities, e.g., water treatment and distribution processes, especially in settings where water is not available through conventional continuous piped systems. Primary prevention of microbial and chemical contamination from the water sources to the point of use, including source protection, infrastructure maintenance as well as promotion of point-of-use drinking water safety, is key to successful health-based interventions.

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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.2022.114060.

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Drinking water and the implications for gender equity and empowerment: A systematic review of qualitative and quantitative evidence

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ABSTRACT

Background: Safe drinking water is a fundamental human right, yet more than 785 million people do not have access to it. The burden of water management disproportionately falls on women and young girls, and they suffer the health, psychosocial, political, educational, and economic effects. While water conditions and disease outcomes have been widely studied, few studies have summarized the research on drinking water and implications for gender equity and empowerment (GEE).

Methods: A systematic review of primary literature published between 1980 and 2019 was conducted on drinking water exposures and management and the implications for GEE. Ten databases were utilized (EMBASE, PubMed, Web of Science, Cochrane, ProQuest, Campbell, the British Library for Development Studies, SSRN, 3ie International Initiative for Impact Evaluation, and clinicaltrials.gov). Drinking water studies with an all-female cohort or disaggregated findings according to gender were included.

Results: A total of 1280 studies were included. GEE outcomes were summarized in five areas: health, psychosocial stress, political power and decision-making, social-educational conditions, and economic and time-use conditions. Water quality exposures and implications for women's health dominated the literature reviewed. Women experienced higher rates of bladder cancer when exposed to arsenic, trihalomethanes, and chlorine in drinking water and higher rates of breast cancer due to arsenic, trichloroethylene, and disinfection byproducts in drinking water, compared to men. Women that were exposed to arsenic experienced higher incidence rates of anemia and adverse pregnancy outcomes compared to those that were not exposed. Water-related skin diseases were associated with increased levels of psychosocial stress and social ostracization among women. Women had fewer decision-making responsibilities, economic independence, and employment opportunities around water compared to men.

Conclusion: This systematic review confirms the interconnected nature of gender and WaSH outcomes. With growing attention directed towards gender equity and empowerment within WaSH, this analysis provides key insights to inform future research and policy.

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1. Introduction

Access to safe drinking water is a fundamental human right; however, the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) report that more than 785 million people do not have access to safe drinking water (United Nations Children's Fund and World Health Organization, 2019). Globally, 29% of the population does not have access to a safely managed water source (United Nations Children's Fund and World Health Organization, 2019). Inadequate access to WaSH services is responsible for 9.1% of the global disease burden and 6.3% of all deaths worldwide (Prüss-Üstün et al., 2008). Additionally, barriers to safe drinking water often lead to various negative health outcomes such as diarrhea, cholera, trachoma, typhoid, shigellosis, and malaria (Beer et al., 2015; Bisung and Elliott, 2017; Hunter et al., 2010; Moura et al., 2019; Mourad et al., 2019; Sengupta, 2013; Tomberge et al., 2021). A global study in 2014 of 145 countries concluded that WaSH-related diarrheal deaths accounted for 1.5% of the total disease burden, 58% of all diarrheal diseases, and 9% of all deaths for children younger than 5 years old (Liu et al., 2012; Prüss-Ustün et al., 2014).

Women and girls are disproportionately affected by inadequate water access because they are largely responsible for household water management (Graham et al., 2016; Kayser et al., 2019; Pouramin et al., 2020; Tomberge et al., 2021) When water sources are not readily accessible at the home, women and girls are responsible for collecting water in 4 out of 5 households worldwide (United Nations Children's FundWorld Health Organization, 2019). Compared to men, women experience many negative WaSH-related health outcomes, some of which have been disaggregated (Stevenson et al., 2012; Wutich and Ragsdale, 2008).Women and girls account for a higher number of deaths due to diarrheal diseases and higher disability adjusted life years (DALYs) caused by inadequate hygiene (Prüss-Ustün et al., 2019; Pal et al., 2018). Contaminated drinking water and water carriage can induce complications during pregnancy, increase perinatal health issues, negatively affect menstrual health, and increase the incidence of reproductive tract infections in women (Ademas et al., 2020; Gall et al., 2015; Geere et al., 2018a; Kayser et al., 2019).

While WaSH-related health inequities have been widely studied, relatively few studies have evaluated how drinking water impacts gender inequities (Kayser et al., 2019). Among studies that have explored the impact of drinking water on gender, the focus has been on water fetching, sanitation, and sexual violence. Women must travel long distances to retrieve drinking water and find a private place to openly defecate due to a lack of proper sanitation facilities (Sommer et al., 2015; Kayser et al., 2021). This puts them at a much higher risk of being physically assaulted, abused, or harassed (Sommer et al., 2015; Kayser et al., 2021). Among the one in three women that suffer from gender-based violence (World Health Organization, 2021), many attribute their struggle to access adequate WaSH services as a contributing factor (Sommer et al., 2015).

Women often suffer the social-educational and economic ramifications associated with finding and accessing safe drinking water (Stevenson et al., 2012, United Nations Children's Fund, 2016). According to UNICEF, one in five girls of primary-school age are not in school, compared to one in six boys (United Nations, 2007). Young girls are often taken out of school to help manage the household while young boys are allowed to continue their education (House et al., 2012; UNICEF and WHO, 2019). Additionally, reported school absences increase when girls are menstruating due to 'inadequate WaSH facilities at school (House et al., 2012; Goodman and Norden, 2005). The lack of education regarding proper menstrual hygiene and the presence of cultural stigma causes girls to miss up to one week of school per month (House et al., 2012, Goodman and Norden, 2005). Studies suggest that such school absences contribute to high drop-out rates for girls (Vanneste et al., 2016).

This systematic review evaluates the current state of the literature regarding drinking water management and exposures and gender equity and empowerment outcomes (GEE). Drinking water management and exposures include elements of accessibility, quality, quantity, reliability, continuity (Kayser et al., 2013). GEE is defined as the association between gender and self-determination (United Nations Development Programme, 2005). Specific GEE outcomes included: psychosocial stress, political power and decision-making, health outcomes, social-educational conditions, and economic conditions. This systematic review includes both quantitative and qualitative published literature. The review assesses the relationship between drinking water and GEE outcomes and highlights areas where future research is needed. The overarching goal is to provide awareness of the connection between drinking water and GEE in order to benefit the health and wellbeing of women and girls, globally.

2. Methods

This study was conducted in accordance with the International Prospective Register of Systematic Reviews (PROSPERO) guidelines and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. Prospero registration number is CRD42021198202.

2.1. Search strategy

Our search strategy identified published studies in the following databases – EMBASE, PubMed, Web of Science, Cochrane, ProQuest, Campbell, the British Library for Development Studies, SSRN, 3ie International Initiative for Impact Evaluation, and clinicaltrials.gov. Key terms used in the search included "water OR sanitation OR hygiene OR WaSH", "drinking-water OR drinking", and "gender OR women OR girl OR girls OR woman OR female OR females". While search terms remained the same, some variability in search term capabilities between databases existed. The primary objective was to identify existing research studies that focused on the relationship between drinking water and gender equity and empowerment.

2.2. Inclusion criteria

Inclusion criteria included peer-reviewed published studies conducted between January 1, 1980–September 30, 2019, written in the English language. Quantitative and qualitative studies were included. Studies were required to explicitly consider a gendered outcome, as demonstrated by a female study population or gender-stratified study outcomes. Additionally, drinking water components, defined as water access, quality, quantity, reliability, or continuity were clearly evaluated. Duplicate articles were removed after the database search was completed. The methodological approach used in this study is outlined in Fig. 1.

2.3. Exclusion criteria

Studies that did not provide primary data or analysis (i.e., commentaries, systematic review articles, periodicals, theses, dissertations, and meta-analyses), did not consider a population (i.e., case reports), or had small sample sizes (<10) were excluded for quality, transparency, and to reduce risk of bias. Studies that were missing either a gender or drinking water component were not included in this systematic review. Studies with small sample sizes (<10) and studies that evaluated outcomes unrelated to GEE or drinking water were excluded. Studies evaluating dental health, exercise, and mineral water were excluded because they were not considered drinking water related.

2.4. Additional criteria

Studies that included sanitation and hygiene components were

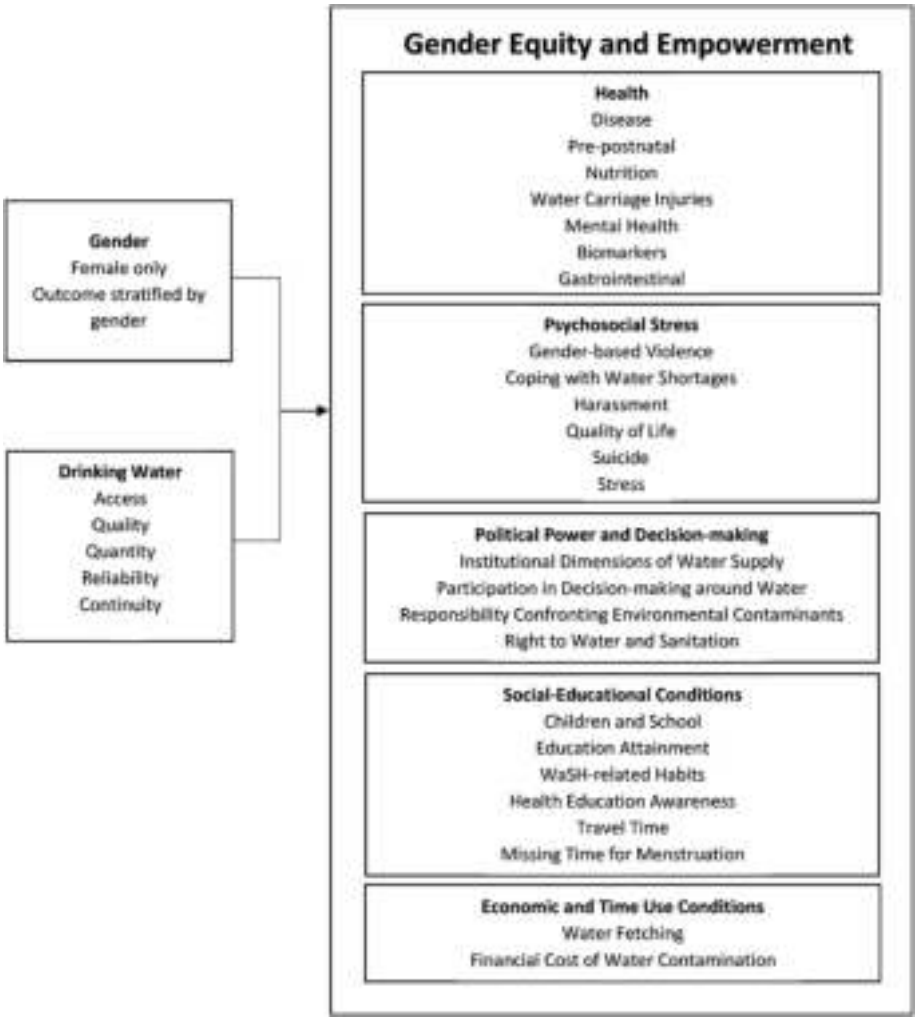

Fig. 1. Study schematic for drinking water and gender equity and empowerment.

included only if drinking water and gender remained a primary focus of the research question. Sanitation was defined as the adequate treatment of human excreta and sewage, including but not limited to sanitation systems, toiletry availability and cleanliness, feces disposal, and open defecation (UNICEF, 2022). Hygiene refers to the behaviors, habits, and actions of individuals that can improve cleanliness and sanitation conditions and decrease the spread of infectious diseases (UNICEF, 2022). Hygiene practices included handwashing, bathing, and menstrual hygiene. While data was collected on whether a study included hygiene and sanitation measures, neither hygiene nor sanitation were part of the inclusion criteria for the systematic review.

2.5. Outcomes

The studies included in the review evaluated the association between drinking water in relation to its implications for GEE. Drinking water's implications for GEE were separated into five main areas–psychosocial stress, political power and decision-making, health, social-educational conditions, and economic and time use conditions–to create a theoretical framework, which developed through our systematic review and analysis. Each GEE category was subdivided and is listed in Fig. 1.

2.6. Data extraction

Three rounds of article review were conducted by the research team. The research team met weekly to discuss study patterns and present articles. The first round analyzed study titles, the second round analyzed study abstracts, and the third round was a full-text review. Using the established exclusion criterion, articles were eliminated at each round.

2.7. Title exclusion

During the first round of exclusion, only publication titles were reviewed. To reduce potential bias, five independent reviewers conducted the title exclusion process and inclusion/exclusion criteria was clearly defined. Article titles needed to explicitly mention either drinking water or water-related outcomes. Gender did not have to be explicitly mentioned in the study title, but studies that included gender in the title were included if present. If mentioned in the title, commentaries were excluded. If a study could not be definitively excluded, all five readers independently decided whether to include or exclude the study during weekly meetings. The final decision to include or exclude was dependent on a majority vote.

2.7.1. Abstract exclusion

Following the title exclusion stage, eligible quantitative and qualitative studies were screened based on the article's abstract where both gender and drinking water were required to be mentioned. To reduce potential bias, five independent reviewers conducted the abstract exclusion. During this stage, commentaries, reviews, and studies with small sample sizes were excluded. All five readers independently decided whether to include or exclude their assigned articles. If a study

could not be definitively excluded, the final decision was dependent on a majority vote during weekly meetings.

2.7.2. Full-text review

In the final round of the review process, a full-text analysis was completed. Both gender and drinking water components were required at this stage for the study to be included. The study team designed an intake survey to extract information on study type, gender component, drinking water component, GEE outcome, and method of data analysis. To reduce potential bias, potential for random error, and to ensure quality, each article was reviewed by two blinded readers. Each reviewer independently completed an intake survey for the same article. Any exclusions at the full-text stage needed to provide justification for exclusion in the intake survey. A third senior reviewer resolved any discrepancies between the two intake surveys and made the final decision if the initial reviewers did not agree.

2.8. Data analysis

The data from the intake surveys were used for data analysis and exported to Microsoft EXCEL. Percentage-based counts and frequency distributions were calculated for study type, gender component, drinking water component, GEE outcome, and method of data analysis. Drinking water was disaggregated according to water access, quality, quantity, reliability, and continuity. Each GEE subcategory was quantitatively assessed and qualitatively evaluated using thematic analysis.

3. Results

A total of 1280 studies were included in this systematic review. The full screening process is outlined in Fig. 2. The initial database search yielded 27,221 studies. After reviewing titles, 20,960 studies were excluded. The abstract review excluded an additional 3181 studies. This left a total of 3080 studies eligible for the full-text review. After full-text review, the final 1280 studies were included. Roughly 87% (n = 1107) of the included studies were published between 2000 and 2020 with approximately 34% (n = 439) published within the last five years. The remaining 173 studies were published between 1980 and 1999. A total of 1202 (93.9%) quantitative studies, one (0.08%) qualitative study, and 47 (3.7%) mixed methods studies were included. The most commonly utilized study designs were cross-sectional (n = 581, 45.4%), observational cohort (n = 329, 25.7%), and case control (n = 195, 15.2%).

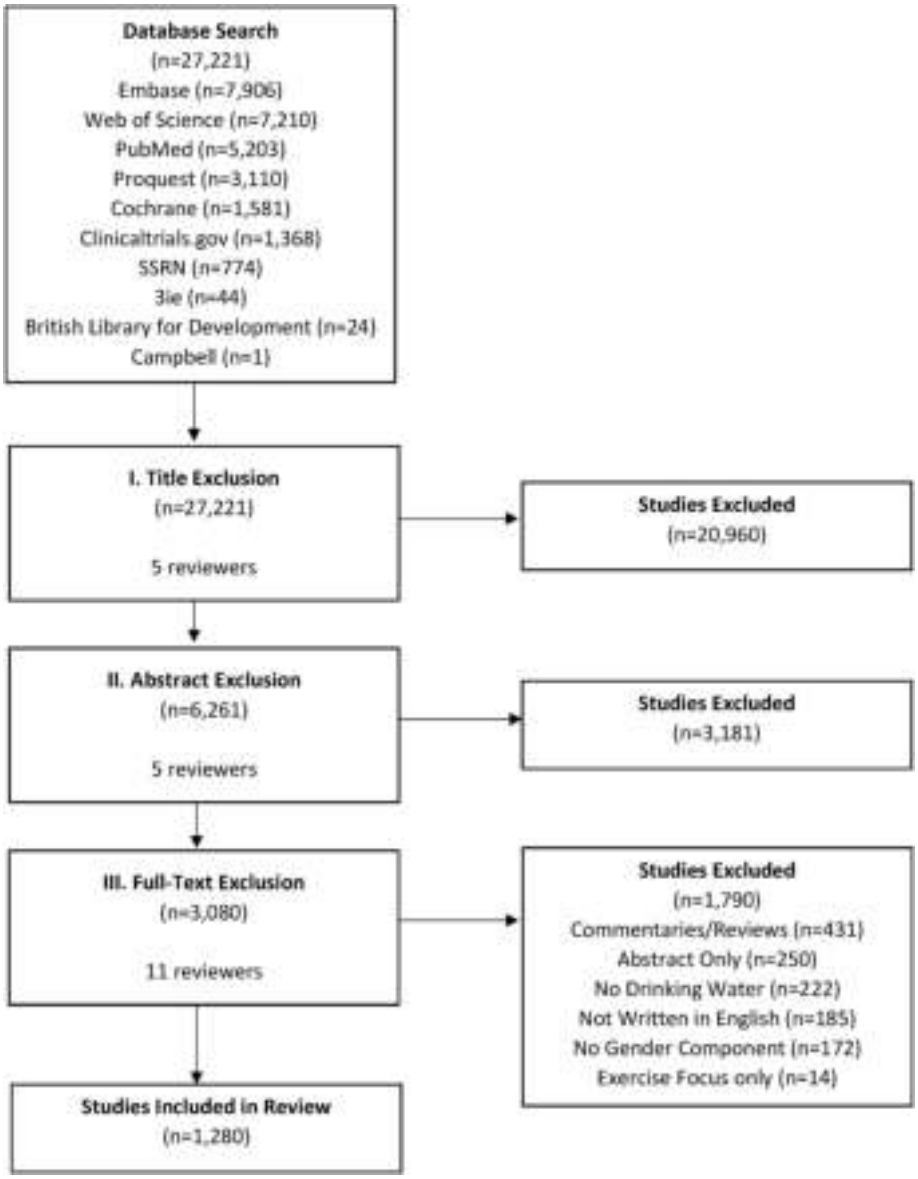

Fig. 2. The identification, screening, and inclusion process for the WaSH systematic review.

3.1. Drinking water

In each study, drinking water was evaluated according to five different parameters – quality, quantity, accessibility, reliability, continuity. Approximately 85.4% (n = 1093) of all studies focused on water quality by specifically examining chemical contaminants (n = 845, 66%) and biological contaminants (n = 252, 19.7%). Chemical contaminants measured across all the studies are listed in Table 1. Roughly 12% of all studies (n = 157) measured water quantity while only 7.6% (n = 97) evaluated water access. Far fewer studies focused on water reliability (n = 17, 1.3%) and water continuity (n = 14, 1%). Table 2.

3.2. Gender

The gender component in each study was categorized as either "study population entirely women" or "data stratified according to gender." Approximately 31.6% (n = 405) of all studies included only female participants while most studies (n = 875, 68.4%) were stratified by gender. While both drinking water and gender components were essential study inclusion criteria for this systematic review, not all studies found a significant association between the two. Significant associations were quantitatively determined ($p < 0.5$) in the individual studies. Most studies (n = 854, 66.7%) noted a gender-based association with water and a GEE outcome.

3.3. Primary GEE outcome

GEE outcomes from the 1280 studies included in this systematic review were separated into five categories: health outcomes, psychosocial stress, political power/decision-making, social-education conditions, and economic/time use conditions. The intake survey that was used to review full-text articles gathered information about the primary GEE outcomes listed in Table 3.

3.4. Health

The health outcomes of study participants were assessed in 91.2% (n = 1167) of all studies. Of those 1167 studies, health was categorized as disease (n = 747, 64%), pre-postnatal conditions (n = 250, 21.4%), gastrointestinal (n = 115, 9.9%), nutrition (n = 113, 9.7%), biomarkers (n = 75, 6.4%), mental health and neurobehavioral conditions (n = 34, 2.9%), and physical injuries related to water-carrying (n = 21, 1.8%). As the most prevalent GEE health outcome, disease was further broken down into 19 categories after the initial categories were established. The association between each disease category and the five drinking water components were determined based on the individual study's findings, along with the presence of a gender association (Table 4).

Cancer – Of the 747 studies that focused on disease, 191 studies identified cancer as the primary health outcome of interest, largely in relation to water quality. The three most widely studied cancer types were bladder (n = 39, 20.4%), lung (n = 38, 19.9%), and breast (23, 12.0%) due to various chemical contaminants such as arsenic, trihalomethane, disinfection byproducts, and asbestos in drinking water.

Arsenic exposure was found to be the main contaminant linked to incidence of bladder cancer. All but one study found a gender-based association between bladder cancer and chemical contamination of drinking water. In cases where the data were stratified and a gender-based association was identified, 70% of the studies found that women had a greater prevalence and increased risk of developing bladder cancer compared to men when exposed to arsenic, trihalomethanes, and chlorine in their drinking water (Hopenhayn-Rich et al., 1996a; Yeh et al., 2015; Llopis-González et al., 2011; Fernandez et al., 2012; Mallin, 1990; Koivusalo et al., 1997; Chen et al., 1985; Steinmaus et al., 2013; Marshall et al., 2007; Smith et al., 2018).

A gender-based association was found between lung cancer and drinking water quality in roughly 80% of all lung cancer studies due to environmental contaminants such as arsenic, uranium, hexavalent chromium, asbestos, and radioactive material. In cases where the data were stratified and a gender-based association was found, 67% of the studies found that men had a greater prevalence and increased risk of developing lung cancer compared to women due to ingestion of arsenic, asbestos, and radioactive material in drinking water (Chen et al., 1992; Argos et al., 2014; Bean et al., 1982a; Buchet and Lison, 1998; Han et al., 2009; Vinceti et al., 2004; Bean et al., 1982b, Kanarek et al., 1980; Lee et al., 2007; Liu et al., 2011; Hopenhayn-Rich et al., 1998; Chung et al., 2013; Smith et al., 2018).

Drinking water contaminated with arsenic, tetrachloroethylene, or disinfection biproducts was associated with an increased risk of developing breast cancer (Aschengrau et al., 2003; Bean et al., 1982b; Aschengrau et al., 1998; Brody et al., 2006; Gallagher et al., 2010; Garland et al., 1996; Michel-Ramirez et al., 2020; Font-Ribera et al., 2018; Vinceti et al., 2004). Although arsenic contamination was associated with an increased risk of breast cancer, the proposed mechanism involving genetic polymorphisms did not appear to be a contributing factor (Michel-Ramirez et al., 2020).

Cardiovascular Disease (CVD), Stroke, and Anemia – Of the 63 studies that focused on cardiovascular disease, anemia, and stroke, 37 studies evaluated CVD, 18 studies evaluated anemia, and 8 studies evaluated stroke as the primary health outcome. Among the CVD studies, 21 studies found a gender-based association with drinking water contaminated with arsenic, magnesium, and calcium. With regards to children, there were higher rates of arsenic-related congenital heart anomalies and cardiovascular deaths among girls (Marie et al., 2018; Rahman et al., 2013). While women were more likely to develop abnormal cardiac rhythms (QT elongation) due to chronic arsenic exposure in drinking water (Mumford et al., 2007; Chen et al., 2013), there were overall higher rates of sudden cardiac death, ischemic heart disease, and carotid atherosclerosis among men (Bernardi et al., 1995; Nerbrand et al., 1992; Huang et al., 2009; Wang et al., 2007) due to magnesium and calcium contamination in drinking water. Only one study uncovered higher incidence rates of CVD for women due to arsenic contamination (Moon et al., 2013).

Out of the 17 studies that evaluated anemia, 15 found a gender-based association with drinking water contaminated with arsenic, iron, and iodine (Kile et al., 2016; Surdu et al., 2015; Henjum et al., 2012; Merrill et al., 2012). Women routinely had higher prevalence rates of arsenic-related anemia compared to men, with an additional association found between maternal anemia and child anemia (Heck et al., 2008; Kang and Kim, 2019). In studies that focused on arsenic contaminated drinking water and stroke, four studies found a gender-based association. Arsenic-contaminated drinking water was associated with an increased stroke mortality risk among women with no improvement in vascular response after switching to low-arsenic water when compared to men (Rahman et al., 2014; Pi et al., 2005). The two remaining studies

Table 1
Water quality chemical contaminants.

Chemical	n (%)
All Chemicals	845
Arsenic	312 (36.9)
Trace Elements(Ca, Mg, Mn, Fe, Cd)	127 (15.0)
Fluoride	67 (7.9)
Disinfectant by-products(Chlorine, Chlorine by-products, Perchlorate)	58 (6.9)
Nitrate/Nitrite	48 (5.7)
Lead	37 (4.4)
Trihalomethanes	37 (4.4)
PFAs/PFOs/PCE	34 (4.0)
Iodine	32 (3.8)
Lithium	17 (2.0)
Phthalates	4 (0.5)
[a]All other	72 (8.5)

[a] All other includes: antibiotics, asbestos, BPA, chloroform, herbicides, or unspecified.

Table 2
Included continuity and reliability studies.

Publication [Author, Date]	Title	Study Design	Country	Main Findings	Association with GEE outcome	Significant GEE association?
Alfredo et al. (2014)	Fluoride contamination in the Bongo District of Ghana, West Africa: geogenic contamination and cultural complexities	Observational	Ghana	Fluoride concentrations in the region are higher than reported and are inversely related to rainfall; women should be involved in decision-making to adequately treat water	Women's participation in decision making; Right to water and sanitation	Yes
Alhassan and Kwakwa (2014)	When water is scarce: the perception of water quality and effects on the vulnerable	Cross-sectional	Ghana	Water insecurity increases burdens and gender-specific hazards on women and children and decreases quality of life	Psychosocial stress	Yes
Baguma et al. (2013)	Safe-water shortages, gender perspectives, and related challenges in developing countries: The case of Uganda	Cross-sectional	Uganda	Efficient water management is related to women's years of water harvesting, family size, and tank operation and maintenance; emphasis should be placed on women-related water management activities	Women's participation in decision-making; Women's responsibility in confronting environmental contaminants	Yes
Habi and Harrouz (2015)	Domestic water conservation practices in Tlemcen City (Algeria)	Cross-sectional	Algeria	Informal water rationing due to scarcity leads to increased tension among women in the community to store enough water and large discrepancies in water distribution	Quality of life; Women's participation in decision-making	Yes
Phiri et al. (2014)	Climate Change Impacts on Rural Based Women: Emerging Evidence on Coping and Adaptation Strategies in Tsholotsho, Zimbabwe	Cross-sectional	Zimbabwe	Impact of climate change affect women's quality of life leading to water and food insecurity and increased poverty, particularly for those in rural areas	Women's responsibility in confronting environmental contaminants; Quality of life	Yes
Stoler et al. (2012)	When urban taps run dry: sachet water consumption and health effects in low income neighborhoods of Accra, Ghana	Cross-sectional	Ghana	Neighborhood rationing is predictive of water sachet uptake. Water sachet uptake in low SES leads to better health outcomes and less diarrheal diseases	Women's responsibility in confronting environmental contaminants; Gastrointestinal	Yes
Khan et al. (2017)	Optimizing household survey methods to monitor the Sustainable Development Goals targets 6.1 and 6.2 on drinking water, sanitation and hygiene: A mixed-methods field-test in Belize	Cross-sectional	Belize	MISC surveys for WASH monitoring should be further refined; safely managed drinking water is underestimated; stored drinking water is more likely to be contaminated	Coping with water shortages; Women's responsibility in confronting environmental contaminants	Yes
Trudeau et al. (2018)	Water system unreliability and diarrhea incidence among children in Guatemala	Retrospective Cohort	Guatemala	Age, female gender, Spanish language, garbage disposal, and interrupted water service is associated with diarrheal incidence in children	Women's responsibility in confronting environmental contaminants; Gastrointestinal	Yes
Abu Morad (2004)	Palestinian refugee conditions associated with intestinal parasites and diarrhea: Nuseirat refugee camp as a case study	Case study	Palestine	Intestinal parasites are associated with low socioeconomic status, interrupted water service, drinking water source, drinking water storage methods, and women's attitude and practice of personal hygiene	Habits (ie. hand washing, open defecation); Women's responsibility in confronting environmental contaminants	Yes
Morgan et al. (2017)	Water, sanitation, and hygiene in schools: Status and implications of low coverage in Ethiopia, Kenya, Mozambique, Rwanda, Uganda, and Zambia	Cross-sectional	Ethiopia, Kenya, Mozambique, Rwanda, Uganda, Zambia	Basic WaSH deficiencies in rural schools in these countries are associated with low attendance and adverse health outcomes	Children and school	Yes
Khodarahimi and DehghaniNikpourian, 2014	Mental Health and Coping Styles of Rural Residents Affected by Drinking Water Shortage in Fars Province: An Ecopsychological Perspective	Cross-sectional	Iran	Rural residents with water shortages had higher a psychopathology indicator, lower rational coping styles, and higher emotion focused avoidant coping styles	Coping with water shortages	No
Prasad et al. (2018)	Epidemiology and risk factors for typhoid fever in Central Division, Fiji, 2014ñ2017: A case-control study	Case-control	Fiji	Salmonella typhii transmission is attributed to water availability, drinking	Disease	No

(*continued on next page*)

Table 2 (*continued*)

Publication [Author, Date]	Title	Study Design	Country	Main Findings	Association with GEE outcome	Significant GEE association?
				contaminated water, habits, and poor sanitation facilities		
Joshi et al. (2013)	Water and sanitation hygiene knowledge attitude practice in urban slum settings.	Cross-sectional	India	Women and girls are primarily responsible for water collection, particularly from long distances during shortages; most respondents believed contaminated water was safe to drink	Habits (ie. hand washing, open defecation)	Yes

Table 3
Primary GEE outcomes for included studies.

Primary GEE Outcome	n (%)
Disease	743 (58.0)
Pre-postnatal	192 (15.0)
Nutrition	60 (4.7)
Gastrointestinal	57 (4.4)
Institutional Dimensions of Water Supply	24 (1.9)
WaSH-related Habits	23 (1.8)
Water Exposure/Water-carrying Injury	21 (1.6)
Women's Participation in Decision-Making	17 (1.3)
Mental Health	17 (1.3)
Contamination in Crops and Food	16 (1.3)
Socioeconomic Status and Disease Risk	15 (1.2)
Children and School	12 (0.9)
Health Education	11 (0.9)
Right to Water and Sanitation	9 (0.7)
Women's Responsibility Confronting Environmental Contaminants	5 (0.4)
Education Attainment	5 (0.4)
Coping with Water Shortages	4 (0.3)
Unspecified	52 (4.1)

found that arsenic concentration in well water was associated with a higher cerebrovascular disease prevalence and mortality rate among men (Chiou et al., 1997; Cheng et al., 2010).

Infectious Diseases – Of the 130 studies that identified an infectious or enteric disease as the primary health outcome from an exposure in water, 22 studies evaluated Toxoplasma gondii, 16 studies evaluated Helicobacter pylori, 12 studies evaluated typhoid, 12 studies evaluated intestinal parasites, and 11 studies evaluated schistosomiasis exposure. Of the 22 Toxoplasma gondii studies, 18 found a gender-based association. However, there were mixed results as to whether women (Fu et al., 2014a; Messier et al., 2009) or men (Sucilathangam and Anna, 2016) had higher Toxoplasma gondii infection rates. Results seemed to vary by study location and population. In the 40 studies that focused on Helicobacter pylori, typhoid, or intestinal parasite infections, no discernible consensus regarding gender-based associations were found. When data were disaggregated by gender, the majority of Helicobacter pylori, typhoid, and intestinal parasite studies found no gender-based association at all. However, in the schistosomiasis studies, nine studies found a gender-based association. Men had an increased risk and higher prevalence rates of schistosomiasis with lower reduction in disease severity after intervention (Lee et al., 2015; Yang et al., 2009, Hajissa et al., 2018).

Arsenicosis and Skin Lesions –A total of 70 studies identified either arsenicosis or skin lesions as the primary health outcome. Fifty-four studies found a gender-based association with arsenic in drinking water and skin lesions. In cases where the data was stratified and a gender-based association was identified, 77% of the studies found that men had a greater prevalence and increased risk of developing skin lesions from arsenicosis compared to women (Adhikari and Ghimire, 2009; Gamble et al., 2005; Nahar, 2009; Hadi and Parveen, 2004; Maharjan et al., 2006; von Ehrenstein et al., 2005; Guha Mazumder

Table 4
Evaluation of disease outcomes with drinking water and gender association.

Disease	*n (%)	+Gender Association n (%)	+Water Quality n (%)	+Water Quantity n (%)	+Water Access n (%)	+Water Continuity n (%)	+Water Reliability n (%)
Cancer	191 (25.6)	137 (71.7)	179 (93.7)	12 (6.3)	1 (0.5)	–	–
Infectious Disease	130 (17.4)	80 (61.5)	126 (96.9)	5 (3.8)	11 (8.5)	2 (1.5)	2 (1.5)
Cardiovascular disease, stroke, anemia	63 (8.4)	40 (63.5)	54 (85.7)	7 (11.1)	1 (1.6)	–	–
Blood Pressure, Obesity, Cholesterol, Diabetes	47 (6.3)	25 (53.2)	42 (89.4)	5 (10.6)	1 (2.1)	–	–
Skin Lesions	42 (5.6)	35 (83.3)	42 (100)	1 (2.9)	–	–	–
Thyroid Disorders	39 (5.2)	27 (69.2)	39 (100)	2 (5.1)	–	–	–
Liver Disorders	32 (4.3)	21 (65.6)	31 (96.9)	1 (3.1)	1 (3.1)	–	–
Bone Disorders	31 (4.1)	21 (67.7)	31 (100)	1 (3.2)	–	–	–
Arsenicosis	28 (3.7)	19 (67.9)	28 (100)	6 (21.4)	2 (7.1)	–	–
Children	24 (3.2)	20 (83.3)	16 (66.7)	1 (4.2)	4 (16.7)	–	–
Kidney Disorders	21 (2.8)	10 (47.6)	16 (76.2)	4 (19.0)	1 (4.8)	–	–
Genetic	19 (2.5)	15 (78.9)	19 (100)	–	–	–	–
Neurobehavioral	19 (2.5)	11 (57.9)	17 (89.5)	2 (10.5)	–	–	–
Women's Health	17 (2.3)	12 (70.6)	13 (76.5)	1 (5.9)	–	–	–
Toxicity	8 (1.1)	5 (62.5)	7 (87.5)	2 (25.0)	–	–	–
Respiratory Illness	5 (0.7)	4 (80.0)	5 (100)	–	–	–	–
Gastrointestinal	4 (0.5)	2 (50.0)	3 (75.0)	1 (25.0)	–	–	–
Urinary Disorders	4 (0.5)	3 (75.0)	1 (25.0)	3 (75.0)	–	–	–
All Other	46 (6.1)	32 (69.6)	40 (87.0)	8 (17.4)	3 (6.5)	–	–

*Percentages were calculated using the 747 studies where disease was indicated as a health GEE outcome; +Percentages were calculated using the total number of studies for the specific disease indicated in that row.

et al., 1998; Liao et al., 2007; Argos et al., 2013; Lindberg et al., 2008a; Lindberg et al., 2008b; Yang et al., 2017; Tondel et al., 1999; Ahsan et al., 2006; Watanabe et al., 2001; Argos et al., 2011; Fu, S et al., 2014; Fu et al., 2014b; Smith et al., 2000; Chen et al., 2006). However, several studies reported increased risk of adverse pregnancy outcomes in women with chronic arsenic exposure and arsenicosis including spontaneous abortion, stillbirth, neonatal death, low birthweight, preterm birth, and congenital abnormalities (Chakraborti et al., 2016a; Chakraborti et al., 2016b; von Ehrenstein et al., 2006; Chakraborti et al., 2003; Marie et al., 2018).

Musculoskeletal Injuries – Of the 21 studies that evaluated physical injuries due to water fetching, 18 studies identified women as the main collectors of household drinking water. Three studies identified water fetching as the responsibility of women and men (Sarkar et al., 2015; Subbaraman et al., 2015; Mercer and Hanrahan, 2017). The act of water carriage, the distance traveled to fetch water, and the number of trips necessary to collect a sufficient amount of water were contributing factors to the incidence of musculoskeletal injuries (Zolnikov and Blodgett Salafia, 2016; Narain 2014; Geere et al., 2018b; Mercer and Hanrahan, 2017). To transport containers of water while walking, women balanced containers on their heads, carried containers in their arms, or utilized both methods of carriage simultaneously (Bisung et al., 2015; Stevenson et al., 2012). These methods of water carriage resulted in head and neck pain, axial compression, upper and lower back pain, and joint pain (Geere et al., 2010, 2018b; Narain 2014; Stevenson et al., 2012; Sarkar et al., 2015). Long distances to water sources and frequent number of trips resulted in exhaustion, neck and hip pain, chronic pain, and foot injuries, especially when walking barefoot (Mercer and Hanrahan, 2017; Zolnikov and Blodgett Salafia, 2016; Sarkar et al., 2015; Bisung et al., 2015).

Health Outcomes in Children – Fifty-two studies found a gender-based association between water access and contamination with arsenic, lead, manganese, and biologic agents on health outcomes in children. Health outcomes in children primarily focused on parasitic and waterborne infections such as cryptosporidiosis, dysentery, giardiasis, helicobacter pylori, schistosomiasis, diarrhea (n = 32) and effects on cognitive function and intelligence quotient (IQ) (n = 8), blood pressure (n = 7), and thyroid function (n = 6). In general, studies found that girls had higher infection rates for helminth, helicobacter pylori, urinary tract infections, and diarrheal disease due to biological contaminants in drinking water compared to boys (Onyido et al., 2017; Awuku et al., 2017; Pirinççioğlu et al., 2018; Zincir et al., 2012; Komarulzaman et al., 2019). While boys were more likely to be infected with cryptosporidium, giardiasis, schistosomiasis, and hookworm compared to girls (Al-Shamiri et al., 2010; Nimri, 1994; Bigwan et al., 2013; Abdulkareem et al., 2018; Lee et al., 2015; Forrer et al., 2018), studies found mixed results for intestinal parasitosis with no general consensus whether girls or boys had higher prevalence rates (Shakya et al., 2012; Fentie et al., 2013).

Higher prevalence rates of waterborne diseases in girls or boys were generally ascribed to higher levels of exposures – often through contaminated drinking water sources (Onyido et al., 2017; Nimri, 1994; Awuku et al., 2017; Bigwan et al., 2013; Shakya et al., 2012). The authors explained these different levels of exposures with the different behaviors of boys and girls. Abdulkareem et al. ascribed higher schistosomiasis infection rates in Nigerian boys to more leisure activities in the water, like swimming, fishing, and playing in rivers (2018). Komarulzaman et al. attributed higher rates of diarrheal disease in females to increased exposure through water-fetching (2018).

Longitudinal studies found gender-based associations with arsenic and manganese contamination in drinking water and IQ. A population-based cohort study in Bangladesh (n = 2853) found that arsenic exposure was negatively associated with IQ for girls at age five, but not for boys (Hamadani et al., 2011). The effects of manganese contamination in drinking water on IQ were mixed. Some studies found that manganese exposure improved performance IQ in boys and decreased performance in girls (Bouchard et al., 2018; Dion et al., 2018), while Rahman et al. found that manganese exposure improved cognitive function in girls and did not affect boys (2017).

3.5. Psychosocial stress

Psychosocial stress was evaluated in 13% (n = 166) of all studies. Of those 166 studies, psychosocial stress encompassed topics such as quality of life (n = 33, 19.9%), coping with water shortages (n = 26, 15.2%), stress (n = 22, 12.9%), suicide (n = 11, 6.4%), harassment (n = 6, 3.5%), and gender-based violence related to water (n = 5, 2.9%).

Gender-based Violence and Harassment – None of the articles analyzed a direct association between gender-based violence and drinking water as their primary focus. Only one qualitative study in Uganda documented gender-based violence experienced by women when they travel to collect drinking water (Pommells et al., 2018). However, eight articles studied gender-based violence in relation to drinking water-related health outcomes such as anemia, arsenicosis, preterm birth, and low birth weight. Most of these studies took place in South Asian countries (Ahmad et al., 2007; Baker et al., 2018; Devasia, 1998; Gautam et al., 2019; Nerkar et al., 2013). In the 2016 Nepal Demographic and Health Survey, 24% of study participants reported having ever experienced physical or sexual violence with a partner and out of these participants 41% were anemic. However, the association between intimate partner violence and water-related anemia was not statistically significant (Gautam et al., 2019, Ministry of Health, Nepal, 2017). Gender-based violence was only discussed in relation to sanitation, which was not the primary focus of this review.

Women and girls with arsenicosis were more likely than men to suffer from socio-emotional difficulties including rejection, discrimination, and ostracization (Ahmad et al., 2007; Sarker, 2010; Syed et al., 2012). In a cross-sectional study of 750 respondents in Bangladesh, women were more likely to experience extensive socio-emotional and economic problems with development of cutaneous arsenicosis, less likely to receive treatment compared to men, and more likely to be denied care (Ahmad et al., 2007). Common barriers to care include lack of transportation to clinics due to long distances from homes, poor transportation availability, social expectations of traveling with men, and a lack of access to female doctors (Ahmad et al., 2007). Due to religious and cultural practices in Bangladesh, physical examinations and treatments need to be either conducted by female doctors or approved via consent from a husband (Ahmad et al., 2007). Additionally, other studies found that women and girls with physical manifestations of arsenicosis experienced rejection from family members and the community (Sarker, 2010; Ahmad et al., 2007; Syed et al., 2012). Women and girls with arsenicosis were seen as contagious and consequently denied marriages, abandoned by their husbands, isolated from society, and barred from social functions (Sarker, 2010; Ahmad et al., 2007; Syed et al., 2012).

3.6. Political power and decision-making power

Women's political power and decision-making were evaluated in 23.9% (n = 306) of all studies. Of those 306 studies, political power and decision-making included topics such as institutional dimensions of water supply (n = 244, 79.7%), participation in decision-making around water (n = 42, 13.7%), responsibility confronting environmental contaminants (n = 40, 13.1%), and the right to water and sanitation (n = 38, 12.4%).

Decision-making Power – Decision-making demonstrates the power dynamic within households and communities and is grounded in cultural practices, norms, and societal expectations (Barker et al., 2016). Decision-making power was defined as a woman's ability to make decisions regarding clean water sources, drinking water collection, and the use of water filters (Adams et al., 2018; Kookana et al., 2016; Mohammed and Rilwanu, 2016; Baker et al., 2018). There were 74 studies that specifically focused on women's decision-making. Most studies were

conducted in Africa and India within the last 5 years. Although women's decision-making was identified as the primary outcome of interest in 17 studies, only 6 directly measured women's decision-making ability. The remaining articles focused on the impact that study findings had on women's decision-making ability, rather than measuring the impact women's decision-making had on drinking water.

Six studies measured women's individual decision-making ability while confirming the existing limitations on women's power in their community. For example, a study conducted by Trinies et al. evaluated the decision-making process surrounding the use of water filters in India. The study found that the successful adoption of water filters would require negotiations led by women in the community (Trinies et al., 2011). However, the study concluded that successful negotiations would also have to include family and community members that had more control and the power to make household purchasing decisions (Trinies et al., 2011).

Similarly, women's low levels of power and decision-making were reinforced in a study conducted in Malawi (Adams et al., 2018). Water User Associations (WUAs) were created to promote women's participation in decision-making regarding drinking water utilization and irrigation systems (Adams et al., 2018). The study found that formalized, elected committees did not increase women's ability to make decisions regarding drinking water as women were relegated to lower positions that afforded them little to no real power (Adams et al., 2018). Even when women were acknowledged in community-based negotiations, women's power and decision-making were not impactful.

3.7. Social-educational conditions

Social-educational conditions were evaluated in 51.3% (n = 657) of all studies. Of those 657 studies, social-educational conditions were related to children and school (n = 88, 13.1%), education attainment of the study participants (n = 559, 85.1%), WaSH-related habits (n = 204, 31.1%), health education/awareness (n = 126, 19.2%), travel time to water source (n = 38, 5.8%), and missing time for menstruation (n = 1, 0.2%).

Children and School – Eighty-eight studies evaluated the relationship that drinking water access and water quality had on children at school. Most studies were conducted in South Asia and Africa (n = 42), while 11 studies were conducted in the U.S. and Canada. Approximately 90% (n = 78) of the studies were conducted after the year 2000 with 30 studies published within the last 5 years. The recency of these studies suggests that the impact of drinking water on children and school is an emerging concern.

Water fetching was discussed in 11% (n = 10) of studies and heavily impacted girls' participation in school when compared to their male counterparts (Kookana et al., 2016; Alhassan and Kwakwa, 2014; Tayeh et al., 1993; Demie et al., 2016; Komarulzaman et al., 2019; Ravichandran and Boopathi, 2005). A cross-sectional study of 500 families in India that looked at school attendance records for children (ages 13–14) found that female students missed school almost twice as often as male students due to domestic responsibilities, including water fetching (Kookana et al., 2016). Female students missed five or more days of school per month, at a rate of 2–10 times more than their male counterparts, when faced with limited water accessibility (Kookana et al., 2016 et al.). Overall, girls spent less time receiving an education and more time collecting water. A mixed-methods study in Ethiopia used multiple stage sampling to collect data on 197 households and found that women and girls spend 3–4 h per day fetching water, which corresponds to the loss of 37–51 days of education per year (Demie et al., 2016). Nearly 50% of girls were not enrolled in school at all due to their domestic responsibilities (Demie et al., 2016). Similarly, a cross-sectional study in India found that girls had more limited options than boys due to their domestic duties (Ravichandran and Boopathi, 2005). During summer months, water fetching was highly dependent on rainwater availability, which led to increased travel time to water sources (Ravichandran and Boopathi, 2005). The burden of water fetching was made easier for young girls if they dropped out of school, as water fetching often conflicted with school hours (Ravichandran and Boopathi, 2005).

3.8. Economic and time-use conditions

Economic and time-use conditions were evaluated in 46.2% (n = 591) of all studies. Economic and time-use conditions were defined as the missed economic opportunities due to time spent fetching water (n = 49, 8.3%) and the financial costs associated with contaminated crops, food, and livestock (n = 161, 27%).

Water Fetching – A total of 49 studies focused on water fetching with the majority of studies published within the last 10 years. Studies evaluated the uneven distribution of domestic tasks between men and women and the negative impact of water fetching on women's participation in the labor market (Hoque and Hope, 2018; Devasia 1998; Nerkar et al., 2013; Alfredo et al., 2014; Demie et al., 2016). The majority of studies identified women and girls as the primary collectors of water with limited contribution from their male counterparts and found that time spent fetching water equated to less time for women to pursue economic ventures (Hoque and Hope, 2018; Devasia 1998; Nerkar et al., 2013; Alfredo et al., 2014; Demie et al., 2016). In Ethiopia, a mixed-methods study of 197 households found that women, on average, traveled greater than 1 km for water, which is equivalent to 5 h collecting water per day (Demie et al., 2016).

When employment opportunities were available in water management, operation and/or maintenance, women were largely responsible for monitoring water distribution for communities that needed to fetch water (Adams et al., 2018). One study in Malawi assessed the economic opportunities available to women in relation to drinking water (Adams et al., 2018). When women were able to find water management jobs, they were relegated to lower-paying positions (ie. Water kiosk attendants), compared to men, and were given lower wages than stated in their contracts (Adams et al., 2018). Women were also given more labor-intensive jobs, such as field inspectors, and had to travel long distances to fetch and monitor and read water meters (Adams et al., 2018).

Climate Change and Water – In Zimbabwe, climate change was responsible for depleting water resources, thereby impacting food reserves and livestock (Phiri et al., 2014). As women are primarily responsible for domestic duties, including water and food preparation, they are affected by climate change's impact on the water supply. Depleted water resources led to sick livestock, more money spent on medications, and less income generated from sick livestock (Phiri et al., 2014) . An additional burden was placed on women due to the climate change-induced migration of the male workforce (Phiri et al., 2014). In Bangladesh, climate change was responsible for the salinization of water sources, river erosion, water logging in homes and loss of agricultural fields resulting in fewer employment opportunities (Beier et al., 2015). Older females were more likely to be negatively impacted by climate-induced changes in employment opportunities and drinking water supplies (Beier et al., 2015). Women were also more likely to suffer negative health outcomes due to food scarcity and agricultural crop destruction (Beier et al., 2015).

4. Discussion

While previous studies have evaluated WaSH conditions and its association with gender roles, this is the first systematic review to assess the management of drinking water and drinking water related disease outcomes and its implications for gender equity and empowerment (GEE). A theoretical framework was created that separates drinking water conditions and GEE outcomes, and divides outcomes into categories – health, psychosocial stress, political power and decision-making, social/educational conditions, and economic and time use

conditions (Fig. 1). We synthesized evidence on drinking water exposures and GEE outcomes resulting in the largest and most comprehensive gender-focused systematic review on drinking water to date. The results span across multiple domains, alluding to the highly gendered nature of drinking water and the intersectionality between gender equity and empowerment. We found that drinking water and GEE were most commonly studied by assessing water quality, specifically its chemical and microbiological contaminants and the relationship with women and girls' health. Within the GEE outcome of health, cancer, cardiovascular disease/stroke/anemia, infectious diseases, and arsenicosis were most commonly studied. Water access, reliability, and continuity as they relate to gender were less well studied.

Health – Over 90% of full text articles reviewed focused on the association between drinking water exposures and health outcomes. Overall, women had higher incidence rates of bladder cancer due to drinking water contaminated with arsenic, trihalomethanes, and chlorine (Hopenhayn-Rich et al., 1996b; Yeh et al., 2015; González-Weller et al., 2012; Fernandez et al., 2012; Mallin et al., 1990; Koivusalo et al., 1997; Koivusalo et al., 1995; Chen et al., 1985; Steinmaus et al., 2013; Marshall et al., 2007; Smith et al., 2018). Arsenic, trichloroethylene, and disinfection byproducts exposure in drinking water was associated with increased incidence of breast cancer in women (Aschengrau et al., 2003; Bean et al., 1982b; Aschengrau et al., 1998; Brody et al., 2006; Gallagher et al., 2010; Garland et al., 1996; Michel-Ramirez et al., 2020; Font-Ribera et al., 2018; Vinceti et al., 2004). While we found some evidence, more research is needed on arsenic and trihalomethanes and the relationship with breast cancer in women, as the results are mixed (Pullella and Kotsopoulos, 2020; Font-Ribera et al., 2018). Women experienced higher incidence rates of anemia when exposed to drinking water contaminated with arsenic and iodine (Kile et al., 2016; Surdu et al., 2015, Henjum et al., 2012). Conversely, we found that men had higher incidence rates for lung cancer due to exposure to arsenic, asbestos, and radioactive material (Chen et al., 1992; Argos et al., 2014; Bean et al., 1982a, Buchet and Lison, 1998; Han et al., 2009; Vinceti et al., 2004; Bean et al., 1982b; Kanarek et al., 1980; Lee et al., 2007; Hopenhayn-Rich et al., 1998; Chung et al., 2013; Smith et al., 2018). Men also had higher incidence rates of cardiovascular disease due to magnesium and calcium exposure in drinking water (Bernardi et al., 1995; Nerbrand et al., 1992; Huang et al., 2009; Wang et al., 2007).

While water-borne diseases are an important area of research for GEE and drinking water, comprising roughly 10% of our data, few studies disaggregated data by gender. Of those that did, results were often mixed with no consensus regarding Helicobacter pylori, typhoid, and intestinal parasitosis for adult populations. This finding aligns with previous systematic reviews, which have found a lack of WaSH-related water-borne disease studies among adult populations; most focus on children (Pouramin et al., 2020). As drinking water is a heavily gendered issue that disproportionately impacts women and is a known cause of many parasitic, infectious, and diarrheal diseases, we recommend future studies also include adult populations and disaggregate data by gender when evaluating water-related disease incidence (Mourad et al., 2019; Hunter et al., 2001; Beer et al., 2015; Tomberge et al., 2021; Graham et al., 2016). Furthermore, it is important to note the lack of research regarding the impact of water-related parasitic, infectious, and diarrheal diseases on pregnant women and newborns.

One area of health that demonstrates a strong correlation between GEE and drinking water is arsenicosis and skin lesions, with 77% of arsenic studies finding a gender-based association. Women's prenatal health outcomes are severely impacted by arsenic exposure. Higher rates of spontaneous abortion, stillbirth, neonatal death, low birth weight, preterm birth, and congenital abnormalities have been found among female cohorts that have been exposed to chronic arsenic contamination in drinking water during pregnancy (Chakraborti et al., 2016a; Chakraborti et al., 2016b; von Ehrenstein et al., 2006; Chakraborti et al., 2003; Marie et al., 2018).

Studies suggest that there is a strong correlation between water fetching, water carriage, and the incidence of musculoskeletal injuries in women (Geere et al., 2010; Stevenson et al., 2012; Bisung et al., 2015; Zolnikov and Blodgett Salafia, 2016; Geere et al., 2018b; Narain, 2014). Common injuries included head and neck pain, axial compression, upper and lower back pain, joint pain, hip pain, and foot injuries (Geere et al., 2010; Geere et al., 2018b; Narain, 2014; Stevenson et al., 2012; Mercer et al. 2017; Zolnikov and Blodgett Salafia, 2016; Sarkar et al., 2015; Bisung et al., 2015). Comparatively fewer studies identified both men and women as household water collectors (Sarkar et al., 2015; Subbaraman et al., 2015; Mercer and Hanrahan, 2017). However, of the studies that did, none compared the types of physical injuries suffered by men and women (Sarkar et al., 2015; Subbaraman et al., 2015; Mercer and Hanrahan, 2017). Additionally, only one study evaluated pregnant women as water collectors, but physical injuries were not evaluated (Ghosh et al., 2016). Although water fetching and water carriage have been found to negatively impact the physical health of water collectors, future research should compare the physical injuries suffered by men and women that fetch water and evaluate the impact water fetching and water carriage have on the physical health of pregnant women.

Psychosocial Stress – Psychologically, we found substantial evidence that women face the added burden of arsenicosis-related harassment that often does not apply to men (Ahmad et al., 2007; Sarker, 2010; Syed et al., 2012). Even though men are more likely than women to develop skin lesions from arsenic (Adhikari and Ghimire, 2009,; Gamble et al., 2005; Nahar, 2009; Hadi and Parveen, 2004; Maharjan et al., 2005; Maharjan et al., 2006; von Ehrenstein et al., 2005; Guha Mazumder et al., 1998; Liao et al., 2007; Argos et al., 2013; Lindberg et al., 2008b; Yang et al., 2017; Tondel et al., 1999; Ahsan et al., 2006; Watanabe et al., 2001; Argos et al., 2011; Fu et al., 2014b; Smith et al., 2000; Chen et al., 2006), women and girls face social ostracization from the community and are pulled out of school, rejected by their families and husbands, denied health care, and lose employment opportunities due to stigma (Brinkel et al., 2009; Ahmad et al., 2007; Sarker, 2010; Syed et al., 2012). While the physical health impacts of water-related arsenicosis may not be as pronounced for women, the psychological burden disproportionately impacts their quality of life. However, in order to adequately address the ramifications of inequitable treatment, we acknowledge that the basic physiological health and safety needs of women and girls must be met first. In accordance with Maslow's hierarchy of needs, health and safety must be established before steps can be taken to improve social connections, self-esteem, and empowerment (Lester et al., 1983). Likewise, the GEE outcomes of psychosocial stress, political power and decision-making, social-educational achievement, economic power, and time use cannot be addressed until the social structures women live in are more accepting of women's empowerment and gender equity. We found little research on psychosocial stress related to water availability, continuity, or reliability of water supply.

None of the studies evaluated the direct association between gender-based violence (GBV) and drinking water, a topic better studied as it relates to sanitation. This may be due to the lack of research that quantifies gender-based violence and harassment as it is associated with drinking water as well as methodological and ethical challenges that arise when conducting research of this nature (Sommer et al., 2015; Cepeda et al., 2021; Caruso et al., 2021). Stigma and shame associated with gender-based violence can negatively impact incident reporting (Barnett et al., 2016). It will be difficult to evaluate the direct association between drinking water, water fetching, gender-based violence, and psychosocial stress without first addressing the need to destigmatize violence against women and developing validated measurement strategies. Recently, there have been efforts to develop quantitative toolkits for gender-based violence in order to uniformly define and measure violence against women (House et al., 2012; Sommer et al., 2015; Raj, 2021). We believe that these are crucial steps towards ensuring that future research can evaluate how and to what extent drinking water and water fetching impact gender-based violence or harassment.

Political Power and Decision-making – The existing literature on

gender and women's participation in water management suggests that when women must walk to collect water, women bear an increased burden compared to men (United Nations Children's Fund, 2016). Furthermore, women's participation in water planning and decision-making is low compared to men despite evidence that their participation may lead to better outcomes (Adams et al., 2018; Andajani-Sutjahjo et al., 2015). While studies acknowledged the important role women play in household water collection and management, they are not typically in positions of power where they are able to exercise their decision-making power in meaningful ways. While we were interested in assessing how women's political power and decision-making impacted drinking water conditions, we found that the vast majority of studies focused on how women's decision-making abilities were impacted by drinking water conditions. For example, women were occupied with water collection duties, which left little time to engage in political matters or offer their insight when decisions needed to be made. While that is an important factor in GEE, it is also critical to study how women's decision-making about drinking water impacts drinking water quality, continuity, reliability, quantity, and cost and subsequent water-related health outcomes. With so few studies quantitatively measuring women's decision-making, future research could focus on the social and political factors that affect how and when women make decisions about drinking water usage. Further examination of women's decision making in water management and how it is associated with water outcomes (quality, continuity, reliability, quantity, and cost) and community health is also needed.

Social-educational Conditions – We found that girl's participation in school was severely impacted by household domestic duties, including water fetching. Young girls were more likely to miss up to two months of schooling per year due to time spent collecting water (Kookana et al., 2016; Demie et al., 2016; Ravichandran and Boopathi, 2005). The studies we reviewed focused solely on the impact water fetching has on young girls' school absenteeism and did not comprehensively study the long-term implications for school dropout rates. Education research suggests that missed educational opportunities, including missed school, can lead to drop out and impact girls' life trajectory by limiting financial independence through income-generating endeavors and basic health knowledge acquisition (U.S. Department of Education, 2019). Future water studies should expand on the effects of girls' school absenteeism while water fetching and dropout on gender roles as they age. Additionally, very few articles mentioned the impact water inequities may have on young girls outside of school. Although most studies did not measure the physical health impact of water collection on young girls, it is likely that young girls experience some of the same water carriage injuries as women that fetch water. These can include physical pain, fatigue, perinatal health problems, uterine prolapse, spinal fractures and dislocations, and cervical compression syndromes, which lead to serious long-term disabilities (Geere et al., 2018a). However, there is limited research regarding the prevalence of these health outcomes in young school-aged girls. This is an important study area for future research due to the degenerative nature of these health problems.

Economic/Time use Conditions – Women and girls were identified as the primary water collectors in the household (Hoque and Hope, 2018; Devasia 1998; Nerkar et al., 2013; Alfredo et al., 2014; Adams et al., 2018). Due to the time-consuming nature of water fetching, some women do not have enough time to pursue careers, thereby limiting their economic opportunities (Hoque and Hope, 2018; Devasia, 1998; Nerkar et al., 2013; Alfredo et al., 2014; Demie et al., 2016). The scarcity of water-related jobs for women was further highlighted by the fact that only one study evaluated employment and economic opportunities for women in relation to drinking water (Adams et al., 2018). When women were able to obtain paid work in drinking water operations, they were subjected to physically demanding conditions with lower wages and lower positions than their male colleagues due to gender roles and expectations (Adams et al., 2018).

Climate Change – Women can face the financial burden of depleted food and water reserves and ill livestock as a result of climate change-induced alterations in water availability (Phiri et al., 2014; United Nations Women, 2022). As household managers, women suffer the consequences of contaminated and diminished water reserves–they may have to purchase additional food and medication for sick livestock and make less profit selling unhealthy livestock (Phiri et al., 2014). However, most disconcerting is the effect climate change has on the labor market and women's domestic duties. In some places, climate change has triggered the migration of the male workforce, thereby increasing the workload for women (Phiri et al., 2014). Additionally, droughts and floods caused by climate change can increase drinking water contamination, and lead to increased incidence of household enteric diseases and reduced availability of water supply (Nounkeu et al., 2019; Khodarahimi and DehghaniNikpourian, 2014; Peragallo Urrutia et al., 2012). Women who are tasked with caring for sick household members can lead to an increase in domestic duties and additional stress (Nounkeu et al., 2019; Khodarahimi and DehghaniNikpourian, 2014; Peragallo Urrutia et al., 2012). Women's roles and responsibilities pertaining to drinking water and management are dependent on the current environment, leaving them vulnerable when environmental change occurs (Devasia 1998; United Nations WomenWatch, 2009).

While the effects of climate change on water and women's responsibilities and economic independence are likely widespread, only one study evaluated the impact of climate change on drinking water and women's livelihoods. Additionally, climate change exacerbates the burden of water fetching, especially in rural areas due to decreased water supply, increasing travel distances triggered by water shortages (Sultana, 2014). We believe that research on the economic impact of climate change on women, as it relates to water supply and quality is both important and time sensitive as climate change continues to become a bigger and more pressing problem. If not addressed, the burden on women will only become more prominent.

Strengths and Limitations – The results of this systematic review give a broad overview of the existing research at the intersection of drinking water and gender equity and empowerment (GEE). We hope that this may provide a framework and guide areas for future investigations to fill in the gaps in knowledge identified in this review. As a large systematic review, there are both strengths and limitations with this study design. During the study identification process, a broad literature review was conducted using 10 different research databases. Teams of reviewers worked in pairs to analyze each study, thereby ensuring a high level of internal validity, and decreasing risk of bias. We also separated WaSH-related drinking water into five different components (quality, quantity, access, continuity, reliability) and GEE outcomes into five different components (health, psychosocial stress, political power and decision-making, social-educational conditions, economic/time use conditions). The large number of studies included in this systematic review allowed us to uncover and credibly support common trends, while using specific water and GEE criteria to identify detailed associations between drinking water and GEE and point out areas where further research is needed to improve the health and wellbeing of women and girls, globally. However, our review did not find articles or assess the relationship between drinking water and non-binary genders. This remains an important area of study for future research. However, studies spanned a large time frame of 40 years and findings from earlier studies may not fully represent the current state of drinking water quantity, accessibility, continuity, and reliability as it affects GEE, as there is limited research related to gender on these topics. Studies were restricted to those published in the English language, which may have limited the sources available for review. Lastly, as studies were not restricted by study location, general findings of this systematic review may not equally apply to every country.

5. Conclusion

Ninety percent of full text studies reviewed studied the association

between drinking water quality and health outcomes. Research on the relationship between drinking water and other equity and empowerment outcomes was sparse. Women had higher incidence of breast cancer due to arsenic, trichloroethylene, and disinfection byproduct exposure and greater incidence of bladder cancer due to arsenic, trihalomethane, and chlorine in drinking water. Men had higher incidence rates of lung cancer due to arsenic, asbestos, and radioactive material exposure, and increased incidence of cardiovascular disease due to magnesium and calcium in drinking water. Importantly, while men had an increased risk of developing arsenicosis and skin lesions compared to women, women were subjected to social ostracization and psychosocial stress, denied healthcare, and pulled out of school and work as a result of arsenicosis. Despite how extensive our findings for drinking water and health outcomes were, there was a notable lack of research regarding drinking water contamination and parasitic, infectious, or diarrheal diseases that disaggregated the data by gender or focused on pregnant women and newborns. Additionally, there were few studies that assessed water carriage injuries, particularly among young girls. As poor water-related health outcomes contracted at young ages can progress and worsen into adulthood, it is important to study how drinking water conditions affect vulnerable populations throughout the life course.

Although we found an association between water fetching and school absenteeism, there is less evidence of the subsequent association with school dropout rates among young girls. Research on the longitudinal impact of water fetching and the impact on girls' economic independence and employment opportunities is sparse. We found that women have poor political power and decision-making ability related to water, few economic opportunities related to water, and limited employment options outside of domestic duties related to water. The lack of economic and decision-making opportunities related to water may also influence women and girls' efforts to obtain independence and self-actualization. Women may face the threat of gender-based violence while collecting drinking water; however, no research in the review studied this relationship. There is underreporting of gender-based violence due to stigma and shame. Further research should be conducted to measure and ensure the safety of girls and women as it relates to water, globally.

This systematic review underscores many ways in which drinking water and gender inequities intersect while identifying critical areas of future research to improve the health and wellbeing of women and girls now and in the future. Findings from this review help us to understand some strategies and solutions to address gender inequities related to drinking water.

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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.2022.114044.

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Effect of Club cell secretory proteins on the association of tobacco smoke and PAH co-exposure with lung function decline: A longitudinal observation of Chinese coke oven workers

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ABSTRACT

Background: Exposure to polycyclic aromatic hydrocarbons (PAH) and tobacco smoke is associated with epithelial damage and reduced lung function. Club cell secretory protein (CC16) is a known biomarker for lung epithelial cells. However, the potential relationships between PAH and tobacco smoke exposure, CC16 levels, and reduced lung function remain unclear.

Objectives: This longitudinal study aimed to explore the potential role of CC16 in the association of tobacco smoke and PAH co-exposure with lung function.

Methods: We enrolled 313 workers from a coking plant in China in 2014 and followed them up in 2019. The concentrations of PAH and nicotine metabolites in urine were determined using high-performance liquid chromatography (HPLC) with a fluorescence detector and HPLC-tandem mass spectrometry, respectively. The plasma CC16 concentration was determined using an enzyme-linked immunosorbent assay.

Results: An analysis of the generalized estimating equation showed that each 1-unit increase in log-transformation of the last tertile of trans-3′-hydroxycotinine (3HC) was associated with a 3.30 ng/ml decrease in CC16. Restricted cubic spline analysis revealed a significant nonlinear dose-effect association between cotinine (COT) and CC16 ($P_{\text{nonlinear}} = 0.018$). In the low- CC16 subgroup, we found a significant association between total nicotine metabolites and forced vital capacity (FVC%) (β: 1.45, 95% CI: 2.87, −0.03), and the associations of nicotine (NIC), COT, and 3HC with FVC% were all of marginal significance. High levels of total hydroxyl polycyclic aromatic hydrocarbons (ΣOH-PAH) and NIC in the urine had an interactive effect on the decline of CC16 ($P < 0.05$). Cross-lagged panel analysis indicated that the decrease in CC16 preceded the decrease in FVC %. CC16 mediated the association between elevated nicotine metabolites and decreased FVC% in the low- CC16 subgroup.

Conclusions: CC16 plays an essential role in the association of PAH and tobacco smoke exposure with reduced lung function. Coke oven workers with low plasma CC16 levels are more likely to experience decreased lung function after tobacco smoke exposure.

Abbreviations: PAH, polycyclic aromatic hydrocarbons; CC16, Club cell secretory protein; HPLC, high-performance liquid chromatography; HPLC-MS, high-performance liquid chromatography-mass spectrometry; NIC, nicotine; COT, cotinine; 3HC, trans-3'-hydroxycotinine; COPD, chronic obstructive pulmonary disease; LOD, limit of detections; 2-OHNAP, 2-hydroxynaphthalene; 9-OHPHE, 9-hydroxyphenanthrene; 2-OHFLU, 2-hydroxyfluorene; 1-OHPYR, 1-hydroxypyrene; Cr, creatinine; FEV_1%, the percentage of predicted forced expiratory volume in the first second; FVC%, the percentage of predicted forced vital capacity; OH-PAHs, Hydroxyl polycyclic aromatic hydrocarbons; ΣOH-PAHs, Total hydroxyl polycyclic aromatic hydrocarbons; ΣLMW OH-PAHs, The low molecular weight hydroxyl polycyclic aromatic hydrocarbons; ΣHMW OH-PAHs, The high molecular weighhydroxyl polycyclic aromatic hydrocarbons; MI, multiple interpolation; RCS, restricted cubic spline; CFI, comparative fit index; RMSEA, root mean square error of approximation.

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1. Introduction

China is the world's largest coke producer, accounting for 60% of the world's total coke production (Liu et al., 2009). Shanxi Province is the largest coke-producing area in the world (Xu et al., 2006). During the coking process, a large amount of coke oven emissions are produced, of which polycyclic aromatic hydrocarbons (PAH) are the main component (Cavallo et al., 2008). Therefore, as an important base of the coal chemical industry, public health problems caused by coal-fired pollution in Shanxi province have attracted great attention from scholars and the government.

Coke oven workers are predominantly male, comprising a high number of smokers; thus, there is considerable tobacco smoke exposure, in addition to long-term exposure to PAH. Numerous studies have shown that long-term PAH exposure is closely related to the occurrence of various respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma (Al-Daghri et al., 2013; Burstyn et al., 2003). Previous studies have found that smoking (including passive smoking) is an important risk factor for respiratory disease, with approximately 30% of smokers developing COPD and 15% developing lung cancer, whereas only 10% of patients with COPD or lung cancer have no clear history of smoking (Govindan et al., 2012). Self-reported smoking levels have been widely used to assess the health effects of smoking; however, they may be inaccurate. Actual smoke inhalation and absorption depend on the mode of smoking and the level of exposure to secondhand smoke, making it particularly important to detect the levels of internal exposure to tobacco (Connor Gorber et al., 2009; Reynolds et al., 2017). A cross-sectional study showed that the interaction between PAH exposure and smoking led to decreased lung function in coke oven workers, suggesting that long-term exposure to PAH and smoking can lead to early lung impairment in workers (Hu et al., 2006). However, the underlying mechanisms linking tobacco smoking and PAH exposure to decreased lung function remain unclear. PAH and tobacco disrupt the structural integrity of lung epithelial cells by oxidative stress and inflammatory responses, which may play an important role in the pathogenesis of reduced lung function (Farzan et al., 2016).

Exposure to several environmental pollutants affects the level of Club cell secretory protein (CC16), a 16 kDa homodimeric protein secreted by cilia-free bronchial Club cells (Wang et al., 2017; Zhou et al., 2018). Some studies have confirmed that CC16 is an important lung epithelial marker because it reflects the process of air-blood barrier disruption and altered lung epithelial permeability (Broeckaert and Bernard, 2000; McAuley and Matthay, 2009). When stimulated by exogenous substances, the airways locally produce large amounts of reactive oxygen species (ROS) and inflammatory factors. Simultaneously, the Club cells secrete CC16 to counteract the oxidative stress and inflammatory responses (Van Vyve et al., 1995). However, the long-term presence of contaminants may lead to a decrease in Club cells as well as mRNA and protein expression of *CC16*, resulting in a decrease in CC16 levels (Lam et al., 2018). Therefore, CC16 levels may have great potential in assessing the health risks of pollutants and early impairment of lung function. Several epidemiological and clinical studies have shown reduced serum CC16 levels in patients with lung function defects, asthma, and COPD (Guerra et al., 2016; Lomas et al., 2008; Rava et al., 2013). However, some epidemiological cohorts have found that low serum concentrations of CC16 predict an accelerated decline in lung function in adulthood (Guerra et al., 2015). Therefore, although CC16 may reflect the degree of lung epithelial damage, a causal relationship between its concentration and altered lung function has been difficult to establish.

Therefore, we hypothesized that exposure to PAH and tobacco smoke in coke oven workers might reduce lung function via decreasing CC16 concentrations. To investigate the associations between PAH and tobacco smoke exposure and the levels of CC16 and lung function, we measured urinary nicotine and PAH metabolite levels, lung function indicators and plasma CC16 levels at baseline among 313 occupational workers in a coke oven plant in Shanxi province, with follow-up after 5 years. We further conducted a cross-lagged panel analysis to demonstrate the causal relationship between CC16 levels and decreased lung function. Finally, a causal mediation model was used to assess the mediating role of plasma CC16 levels in the association of PAH and tobacco smoke exposure with decreased lung function.

2. Materials and methods

2.1. Study design and population

In 2014, a baseline investigation was conducted among 313 coke oven workers aged 18–60 years who had been working for more than one year. The participants completed a standardized questionnaire and a physical examination. Face-to-face interviews were conducted by specially trained interviewers using standardized questionnaires for demographic information, including age, sex, education, alcohol drinking and smoking status, dietary habits, physical activity, and occupational exposure history. After signing the informed consent form, morning urine (20 ml) and blood samples (5 ml) were collected from the participants. A total of 307 workers completed a physical examination and provided blood and urine samples during the follow-up visit in 2019. Five workers had relocated and were lost to follow-up. None of the participants had been exposed to known mutagens, such as radiotherapy or chemotherapy, in the past three months. This study was approved by the Medical Ethics Committee of the Shanxi Medical University.

2.2. Determination of urinary nicotine metabolites

At the end of the workweek, a 20 ml morning urine sample of workers was collected and stored at −80 °C until further processing, as recommended by the American Conference of Governmental Industrial Hygienists (ACGIH). Nicotine (NIC), cotinine (COT) and trans-3′-hydroxycotinine (3HC), three nicotine metabolites in urine at baseline, were detected by high-performance liquid chromatography-mass spectrometry (HPLC-MS) (De Cremer et al., 2013). Detailed test methods can be found in our previously published article (Zhao et al., 2022). Briefly, urine samples were thawed at room temperature before the detection of nicotine metabolites. Then, 0.1% NH_4OH was prepared using double distilled water (solvent A) and acetonitrile (solvent B). The supernatants of urine samples from smokers and nonsmokers were diluted with solvent A in appropriate proportions. After equilibration of the solid phase extraction (SPE) C18 sorbent column (Agela Technology, China) with 3 mL of methanol, 3 mL of deionized water, and 3 mL of solvent A, the samples were dripped through the column. The SPE column was washed with 3 mL of deionized water, drained, and eluted with 5 mL of solvent B. The eluate was concentrated under a weak flow of nitrogen gas. Finally, the concentrated solution was adjusted to 1 mL with a mixture of 100 μL of solvent B and 900 μL of solvent A, filtered through a needle filter (0.22 μm), and loaded into a 1.5 mL injection vial for analysis.

Table S1 details the quality control information for nicotine metabolites. The effective concentration of nicotine metabolites in urine were calibrated with urine specific gravity. Metabolite concentrations below the limit of detections (LOD) were replaced by LOD/2.

2.3. Determination of urinary PAH metabolites

HPLC equipped with a fluorescence detector was used to detect the concentrations of PAH metabolites in urine at baseline, including 2-hydroxynaphthalene (2-OHNAP), 9-hydroxyphenanthrene (9-OHPHE), 2-hydroxyfluorene (2-OHFLU) and 1-hydroxypyrene (1-OHPYR) (Elovaara et al., 2003). Urine concentrations of hydroxyl polycyclic aromatic hydrocarbons (OH-PAH) were calibrated with the levels of urinary creatinine (Cr) and expressed as μg/mmoL Cr. Quality control data and testing methods are available in our previously published article (Fu et al., 2019). Metabolite concentrations below the LOD were replaced

with LOD/2.

2.4. Lung function test

The lung function of the workers at baseline and follow-up was measured by professionally trained nurses using a lung function analyzer (CHESTGRAPH HI-101, Japan). Measurements were based on the spirometry guidelines (Pellegrino, 2005) issued jointly by the American Thoracic Society (ATS) and the European Respiratory Society (ERS). Pulmonary function indicators included forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC). The maximum values after three tests were considered as lung function parameters.

2.5. Plasma CC16 determination

Plasma CC16 concentrations at baseline and follow-up were determined using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minnesota, USA). The assay range was 0.8–50 ng/mL according to the manufacturer's instructions. The minimum detectable dose (MDD) was 0.014–0.217 ng/ml and the mean MDD was 0.070 ng/ml. All the samples were tested three times. Plasma from ten samples was randomly selected and mixed thoroughly as a mixed standard to control for inter-assay variation. The intra- and inter-assay coefficients of variation were 3.1% and 3.7%, respectively.

2.6. Definitions

Participants who drank alcohol at least once a week for more than 6 months were defined as current drinkers. Those who had abstained from drinking for more than one year were defined as former drinkers, while others were defined as nondrinkers. The total nicotine metabolites included NIC, COT and 3HC. Total OH-PAHs (ΣOH-PAHs) included 2-OHNAP, 9-OHPHE, 2-OHFLU and 1-OHPYR. The low molecular weight OH-PAHs (ΣLMW OH-PAHs) included 2-OHNAP, 9-OHPHE and 2-OHFLU. The high molecular weight OH-PAHs (ΣHMW OH-PAHs) included only 1-OHPYR. Pulmonary function indicators were expressed as the percentage of predicted forced expiratory volume in the first second (FEV_1%) and the percentage of predicted forced vital capacity (FVC%) (measured/predicted value) to exclude the effects of sex, age, and height.

2.7. Statistical analysis

The concentrations of nicotine and PAH metabolites were log-transformed because of the severely skewed distribution. The 307 coke oven workers were classified into two subgroups (low- CC16/high-CC16) based on the median CC16 concentrations at baseline, and their basic characteristics were described by frequency (proportion) and median (first quartile, last quartile). The Chi-square test and Kruskal–Wallis H- test were used to test for differences in the categorical and numerical variables, respectively. We used multiple interpolation (MI), based on 5 replications and the 'mice' package in Rstudio, to estimate missing data for all exposures and covariates in the database (Austin et al., 2021). The missing data and the comparison of differences before and after MI are shown in Table S2. Spearman's correlation coefficient was used to assess the correlation between PAH and nicotine metabolites (Table S3).

Generalized linear regression model and generalized estimating equations were used to investigate the associations of urinary PAH and nicotine metabolite concentrations with plasma CC16 concentrations and indicators of lung function after adjusting for sex, age (years), working years, education level (<9 years/9–12 years/> 12 years), drinking status (ever/present/never), physical activity level (never/sometimes/often), night shift status (yes/no), smoked food intake (never/sometimes/often), and COT or/and PAH metabolite levels [additional adjustment for visits (1st, 2nd) in repeated measurement analysis]. Subsequently, we estimated the associations of PAH and tobacco smoke exposure with plasma CC16 concentration at baseline using restricted cubic spline (RCS) analysis. We further investigated the associations of urinary PAH and nicotine metabolite concentrations with indicators of lung function in the two subgroups (low- CC16/high-CC16). Interaction analysis was used to explore the interactive effects of PAH and tobacco co-exposure on plasma CC16 concentrations.

Cross-lagged panel analysis was used to investigate the causal relationship between plasma CC16 levels and lung function indicators (Kivimäki et al., 2000; Sweeney et al., 1982). A specific introduction of this method can be found in our previously published article (Liu et al., 2022). Fig. 1 shows the schematic of this model. The β_1 pathway describes the effect of baseline lung function indicators on follow-up plasma CC16 levels, whereas the β_2 pathway describes the effect of baseline plasma CC16 levels on follow-up lung function indicators. The cross-lagged path coefficients β_1 and β_2 were estimated simultaneously using the R package 'lavaan'. The comparative fit index (CFI) and root mean square error of approximation (RMSEA) were used to determine the validity of the model fit. CFI >0.90 and RMSEA <0.05, were considered to be a very good fit (Leung et al., 2018; Zhang et al., 2016). Fisher's Z-test was used to test the difference between β_1 and β_2 derived from standard variables (Z-scores) (Chen et al., 2008).

Once the temporal sequence between CC16 and lung function was determined, it was possible to explore whether the association between tobacco or PAH exposure and lung function is mediated by CC16 by constructing a causal mediator model. The level of tobacco or PAH metabolites was the predictor (X), CC16 was the mediator (M), and the level of lung function was the outcome (Y). Mediating analysis was performed as described earlier (Li et al., 2018). Mediated analyses were performed using the 'mediation' package of Rstudio, adjusted for sex, age (years), working years, education level (<9 years/9–12 years/> 12 years), drinking status (ever/present/never), physical activity level (never/sometimes/often), night shift status (yes/no), intake of smoked foods (never/sometimes/often), and levels of tobacco or PAH metabolites. All statistical analyses were performed using Rstudio (R Version 4.0.0) and SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

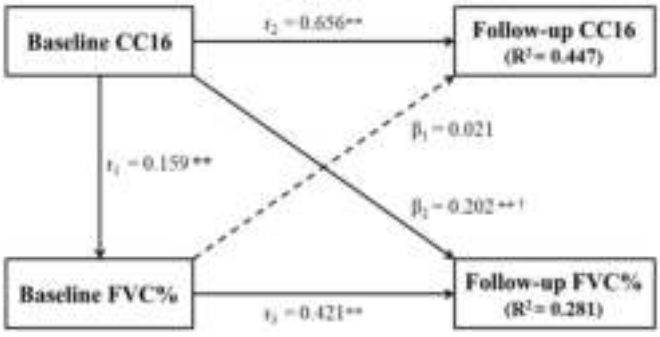

Fig. 1. Cross-lagged panel analysis of CC16 and FVC% (2014–2019)
Adjusted for sex, age (years), working year, education level (<9 years/9–12 years/> 12 years), drinking status (ever/present/never), physical activity level (never/sometimes/often), night shift status (yes/no), intake of smoked foods (never/sometimes/often), as well as levels of cotinine and ΣOH-PAH.
β_1, β_2 = cross-lagged path coefficients; r_1 = synchronous correlations; r_2, r_3 = tracking correlations; R^2 = variance explained.
Goodness-of-fit: root mean square error of approximation (RMSEA) = 0.065 and comparative fit index (CFI) = 0.884.
Coefficients different from 0: *$P < 0.05$, **$P < 0.01$.
†, $P < 0.05$ for difference between β_1 and β_2.

3. Results

3.1. Basic characteristics of the study participants

Table 1 shows the basic characteristics of the 307 participants. In this study, the median age of the participants was 40 years, of which 93.81% were male. The participants were divided into two subgroups (low-CC16/high- CC16) based on the median plasma CC16 concentration of 22.71 ng/mL at baseline, which showed that the participants in the low-CC16 subgroup were older, worked longer, were less educated, had higher cigarette pack-years, and had a higher proportion of current smokers and lower lung function levels. The median plasma CC16 concentration at follow-up was 22.55 ng/mL. Urinary nicotine metabolites levels were higher in the low- CC16 subgroup than in the high-CC16 subgroup. However, there was no significant difference in the concentration of PAH metabolites, between the two subgroups.

3.2. Associations of PAH and nicotine metabolites with CC16 concentration

In the categorical analysis of generalized linear regression model and generalized estimating equation, we found that each 1-unit increase in log-transformation of the last tertile of 2-OHNAP was associated with a 4.20 ng/mL decrease in CC16 of the baseline study, and a 3.36 ng/mL decrease in repeated measurement analysis (Fig. S1, Table S4). In the categorical analysis of generalized estimating equation, we found that each one unit increase in log-transformation of the last tertile of 3HC was associated with a 3.30 ng/mL decrease in CC16 in repeated measurement analysis (Fig. 2, Table S4).

The results of further RCS analysis showed a significant non-linear dose-effect association between urinary COT concentrations and plasma CC16 levels. The increase in COT levels and the degree of decrease in CC16 showed an inverted U-shaped association ($P_{\text{non-linear}} = 0.018$), and the highest point of the curve occurred near the median of COT (Fig. 3). The non-linear association trends of NIC, 3HC, and total nicotine metabolite levels with those of CC16 were consistent with COT (Fig. 3). There was no nonlinear dose-effect association between PAH metabolites and CC16 (Fig. S2).

3.3. Association of PAH and nicotine metabolites with lung function

In the categorical analysis of generalized estimating equation, we found that each 1-unit increase in log-transformation of the second or last tertile of ΣLMW OH-PAHs was associated with a 2.50 or 3.62 decrease in FVC% in repeated measurement analysis (Fig. S1, Table S5). In the categorical analysis of generalized linear regression model and generalized estimating equation, the results showed that each 1-unit increase in log-transformation of the second tertile of total nicotine metabolite was associated with a 3.71 decrease in FVC% in the cross-sectional study and a 2.66 decrease in the repeated measurement analysis, after adjusting for possible covariates (Fig. 2, Table S5).

The associations of PAH and nicotine metabolites with lung function were further quantified after stratification with median CC16. We observed that the associations of ΣLMW OH-PAHs (β: 7.38, 95% CI: 13.77, −0.98) and ΣOH-PAHs (β: 8.09, 95% CI: 14.62, −1.56) with FVC % were significantly negative at baseline only in the low- CC16 subgroup. In repeated measurement analysis, the associations of ΣLMW OH-PAHs and ΣOH-PAHs with FVC% in the low- CC16 subgroup were both of marginal significance (Fig. 4). Repeated measurement analysis showed that the association between total nicotine metabolites and FVC % was significantly negative only in the low- CC16 subgroup (β: 1.45, 95% CI: 2.87, −0.03). In addition, the associations of NIC, COT, and 3HC with FVC% were all marginally significant in the low- CC16 subgroup (Fig. 4). However, no associations were found between PAH and nicotine metabolites and FEV_1% (Table S6).

Table 1
Characteristics of participants by median of baseline plasma CC16 level (N = 307).

Variable[a]	All participants	Median of baseline plasma CC16, ng/ml		P[b]
		<22.71	≥22.71	
No. participants	307	154	153	
Age, years (2014)	40 (32, 45)	41 (37, 47)	38 (26, 45)	<0.001
Working years (2014)	21 (13, 28)	23 (19, 28)	19 (6, 27)	<0.001
Sex				
Male	288 (93.81)	143 (92.86)	145 (94.77)	0.487
Female	19 (6.19)	11 (7.14)	8 (5.23)	
BMI, kg/m^2 (2014)	24.86 (22.32, 27.64)	24.77 (21.95, 27.94)	25.26 (22.65, 27.46)	0.466
Education, years				
<9	82 (26.71)	50 (32.47)	32 (20.92)	<0.001
9–12	108 (35.18)	61 (39.61)	47 (30.72)	
>12 years	117 (38.11)	43 (27.92)	74 (48.37)	
Smoking status				
Never	109 (35.50)	51 (33.12)	58 (37.91)	0.039
Present	174 (56.68)	96 (62.34)	78 (50.98)	
Ever	24 (7.82)	7 (4.55)	17 (11.11)	
Cigarette pack-years				
2014	3.00 (0.00, 15.00)	6.05 (0.00, 18.00)	1.25 (0.00, 10.00)	0.011
2019	6.30 (0.00, 22.50)	11.00 (0.00, 28.00)	3.60 (0.00, 17.00)	0.010
Drinking status				
Never	176 (57.33)	83 (53.90)	93 (60.78)	0.172
Present	115 (37.46)	65 (42.21)	50 (32.68)	
Ever	16 (5.21)	6 (3.90)	10 (6.54)	
Physical activity				
Never	96 (31.48)	48 (31.17)	48 (31.79)	0.991
Sometimes	101 (33.11)	51 (33.12)	50 (33.11)	
Often	108 (35.41)	55 (35.71)	53 (35.10)	
Night shift				
No	95 (30.94)	43 (27.92)	52 (33.99)	0.250
Yes	212 (69.06)	111 (72.08)	101 (66.01)	
Smoked food intake				
Never	176 (57.33)	84 (54.55)	92 (60.13)	0.526
Sometimes	118 (38.44)	64 (41.56)	54 (35.29)	
Often	13 (4.23)	6 (3.90)	7 (4.58)	
CC16 (2019, ng/ml)	22.55 (16.31, 28.79)	17.68 (14.32, 22.48)	27.06 (22.78, 34.67)	<0.001
Pulmonary function				
FVC% (2014)	79.00 (72.00, 86.00)	77.00 (70.00, 84.00)	82.00 (73.00, 89.00)	0.008
FEV_1% (2014)	93.00 (88.00, 100.00)	93.00 (88.00, 100.00)	94.00 (88.00, 99.00)	0.883
FVC% (2019)	84.6 (79.30, 91.60)	84.65 (78.40, 90.00)	84.20 (81.10, 93.10)	0.078
FEV_1% (2019)	84.40 (76.90, 89.80)	83.55 (75.80, 88.30)	85.50 (79.30, 91.00)	0.008
Nicotine metabolites (nmol/ml)[c]				
Nicotine	1.90 (0.05, 14.19)	2.14 (0.06, 16.37)	1.71 (0.04, 10.89)	0.323
Cotinine	2.88 (0.06, 9.57)	3.66 (0.08, 11.55)	2.45 (0.05, 8.41)	0.068
Trans-3′-hydroxycotinine	4.01 (0.08, 16.10)	5.68 (0.10, 21.64)	2.63 (0.06, 13.09)	0.008
Total	13.04 (0.27, 44.57)	17.62 (0.42, 52.29)	10.82 (0.18, 34.52)	0.042
PAH metabolites (μg/mmol Cr)[d]				
2-OHNAP	0.72 (0.43, 1.20)	0.84 (0.43, 1.25)	0.62 (0.44, 0.97)	0.059
2-OHFLU				0.448

(continued on next page)

Table 1 (*continued*)

Variable[a]	All participants	Median of baseline plasma CC16, ng/ml		P^b
		<22.71	≥22.71	
	0.30 (0.22, 0.48)	0.30 (0.23, 0.47)	0.28 (0.20, 0.51)	
9-OHPHE	0.09 (0.06, 0.17)	0.09 (0.06, 0.15)	0.10 (0.07, 0.18)	0.174
1-OHPYR	0.06 (0.04, 0.11)	0.06 (0.04, 0.12)	0.05 (0.03, 0.10)	0.117
ΣLMW OH-PAHs	1.16 (0.84, 1.86)	1.37 (0.88, 1.93)	1.06 (0.83, 1.68)	0.089
ΣOH-PAHs	1.25 (0.88, 1.95)	1.47 (0.93, 2.10)	1.12 (0.85, 1.73)	0.078

Abbreviations: FVC%, the percentage of predicted forced vital capacity; FEV_1%, the percentage of predicted forced expiratory volume in the first second; CC16, Club cell secretory protein; Total, the total nicotine metabolites included nicotine, cotinine and trans-3′-hydroxycotinine; ΣOH-PAHs, the total OH-PAHs included 2-OHNAP, 2-OHFLU, 9-OHPHE and 1-OHPYR. ΣLMW OH-PAHs, the low molecular weight OH-PAHs included 2-OHNAP, 2-OHFLU and 9-OHPHE.

[a] Data were presented as n (%) or Med (25th, 75th).

[b] *P*-values were calculated from Chi-square ($\chi 2$) test for categorical variables and Kruskal-Wallis H-test for numerical variables.

[c] The effective urine concentrations of nicotine metabolites were calibrated by urinary specific gravity.

[d] The effective urine concentrations of PAH metabolites were calibrated by urinary creatinine.

3.4. Effects of tobacco smoke and PAH co-exposure on CC16 and lung function

At baseline, we found that among workers with high exposure to ΣOH-PAHs, plasma CC16 concentrations decreased by 1.70 and 1.78 ng/ml with increasing levels of log-transformed 3HC and total nicotine metabolites ($P < 0.05$), respectively. Such associations were more significant in the repeated measurement analysis. However, among workers with low exposure to ΣOH-PAHs, the concentration of CC16 increased with increasing levels of log-transformed nicotine metabolites ($P < 0.05$) (Table 2). In addition, a slight decrease in the level of FVC% was found with increasing log-transformed 3HC concentrations (β: 1.73, 95% CI: 3.14, −0.32) only in a baseline study among workers with low exposure to ΣOH-PAHs (Table 2). Associations between nicotine metabolites and FEV_1% were not found in workers with low or high exposure to ΣOH-PAHs (Table S7).

Interaction analysis at baseline showed an interaction between high exposure to ΣOH-PAHs and high urinary NIC levels, with a decrease in FVC% (Fig. 5). The interaction effect of high exposure to ΣOH-PAHs and high urinary levels of total nicotine metabolites on the decrease in FVC% was of marginal significant (Fig. 5).

3.5. Cross-lagged panel analysis

Fig. 1 shows the cross-lagged pathway analysis of CC16 and FVC%. After adjusting for sex, age, working years, education level, drinking status, physical activity level, night shift status, intake of smoked foods, and levels of COT and ΣOH-PAH, the absolute value of the path coefficient from baseline FVC% to follow-up CC16 ($\beta_1 = 0.021$, $P = 0.644$) was significantly lower than the absolute value of the path coefficient from baseline CC16 to follow-up FVC% ($\beta_2 = 0.202$, $P = 0.001$). The difference between β_1 and β_2 was statistically significant ($P = 0.012$). Both the synchronous correlation (r_1) and autocorrelation coefficients (r_2, r_3) were significant ($P < 0.01$). The CFI and RMSEA were 0.884 and 0.065, respectively, indicating a relatively good fit of the model based on the criteria of CFI >0.90 and RMSEA <0.05.

3.6. Role of CC16 on the association between nicotine metabolites and FVC%

Table 3 shows the mediating effect of CC16 levels on the association between nicotine metabolites and FVC% at follow-up among the low-CC16 subgroup, adjusted for age, working years, education level, alcohol drinking status, physical activity level, night shift status, intake of smoked foods, and levels of ΣOH-PAH. The mediating effect of CC16 on the effect of NIC, COT, and total nicotine metabolites on FVC% is of marginal significance. The mediating effect of CC16 on the association between NIC and FVC%, between COT and FVC%, and between total nicotine metabolite levels and FVC% was 17.01%, 16.10%, and 23.00%, respectively.

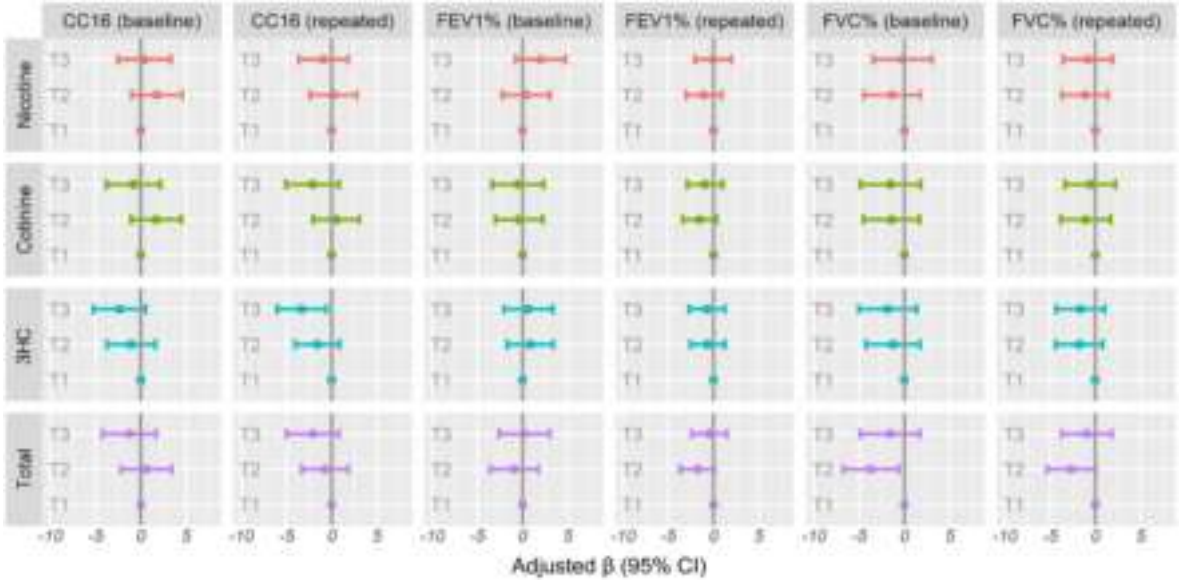

Fig. 2. Associations of urinary nicotine metabolite concentrations with CC16 and lung function indicators (N = 307)
Total, the total nicotine metabolite included nicotine, cotinine and trans-3′-hydroxycotinine; T = tertile.
Adjusted for sex, age (years), working year, education level (<9 years/9–12 years/> 12 years), drinking status (ever/present/never), physical activity level (never/sometimes/often), night shift status (yes/no), intake of smoked foods (never/sometimes/often), and levels of ΣOH-PAH. Repeated measures analysis additionally adjusted for visit events (1st, 2nd).

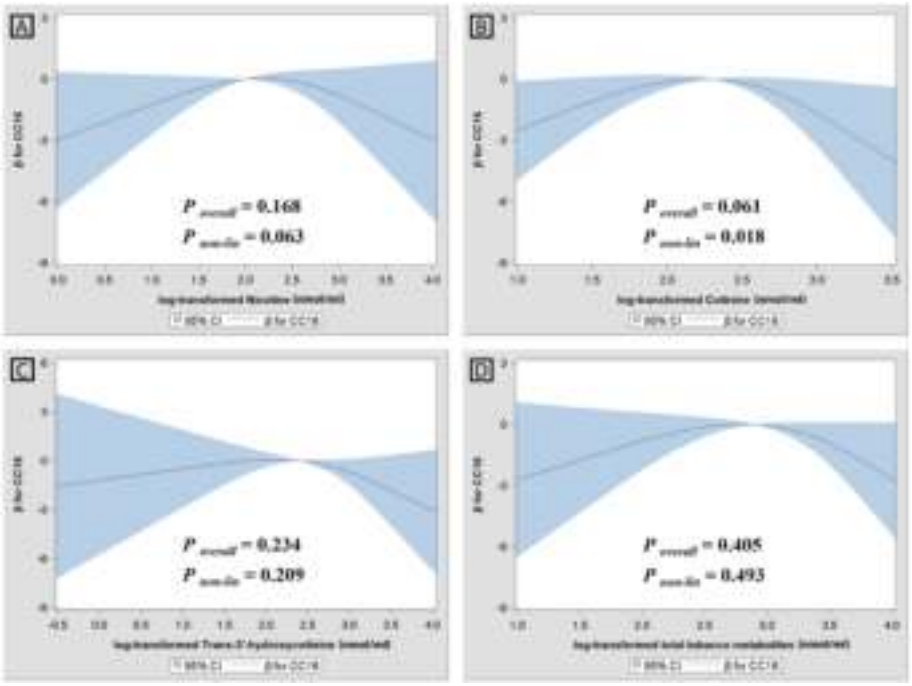

Fig. 3. Restricted cubic spline analysis of the nonlinear dose-effect associations of urinary nicotine metabolites with CC16 at baseline (N = 307)
(A)Between nicotine and CC16; (B) Between cotinine and CC16; (C) Between trans-3′-hydroxycotinine and CC16; (D) Between the total nicotine metabolite and CC16.
The reference point is the median of nicotine metabolite levels in urine; blue shading represents the 95% CI; the 25th, 50th, and 75th percentile of nicotine metabolites in urine were selected as knots, respectively.
Adjusted for sex, age (years), working year, education level (<9 years/9–12 years/> 12 years), drinking status (ever/present/never), physical activity level (never/sometimes/often), night shift status (yes/no), intake of smoked foods (never/sometimes/often), and levels of ΣOH-PAH.

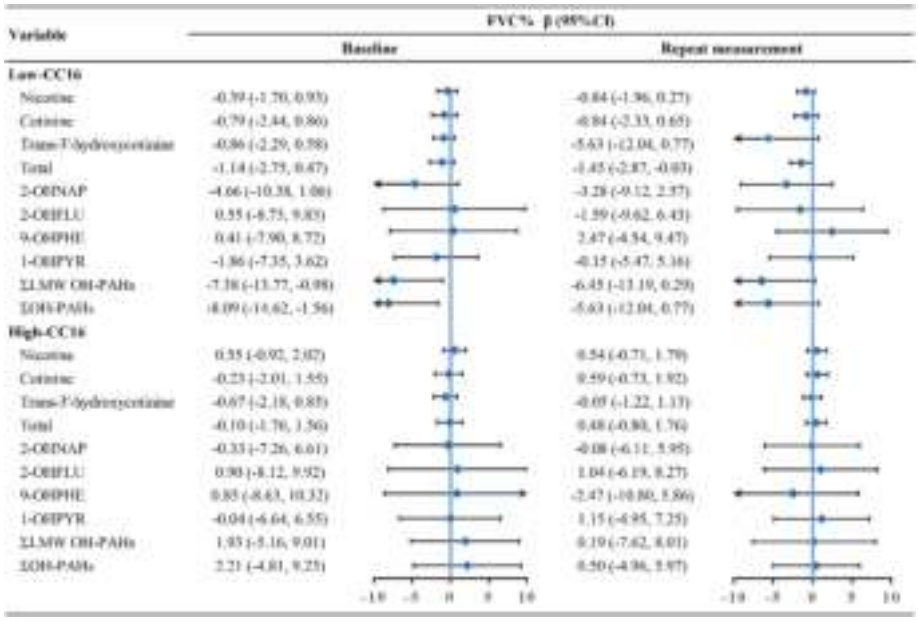

Fig. 4. Associations of urinary nicotine and PAH metabolite concentrations with FVC% stratified by the median of CC16 (N = 307)
Total, the total nicotine metabolite included nicotine, cotinine and trans-3′-hydroxycotinine.
Adjusted for sex, age (years), working year, education level (<9 years/9–12 years/> 12 years), drinking status (ever/present/never), physical activity level (never/sometimes/often), night shift status (yes/no), intake of smoked foods (never/sometimes/often), and levels of cotinine or PAH metabolites. Repeated measurement analysis additionally adjusted for visit events (1st, 2nd).

4. Discussion

The current study found a decrease in FVC% with increasing urinary concentrations of nicotine and PAH metabolites, and these associations were more significant in the low- CC16 subgroup of coke oven workers. We also observed a nonlinear dose-effect association between the concentrations of COT and plasma CC16 levels. Exposure to higher levels of PAH and tobacco had an interactive effect on the decline in plasma CC16 levels. Cross-lagged panel analysis showed that the decrease in CC16 levels preceded the decrease in FVC%. Based on the established CC16-FVC% direction, a causal mediation analysis model was constructed, and it was discovered that in the low- CC16 subgroup, CC16 mediated the association between elevated nicotine metabolites and decreased FVC%.

Previous studies have shown that exposure to PAH and long-term smoking can lead to reduced lung function in the general population. A study in the Wuhan-Zhuhai cohort of 3367 adults found that each one-percentage increase in urinary ΣOH-PAHs, ΣHMW OH-PAHs or ΣLMW OH-PAHs was associated with a 0.22, 0.22, or 0.20 ml decrease in FEV_1, and 0.19, 0.22, or 0.16 ml decrease in FVC, respectively (Cao et al., 2020). A longitudinal study in coke oven workers followed for four years showed that higher baseline PAH exposure levels may lead to a greater decline in lung function (Wang et al., 2016). Mu et al. found similar results of reduced FVC and FEV_1 in participants with high levels of urinary OH-PAHs, and this reduction was more evident in smokers and men (Mu et al., 2019). A prospective birth cohort study from the United States found that parental smoking and active smoking, act synergistically to influence lung function deficits in early adulthood (Guerra et al., 2013). Another cohort study conducted in the United Kingdom found

Table 2
Associations between urinary nicotine metabolite concentrations and CC16 and FVC% stratified by the median of ΣOH-PAHs (N = 307).

Group	CC16 β (95% CI)		FVC% β (95% CI)	
	Baseline[a]	Repeat measurement[b]	Baseline[a]	Repeat measurement[b]
Low-ΣOH-PAHs				
Nicotine	1.79 (0.49, 3.09)	1.17 (0.00, 2.33)	−0.37 (−1.80, 1.07)	0.08 (−1.16, 1.31)
Cotinine	1.51 (−0.04, 3.07)	0.85 (−0.58, 2.28)	−0.86 (−2.55, 0.84)	0.06 (−1.38, 1.50)
Trans-3′-hydroxycotinine	0.54 (−0.79, 1.87)	0.22 (−0.98, 1.43)	−1.73 (−3.14, −0.32)	−0.80 (−1.98, 0.38)
Total	1.47 (0.00, 2.94)	0.78 (−0.55, 2.11)	−1.27 (−2.86, 0.33)	−0.35 (−1.67, 0.98)
High-ΣOH-PAHs				
Nicotine	−0.92 (−2.07, 0.23)	−1.32 (−2.50, −0.13)	0.35 (−1.01, 1.72)	−0.33 (−1.41, 0.75)
Cotinine	−1.38 (−2.85, 0.09)	−1.73 (−3.28, −0.17)	−0.18 (−1.93, 1.57)	−0.21 (−1.70, 1.28)
Trans-3′-hydroxycotinine	−1.70 (−3.01, −0.39)	−2.11 (−3.46, −0.77)	0.13 (−1.44, 1.71)	−0.24 (−1.64, 1.16)
Total	−1.78 (−3.20, −0.36)	−2.21 (−3.69, −0.74)	−0.03 (−1.74, 1.67)	−0.57 (−1.94, 0.80)

Abbreviations: FVC%, the percentage of predicted forced vital capacity; CC16, Club cell secretory protein; Total, the total nicotine metabolites included nicotine, cotinine and trans-3′-hydroxycotinine; ΣOH-PAHs, the total OH-PAHs included 2-OHNAP, 2-OHFLU, 9-OHPHE and 1-OHPYR.
[a] Adjusted for sex, age (years), working year, education level (<9 years/9–12 years/> 12 years), drinking status (ever/present/never), physical activity level (never/sometimes/often), night shift status (yes/no) and intake of smoked foods (never/sometimes/often).
[b] Additionally adjusted for visit events (1st, 2nd).

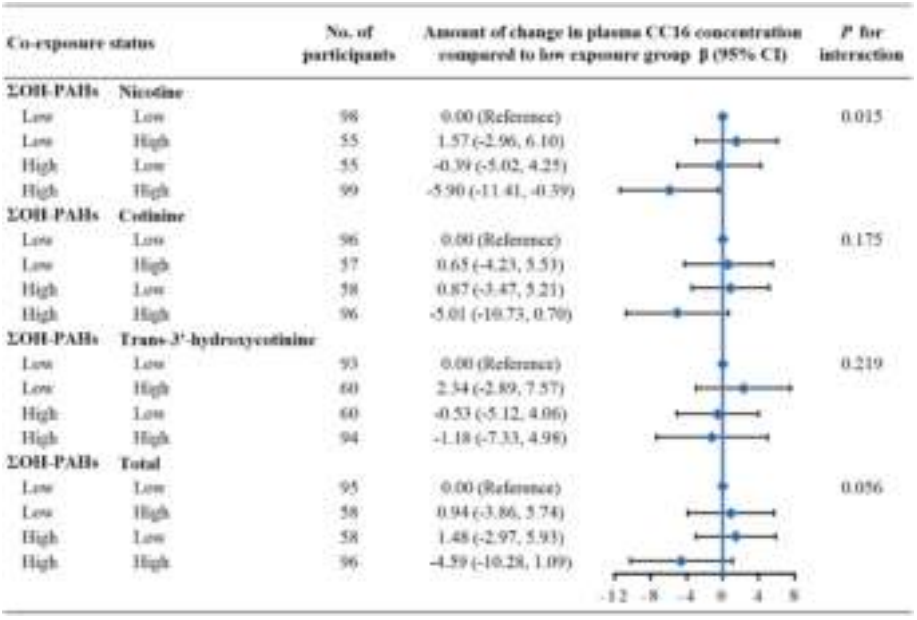

Fig. 5. Co-exposure effects of urine nicotine and PAH metabolites on CC16
Total, the total nicotine metabolite included nicotine, cotinine and trans-3′-hydroxycotinine.
Urinary levels of PAH and nicotine metabolites were stratified by the median into low exposure (<median) and high exposure (≥median), respectively.
Adjusted for sex, age (years), working year, education level (<9 years/9–12 years/> 12 years), drinking status (ever/present/never), physical activity level (never/sometimes/often), night shift status (yes/no) and intake of smoked foods (never/sometimes/often).

Table 3
Mediation effects of CC16 on associations between nicotine metabolites and FVC% among low-CC16 subgroup (N = 154).

Nicotine metabolites[a]	Total effect β (95% CI)	Direct effect β (95% CI)	Mediating effect β (95% CI)	Proportion mediated (%)
Nicotine	−1.37 (−2.60, −0.11)	−1.11 (−2.32, 0.21)	−0.26 (−0.66, 0.01)	17.01
Cotinine	−1.53 (−2.97, 0.02)	−1.26 (−2.80, 0.22)	−0.27 (−0.70, 0.00)	16.10
Trans-3′-hydroxycotinine	−0.73 (−2.09, 0.54)	-[b]	-[b]	-[b]
Total	−1.43 (−2.94, 0.09)	−1.07 (−2.56, 0.47)	−0.37 (−0.91, 0.00)	23.00

Abbreviations: FVC%, the percentage of predicted forced vital capacity; CC16, Club cell secretory protein; Total, the total nicotine metabolites included nicotine, cotinine and trans-3′-hydroxycotinine; CI, confidence interval.
[a] Adjusted for age (years), working year, education level (< 9 years/9 - 12 years/> 12 years), drinking status (ever/present/never), physical activity level (never/sometimes/often), night shift status (yes/no), intake of smoked foods (never/sometimes/often), and levels of ΣOH-PAHs.
[b] Direct and mediating effects could not be tested because the total effect was not significant.

that smoking not only accelerated the decline in adult FEV_1 but also modified the effects of early life exposure on FEV_1 and FVC at midlife (Allinson et al., 2017). Our study found that the total metabolite levels of both PAH and nicotine metabolites had a greater impact on the decline in FVC%. This may be due to the fact that PAHs are a large class of environmental pollutants that can be metabolized to a variety of different hydroxyl compounds in vivo, and the ΣOH-PAH concentration is more representative of the true exposure level (Kim et al., 2013). In addition, it has been proposed that the sum of urinary NIC, COT, and 3HC is a strong biomarker of daily nicotine intake, independent of individual metabolic differences (Benowitz et al., 2020). In contrast to existing studies, no association of PAH and nicotine metabolites with FEV_1% was found in this study. However, the results in Table 1 show a nearly 10% decrease in FEV_1% at the 5-year follow-up (93.00% in 2014 vs. 84.40% in 2019). This may indicate that factors other than PAH and tobacco smoking may contribute to the decrease in FEV_1%.

Interestingly, we found that the association of PAH and nicotine metabolites with FVC% were more significant in participants with low plasma CC16 levels. These results are consistent with the findings of Zhou et al. (2018). Therefore, it is essential to investigate the changes in CC16 concentrations following tobacco smoke and PAH exposure, and the causal relationship between CC16 levels and decreased lung function in a longitudinal study.

The current study provides evidence that PAH and tobacco smoke exposure may have adverse effects on Club cells, which are the primary site of exogenous detoxification in the lung and have the highest cytochrome P450 levels in lung cells (Plopper et al., 1987). CC16 is an abundant protein secreted by Club cells in the fine respiratory bronchi and is a specific marker of acute or chronic lung injury (Lakind et al., 2007). In a cross-sectional study of chromium-exposed occupational workers in Henan Province, the levels of CC16 in the peripheral blood of the exposed group of workers were similar to the present study (Li et al., 2015). However, in a study of the Wuhan-Zhuhai cohort, peripheral blood CC16 levels were slightly lower in the general population than that in our study, which may be due to differences between different geographical populations, or possibly because the age range of the study population was 18–80 years, which included participants who were older than 60 years (Zhou et al., 2018). Regarding the different types of PAH metabolites, the present study only found a significant decrease in CC16 with increasing concentrations of 2-OHNAP, which verifies the findings of Van Winkle et al. The study published by Van Winkle et al. showed that murine Club cells were sensitive to damage following naphthalene inhalation (Van Winkle et al., 1999). These results suggest that, among the four PAH metabolites examined in this study, 2-OHNAP is the main metabolite affecting plasma CC16 concentrations. However, the specific mechanisms involved require further exploration. Zhu et al. found that airway CC16 expression was significantly suppressed in a monkey cigarette smoke exposure model as well as in a cigarette smoke-induced COPD mouse model (Zhu et al., 2015). Our results showed that total nicotine metabolite concentrations were significantly higher in the low- CC16 subgroup than in the high- CC16 subgroup. We also found significant associations between increased concentrations of nicotine metabolites and decreased plasma CC16 concentrations and an interactive effect of high co-exposure to PAH and tobacco on the decrease of CC16. The chronic decline in CC16 may be due to the presence of prolonged or high levels of stimulation/cytotoxicity, resulting in a decrease in Club cells as well as mRNA and protein expression of *CC16* (Lam et al., 2018; Ma et al., 2015; Yildirim et al., 2008). However, subgroup analysis showed that in the high PAH exposure subgroup, plasma CC16 concentrations increased slightly with increasing levels of nicotine metabolites but decreased significantly in the low PAH exposure subgroup. This suggests that when exposed to low concentrations of environmental pollutants, Club cells may produce sufficient CC16 to counteract the pollutant-induced inflammation. The most likely driving mechanism for elevated CC16 levels in the context of short-term and low-concentration exposure is pulmonary hyperpermeability (Van Miert et al., 2012). Furthermore, in the subgroup analysis, we found that the effect of nicotine metabolites on lung function was not significant. This may be due to the presence of a healthy worker effect, while the alteration in plasma CC16 concentrations may have preceded the decline in lung function. Thus, the current study strengthens the view that CC16 is a useful biomarker with many biological functions that may contribute to the underlying mechanisms of reduced lung function (Lakind et al., 2007; Ma et al., 2015).

Several epidemiological and clinical studies have demonstrated reduced serum CC16 levels in patients with lung function deficits (Rava et al., 2013), asthma (Guerra et al., 2016), and COPD (Lomas et al., 2008). However, a few independent epidemiological cohorts have found that low serum concentrations of CC16 predict an accelerated decline in lung function in adulthood (Guerra et al., 2015). Despite the previously mentioned epidemiological evidence, the causal relationship between CC16 concentrations and altered lung function remains elusive. Previous studies have used traditional longitudinal analysis models (Guerra et al., 2015; Wang et al., 2017; Zhou et al., 2018), which have limited ability to infer the temporal sequence of altered CC16 concentrations and decline in lung function. This study investigated the temporal relationship between plasma CC16 levels and lung function in coke oven workers by using a cross-lagged panel analysis model in a longitudinal cohort. The model is a powerful statistical method for analyzing causal relationships between correlated variables (Kivimäki et al., 2000; Li et al., 2018). The current study found that a decrease in plasma CC16 concentration preceded a decrease in FVC%. Our results are similar to those of many previous studies, which showed that plasma CC16 concentrations are positively associated with lung function in a unidirectional CC16-FVC% relationship (Guerra et al., 2015; Zhou et al., 2018). Although the mechanisms linking CC16 to lung function remain unclear, some major pathways have been identified based on epidemiological and experimental evidence. Experimental evidence has suggested that CC16 is associated with immunosuppressive and immunomodulatory mechanisms in epithelial cells exposed to toxicants (Bernard and Lauwerys, 1995). In addition, CC16 protects the respiratory tract from inflammatory responses and has inhibitory effects on several cytokines, including interleukin (IL)-4, IL-5, and IL-13 (Wang et al., 2001). CC16 also exerts direct anti-inflammatory effects by inhibiting phospholipase A2 (PLA2) and prostaglandin D2 proteins (Mandal et al., 2004; Sohn et al., 2003).

Notably, the current study only found an association between CC16 and FVC%, but not between CC16 and FEV_1%, which is consistent with many previous studies (Ma et al., 2015; Zhou et al., 2018). FVC can help identify pulmonary fibrosis in restrictive airway disease and is considered a key lung function parameter (Zhou et al., 2018). In vitro studies have shown that CC16 can block PLA2 activity in the cytoplasm after fine bronchial injury, thereby inhibiting fibroblast chemotaxis, which may be an important mechanism in the development of pulmonary fibrosis (Lesur et al., 1995). Although smoking is a major risk factor for obstructive lung disease, the current results suggest that smoking may also be an important factor in restrictive lung disease. Interestingly, our findings showed that CC16 has a role in the association between nicotine metabolites and FVC% rather than FEV_1%. This suggests that the PLA2 pathway may be involved in nicotine metabolite-induced lung injury and can play a mitigating role. However, the specific mechanisms involved require further exploration.

There was no significant difference in the baseline and follow-up CC16 levels, probably because of the following reasons. Although this study found that a decrease in plasma CC16 levels with increasing PAH levels and tobacco exposure, the majority of workers consistently had lung function levels within the normal range. The median age of the workers in this study was 40 years at baseline and 45 years at follow-up, with a younger age range overall. In addition, the presence of the healthy worker effect could explain why CC16 concentrations did not change significantly over the 5-year study period.

Based on the above mechanism, CC16 concentration may be a critical link between tobacco smoke exposure and a decrease in FVC%. The results of causal mediation analysis showed that the associations of NIC, COT, and total nicotine metabolite levels at baseline with decreased FVC % at follow-up were mediated by decreased plasma CC16 levels in the low- CC16 subgroup. Laucho-Contreras et al. reported that mice exposed for 6 months to cigarette smoke and with *CC16* gene knockout not only had an increased susceptibility to emphysema and peribronchial fibrosis, but also exhibited excessive airway inflammation, increased alveolar septa, apoptosis of bronchial cells, and chemotaxis of cupped cells (Laucho-Contreras et al., 2015). However, overexpression of CC16 by inhalation transfected adenovirus could rescue these animals from the hazards of cigarette exposure (Laucho-Contreras et al., 2015). Interestingly, our study also found that CC16 concentrations mediated a higher proportion of the association between total nicotine metabolites and FVC% than the association of NIC and COT with FVC%. This suggests that the total urinary nicotine metabolite concentrations are more applicable as validated biomarkers of tobacco smoke exposure

(Benowitz et al., 2020). However, only 23.00% of the total nicotine metabolite association with FVC% was mediated by CC16, suggesting that other possible mechanisms are involved in the changes in lung function after tobacco smoke exposure. The current study found no female workers in the low- CC16 subgroup. The reason for this phenomenon may be the low number of females in this population. Another possible reason is that all female workers in this population were non-smokers and had low levels of tobacco smoke exposure; therefore, the CC16 levels were above the median. The specific reasons for this should be further explored using larger sample sizes or other populations.

Our study has several advantages. First, we measured the urinary levels of nicotine metabolites, allowing for a more accurate estimation of the true level of tobacco smoke exposure in individuals. Second, we explored the causal relationship between CC16 and reduced lung function in a longitudinal cohort using cross-lagged panel analysis and the role played by plasma CC16 levels in the association between tobacco smoke exposure and reduced lung function, which provides an epidemiological basis for studies on the mechanisms of tobacco smoke exposure-induced lung function decline. Our findings have potential implications for occupational health. PAH exposure is an important occupational health concern for coke oven workers. These findings suggest that co-exposure to PAH and tobacco can have severe adverse effects on the respiratory system. In addition, tobacco exposure may have additional adverse effects on the respiratory system of individuals with low plasma CC16 concentrations. To protect the lung function of coke oven workers, especially individuals with low plasma CC16 levels, they should be encouraged to quit smoking and reduce their exposure to secondhand smoke and occupational exposure to coke oven emissions.

Our study has some limitations. First, the sample size of the present study was small; therefore, only marginal significance was observed in the causal mediation analysis. Future studies should be performed with an increased sample size. Second, the participants were coke oven workers, and the majority of them were males (288/307); thus, the results would be inappropriate for direct extrapolation to the general population and females. Third, this study only provides an epidemiological basis, and further vitro and in vivo studies are required to confirm this finding. Fourth, we only used single-spot urine samples at baseline to determine the concentrations of metabolites. However, the exposure levels in the population we studied were relatively stable, because the workers in our study had prolonged exposure, did not change their operating post over five years, and did not change their smoking habits. Fifth, other pollutants from coke oven emissions, such as metals, nitrogen dioxide, and ozone, as well as other air pollutants that the workers were exposed to in their daily lives, are possible confounding factors. Finally, different normalization methods were used for PAH and nicotine metabolites in this study. However, a number of studies have shown that there is a strong correlation between urine specific gravity and creatinine (Anestis et al., 2009; Miller et al., 2004) that does not affect the association between exposure and outcome. The normalization methods used in this study have also been used in the published articles (Zhong et al., 2022; Zhou et al., 2018).

5. Conclusions

Our findings provide relevant evidence for an association between tobacco smoke exposure, reduced plasma CC16 levels, and reduced lung function in coke oven workers. A decrease in plasma CC16 levels precedes the decline in lung function and can be used as an early biomarker of lung function. Reduced CC16 levels play an essential role in the association between tobacco smoke exposure and reduced lung function. Coke oven workers with low plasma CC16 levels are more likely to experience decreased lung function after tobacco smoke exposure. Our study provides an epidemiological basis for mechanistic studies and the clinical treatment of diseases related to lung function impairment caused by environmental pollutants.

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.114058.

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Exposure to flame retardants in European children — Results from the HBM4EU aligned studies

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ABSTRACT

Many legacy and emerging flame retardants (FRs) have adverse human and environmental health effects. This study reports legacy and emerging FRs in children from nine European countries from the HBM4EU aligned studies. Studies from Belgium, Czech Republic, Germany, Denmark, France, Greece, Slovenia, Slovakia, and Norway conducted between 2014 and 2021 provided data on FRs in blood and urine from 2136 children. All samples were collected and analyzed in alignment with the HBM4EU protocols. Ten halogenated FRs were quantified in blood, and four organophosphate flame retardants (OPFR) metabolites quantified in urine. Hexabromocyclododecane (HBCDD) and decabromodiphenyl ethane (DBDPE) were infrequently detected (<16% of samples). BDE-47 was quantified in blood from Greece, France, and Norway, with France (0.36 ng/g lipid) having the highest concentrations. BDE-153 and -209 were detected in <40% of samples. Dechlorane Plus (DP) was quantified in blood from four countries, with notably high median concentrations of 16 ng/g lipid in Slovenian children. OPFR metabolites had a higher detection frequency than other halogenated FRs. Diphenyl phosphate (DPHP) was quantified in 99% of samples across 8 countries at levels ~5 times higher than other OPFR metabolites (highest median in Slovenia of 2.43 ng/g lipid). FR concentrations were associated with lifestyle factors such as cleaning frequency, employment status of the father of the household, and renovation status of the house, among others. The concentrations of BDE-47 in children from this study were similar to or

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lower than FRs found in adult matrices in previous studies, suggesting lower recent exposure and effectiveness of PBDE restrictions.

1. Introduction

Human biomonitoring (HBM) is the analysis of substances and/or their respective metabolites in human matrices. HBM is a crucial tool to evaluate and monitor internal chemical exposure in specific population samples, identify chemicals of concern, study determinants of exposure, or evaluate the efficacy of regulations in place to mitigate chemical exposure (Angerer et al., 2007, 2011).

Children often have elevated exposure to many chemicals (Gibson et al., 2019; Koppen et al., 2019), including industrial compounds such as flame retardants (FRs), due to an increased inhalation rate, different breathing zone, increased hand-to-mouth activity, and faster metabolism (van den Eede et al., 2015). Several FRs are known endocrine disruptors that disrupt thyroid function in children, resulting in adverse effects on the individual's long-term mental health, cognitive ability, metabolism, and reproduction (Preston et al., 2017). Since children are the most vulnerable age group to chemical exposure, they should be a priority for human and environmental health policy (Au, 2002).

The European Human Biomonitoring Initiative (HBM4EU) is a large-scale HBM project co-funded under the European Commission's Research and Innovation Program Horizon 2020 that includes 30 European countries and the European Environment Agency. One of its major aims is to enhance the body of evidence of European citizens' internal exposure to chemicals (David et al., 2020; Lange et al., 2021). Flame retardants were identified by European Union (EU) institutions and HBM4EU partner countries as priority substances to be studied as knowledge gaps with an impact on regulation still exist (Louro et al., 2019). Several detrimental ecological and human health issues have been associated with elevated FR concentrations, including environmental persistence, long-range transport, bioaccumulation, and endocrine and neurologically disruptive effects on organisms, including humans (Aznar-Alemany et al., 2019; Bajard et al., 2019, 2021; Stieger et al., 2014; Sverko et al., 2011)

Flame retardants have been widely used since the 1970s in textiles, furnishing, plastic, and electronic equipment (Horrocks, 2011; McGrath et al., 2018; Morel et al., 2022). Brominated flame retardants (BFRs), including polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDDs), were the primary FRs for more than thirty years (Pantelaki and Voutsa, 2019). PBDEs and HBCDDs have been strictly regulated by several international bodies, including the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH), the United States Environmental Protection Agency (USEPA), and the Stockholm Convention (Guo et al., 2016; Kemmlein et al., 2009; Sharkey et al., 2020) since 2004. These compounds are often referred to as legacy FRs given their limited new use. Since the restrictions on the use and production of legacy FRs came into force, the production of alternative FRs, such as organophosphate flame retardants (OPFRs) and novel halogenated flame retardants (NFRs), has increased (Covaci et al., 2011; He et al., 2021). Some commonly used halogenated NFRs are decabromodiphenyl ethane (DBDPE), bis(2-ethylhexyl) tetrabromophthalate (BEH-TEBP), tetrabromobisphenol A (TBBPA) and Dechlorane Plus (DP). Collectively, NFRs and OPFRs are referred to as emerging FRs.

Because OPFRs are less persistent than legacy FRs and have shorter elimination half-lives, it was assumed that these compounds are less harmful to human and environmental health (Blum et al., 2019). However, this assumption has recently been questioned, as several OPFRs are associated with endocrine disruptive effects at the same levels as PBDEs (Behl et al., 2016).

Since the twin projects COPHES (Consortium to Perform Human Biomonitoring on a European Scale) (Joas et al., 2012) and the feasibility study DEMOCOPHES (DEMOnstration of a study to COordinate and Perform Human Biomonitoring on a European Scale) from 2009 to 2012 (Den Hond et al., 2015), no large-scale multi-country HBM project has been conducted to determine chemical compound concentrations in the biological matrices of children or quantified emerging FRs in children. Our study aims to quantify legacy and emerging FRs in children from nine European countries through information obtained by the HBM4EU aligned studies. This activity will enable concentrations of different countries to be comparable to each other. To understand the potential sources of flame retardant exposure, several lifestyle factors were investigated based on ancillary data gathered by questionnaires.

2. Materials and methods

2.1. Study alignment and participation

One of the aims of the HBM4EU aligned studies is to harmonize chemical and data analyses of human biological samples throughout Europe (Ganzleben et al., 2017; Gilles et al., 2021). The project builds on existing capacities within the EU by aligning already existing studies targeting different populations or regions (Gilles et al., 2021). Eight countries and six HBM4EU qualified laboratories were involved in the study of FRs in children (Table 1). The CELSPAC cohort from the Czech Republic was not part of the HBM4EU study. However, the collection, analyses, and QA/QC protocols were aligned with the HBM4EU study as other CELSPAC age groups were used for HBM4EU aligned studies, thus the data collected from the CELSPAC study can be compared with the cohorts participating in the HBM4EU study.

The HBM4EU defined "current exposure" as samples collected between 2014 and 2020 (Gilles et al., 2021), and as such, urine and blood samples from 2136 children aged 6–13 between 2014 and 2021 were used to evaluate current FR exposure in European children (Gilles et al., 2021). Blood and urine (both spot and morning void) samples collected before 2017 were acquired from the biobank of the specific study, while samples from 2017 to 2020 were new collection. Table S1 presents a complete description of the studies, matrices, and institutes involved.

2.2. Sample analysis

OPFRs have half-lives of hours to days, as opposed to most halogenated FRs with half-lives of weeks to years (Dvorakova et al., 2021). Due to the rapid rate of metabolization and elimination of OPFRs, it is most appropriate to quantify metabolites in urine instead of the parent OPFR compounds. OPFR metabolites were quantified in children's urine, while the persistent brominated and chlorinated flame retardants were quantified in children's blood samples (Table 2).

Eighty-six laboratories from 26 countries were invited to participate in HBM4EU proficiency tests. Seventy-four of these laboratories successfully quantified at least one biomarker and were selected to participate in three rounds of interlaboratory comparison investigations (Esteban López et al., 2021). In this study, all laboratories responsible for the analysis of FR biomarkers in urine and/or blood successfully completed the interlaboratory comparison investigations and external quality assurance schemes, described in detail by Dvorakova et al. (2021).

Six HBM4EU qualified laboratories were involved in the FR analyses. The institutes that participated in the analyses are: the University of Chemistry and Technology, Prague (Czech Republic), Department of Environment and Health, Vrije Universiteit Amsterdam (Netherlands), RECETOX, Masaryk University (Czech Republic), Norwegian Institute of Public Health (Norway), Toxicological Center, University of Antwerp (Belgium), and Friedrich-Alexander-Universität Erlangen-Nürnberg

(Germany). The analytical procedures used by the laboratories are presented in the Supplementary Information (Supplementary Table S1 and Text S1). All participating studies in the HBM4EU survey adhered to national and European ethics regulations (Gilles et al., 2021). The ethical information of all participating studies is provided in Table S2 (Gilles et al., 2022). Three laboratories analyzed brominated FRs and DPs using gas chromatography-tandem mass spectrometry (GC-MS/MS). Four laboratories analyzed OPFR metabolites using liquid chromatography-tandem mass spectrometry (LC-MS/MS), and one laboratory used GC-MS. Limits of quantification (LOQs) are given in Tables S3 and S4. With the stringent interlaboratory comparison described above, the concentrations of FRs were deemed comparable, despite differences in analytical methods across laboratories.

2.3. Data analyses

In cases where less than 20% of individuals were represented by left-censored data (below limits of detection/quantification), imputation was performed to replace censored data. Imputation can provide more realistic distributions of data than those relying on simple substitution of a fixed value, e.g., 0.5*LOQ (Bernhardt et al., 2015), and was selected for use in the aligned studies of HBM4EU (Govarts et al., 2021). The maximum-likelihood estimation (MLE) method was used to find a distribution best fitting the non-censored data (R Core Team, 2019). From an infinite set of all theoretical log-normal distributions, the MLE method identifies the one for which the probability that given set of values comes from that distribution is maximal. This is done by maximizing a likelihood function of that distribution by computing first derivative of that function. Based on that distribution, random values were generated for the intervals from 0 to LOD (limit of detection) and from LOD to LOQ (limit of quantification).

After imputation, the urinary data were standardized for creatinine, by dividing metabolite concentrations by the known creatinine concentration, while blood serum/plasma data were standardized for lipid content according to the harmonized level of lipid enzymes calculated as Total lipids (mg/dL) = 2.27* (Total cholesterol) + triglycerides + 62.3 mg/dL (Bernert et al., 2007)

A set of univariate analyses were conducted to check the distribution of both the continuous and discrete (categorical) exposure determinants, typically lifestyle factors. If an exposure determinant was not available for more than 40% of the individuals with a known concentration of a compound, the determinant was excluded from further analyses. Discrete exposure determinants with only one factor level, e.g. when all respondents answered “no” to a particular question, or exposure determinants linearly dependent on other determinants were also excluded from the subsequent steps.

Differences between countries were tested using Kruskal-Wallis one-way analysis of variance on the log-transformed concentration data. Due to the sparse exposure determinants matrix, data were only adjusted for sex and age of the participants using the mean value as the reference, and the investigation of exposure determinants was reserved for within-country analyses only.

Finally, a single effect linear regression was conducted on log-transformed data taking all non-excluded exposure determinants using least squares linear regression (Table S7) based on both the variance inflation factor (VIF) and the determinant p-value (Govarts et al., 2021). Exposure determinants with VIF $\leq$10 or p-value $\leq$ 0.05 were considered as significantly associated with the flame retardant concentrations. Continuous exposure determinants were investigated using Spearman correlation tests and visualized using scatterplots with 95% confidence level.

Table 2
Flame retardants and metabolites analyzed.

Persistent FRs (in blood)	Metabolized FRs (in urine) (Parent compound/ metabolite)
PBDE-47, 153, −209 syn-DP, anti-DP α-HBCDD, γ-HBCDD DBDPE BEH-TEBP TBBPA	tris(2-chloroethyl) phosphate (TCEP)/Bis(2-chloroethyl) phosphate (BCEP) tris(1,3-dichloroisopropyl) phosphate (TDCIPP)/Bis (1,3-dicholoro-2-propyl) phosphate (BDCIPP) tris(1-chloro-2-propyl) phosphate (TCIPP)/Bis(1-chloro-2-propyl) phosphate (BCIPP) triphenyl phosphate (TPHP)/Diphenyl phosphate (DPHP)[a]

[a] DPHP can be a product by itself, a primary metabolite of TPHP, or a secondary metabolite of several OPFRs (Liu et al., 2021).

2.4. Study limitations

The sampling and analytical procedures were aligned according to the HBM4EU standards. However, the aligned study designs were not identical. Each country had different sample sizes (studies ranged from 55 to 413 participants) and prioritized different compounds, as well as different timing of sample collection. Although the sampling years slightly differed, most overlapped and all were within a five-year range (Table 1). Six of the studies provided newly collected samples, while the remainded relied on biobanked samples that had been stored at stable conditions (−80 °C). The difference in storage time is not expected to impact comparability, as storage time has not been found to impact levels of the reported OPFRs in urine (Carignan et al., 2017).

Table 1
Descriptive statistics of the participants of the study and the compounds analyzed in each country.

COUNTRY (COUNTRY CODE)[a]	STUDY	TOTAL PARTICIPANTS	FEMALE	MALE	AGE RANGE (YEARS)	SAMPLING YEARS	COMPOUNDS ANALYZED	STUDY REFERENCE, WHERE AVAILABLE
BELGIUM (BE)	3xG	133	67	66	7–8	2019–2020	BDCIPP, DPHP, BCIPP	Govarts et al. (2020)
CZECH REPUBLIC (CZ)	CELSPAC	195	106	89	11–12	2019	BCEP, BCIPP. BDCIPP, DPHP	Not available
GERMANY (DE)	GerES V	300	150	150	6–12	2015–2016	BCEP, BCIPP, BDCIPP, DPHP	European Commission (2021a)
DENMARK (DK)	OCC	291	130	161	7	2018–2019	BDCIPP, DPHP, BCIPP	Kyhl et al. (2015)
GREECE (EL)	CROME	55	31	24	6–11	2020–2021	PBDE-47, -153, −209, DP, HBCDD, DBDPE	(EnvE Lab, 2022; Gilles et al., 2022)
FRANCE (FR)	ESTEBAN	413	191	222	7–13	2014–2016	PBDE-47, -153, −209, DP, HBCDD, DBDPE, BEH-TEBP, TBBPA, BCIPP, BDCIPP, DPHP	European Commission (2021b)
NORWAY (NO)	NEB II	300	140	160	8–12	2016–2017	PBDE-47, -153, DP, BDCIPP, DPHP	Caspersen et al. (2019)
SLOVENIA (SI)	SLO CRP	149	82	67	7–10	2018	PBDE-47, -153, −209, DP, HBCDD, DBDPE, BDCIPP, DPHP, BCIPP, BCEP	Stajnko et al. (2020)
SLOVAKIA (SK)	PCB cohort	300	167	133	10–13	2014–2017	BDCIPP, DPHP, BCEP, BCIPP	Hertz-Picciotto et al. (2003)

[a] Not all studies are nationally representative.

All countries except FR and DE had 100% participants with a European country of birth; DE had 99.3% and FR 95% European birth country representation. In FR, 18 participants were not born in Europe. However, the results of the study are still deemed to be indicative of the European population. The age range of children participating in the study differed (Table 1), and some studies (e.g., CELSPAC (CZ), PCB cohort (SK)) had children on the upper end of the age bracket (10–13 years), while others (e.g., OCC (DK), 3xG (BE)) covered only the lower end of the age bracket (7–8 years). Urine was collected as both morning void and spot samples. In the NEB II study (NO) analyzed BFRs and DP were analyzed in blood plasma, while in the other cohorts they were analyzed blood serum (Table S1). The questionnaire regarding lifestyle factors also differed between countries. Because most lifestyle factors were reported by all countries, or were incompletely reported, multivariate analyses could not be conducted across the full dataset. Although the laboratories participated in a complete QA/QC programme to ensure the quality and comparability of the analytical results (Dvorakova et al., 2021), they had different LODs and LOQs (Tables S3 and S4). However, all laboratories passed the two-stage evaluation process (Dvorakova et al., 2021; Esteban López et al., 2021) and were declared proficient by the HBM4EU project.

3. Results and discussions

3.1. FR concentrations in children's blood from different European countries

The concentrations of halogenated FRs in children's blood are presented in Table 3. The detection frequencies of all halogenated FR quantified in children's blood are presented in Table S5. Only compounds with detection frequency >40% were considered for statistical analyses. α-HBCDD, γ-HBCDD, and DBDPE were analyzed in blood samples from France, Greece, and Slovenia, but were not present at more than 40% detection frequency (Table S5). BEH-TEBP and TBBPA were only investigated in blood from France but were not present above quantifiable concentrations in any sample (Table S5).

PBDE-47 was present at quantifiable concentrations in France, Greece, Norway, and Slovenia. Slovenia had a 15% detection frequency of BDE-47 and is not included in the inter-country comparison, although we note that the 95th percentile is similar to that of Greece, the other country from the Southern European region (Table 3). When comparing levels across the three countries, Greece had significantly lower concentrations of BDE-47 than either France or Norway (Kruskal-Wallis test, $p < 0.001$, Fig. 1), in both the unadjusted data as well as in data adjusted for age and sex. BDE-153 was detected in more than 40% of the blood samples from Greece and Slovenia (Fig. 2). The compound was analyzed in blood from France and Norway as well but was detected in only 35% and 19% of the samples, respectively. Greece had significantly lower

Table 3
Median concentrations and 95th percentiles (in parentheses) of halogenated flame retardants in children's blood (ng/g lipid).

Country	PBDE-47	PBDE-153	PBDE-209	ΣDP
France	0.36 (2.81)	35% DF (1.50)	8.73 (159)	34% DF (1.29)
Greece	0.03 (0.43)	0.09 (0.82)	0% DF	2% DF
Slovenia	[a]15% DF (0.46)	0.29 (1.02)	8% DF (10.10)	16.0 (24.8)
Norway	0.34 (0.93)	19% DF (0.1)	[b]-	3.17 (33.1)
All countries	[c]**0.32 (1.62)**	**40% DF (0.99)**	**38% DF (115)**	**2.13 (23.6)**

[a] Where a compound had <40% detection frequency (DF), the DF and 95th percentiles are reported.
[b] A dash (-) indicates that the compound was not analyzed in blood from that country.
[c] Values in bold indicate the median and 95th percentile of all data.

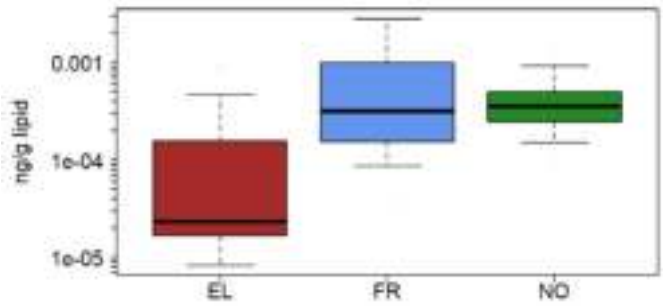

Fig. 1. Box plot of lipid-adjusted concentrations of BDE-47 for France (FR), Greece (EL) and Norway (NO). The box indicates median, 25% and 75% percentiles. Whiskers indicate 5% and 95% percentiles.

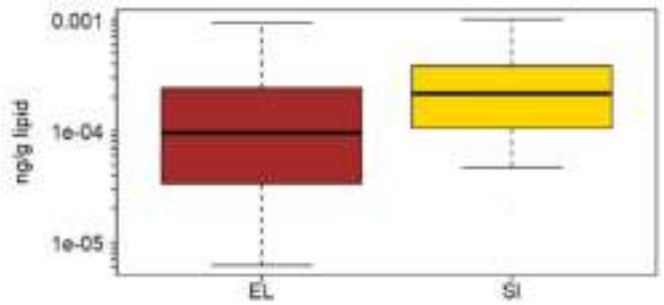

Fig. 2. Box plot of lipid-adjusted concentrations of BDE-153 in blood of children from Greece (EL) and Slovenia (SI). The box indicates median, 25% and 75% percentiles. Whiskers indicate 5% and 95% percentiles.

concentrations of BDE-153 than Slovenia ($p < 0.001$), both in the adjusted and unadjusted data. Lipophilic compounds such as halogenated FRs are typically associated with dietary exposure and breastfeeding (Pan et al., 2020; Poma et al., 2017). Norway is one of the countries with the highest breastfeeding rate for the first six months of infancy (Theurich et al., 2019), while Greece is known to have low exclusive breastfeeding (Pechlivani et al., 2005), which could be reflected in the concentration differences of PBDEs (Figs. 1 and 2).

BDE-209 was only present above 40% detection frequency in France, although it was analyzed in blood from Greece and Slovenia as well. Quantification limits for BDE-209 for Greece and Slovenia were comparable to or lower than those for France (Table S3), suggesting that the differences in detection are due to population exposures rather than analytical differences. BDE-209 was found at the highest concentration for a single compound in blood compared with all quantified FRs in our study (8.73 ng/g lipid), as expected based on the timing of restrictions and relatively higher recent use of BDE-209 (Abbasi et al., 2019). BDE-47 and BDE-153 were banned from production and use by the Stockholm Convention in 2004, while BDE-209 was only included in the Stockholm Convention in 2017 (Sharkey et al., 2020).

BDE-209 is a compound that can be difficult to quantify due to its high molecular mass (Pietroń and Małagocki, 2017; Stapleton, 2006) and often has low detection frequencies in other studies, typically because of high limits of detections and issues with blank contamination (Crosse et al., 2013; Darrow et al., 2017; Johnson et al., 2010; Wu et al., 2007). In our study, three countries analyzed BDE-209, but the compound was only quantifiable above detection limits in blood from France. Although Dvorakova et al. (2021) stated that Europe has ample laboratories with the capacity to quantify BDE-209, the limitation may be a disconnect between detection and quantification limits and concentrations in biological matrices. DBDPE, which is often considered a replacement for BDE-209, suffers from even more serious analytical challenges due to poorer instrument sensitivity and higher LOQs

(Melymuk et al., 2015).

Both *syn*- and *anti*-DP congeners were analyzed in blood from France, Slovenia, Greece, and Norway. Blood from France had detection frequencies below 40%. Only one blood sample from Greece had detectable concentrations of *syn*-DP and no *anti-DP* was detected. Slovenia had a median ΣDP concentration of 16 ng/g lipid, which was the highest concentration quantified in our study (Fig. 3). *Anti*-DP was present in 41% of the Slovenian blood samples, and *syn*-DP in only 12%. Norway had 100% and 99% detection frequencies for *syn*- and *anti*-DP, respectively. The median concentration of ΣDP was 3.17 ng/g lipid in blood from Norway, which was significantly lower than concentrations in children's blood from Slovenia ($p < 0.001$), both for adjusted and unadjusted data. Other countries may also have elevated DP concentrations, but it is difficult to determine due to high LODs (Table S3).

The French ESTEBAN study presented a unique profile in BDE-209 and DP compared with other studies. While detection frequencies are limited for both of these compounds, the comparison of medians, (where available) and 95th percentiles suggest higher exposure to BDE-209 in France and lower exposure to DP compared with Slovenia and Norway. DP has been suggested to be used as a replacement for BDE-209 (Barón et al., 2016) and the dominance of BDE-209 in France compared to DP in other regions may suggest countries are at different points in the transition away from BDE-209. We also note that the French data were among the earliest collected out of all studies, which may be an additional reason for the dominance of BDE-209.

3.2. OPFR metabolite concentrations in children's urine from different European countries

The detection frequencies of OPFR metabolites in children's urine are presented in Table S6. The concentrations of OPFR metabolites in children's urine are presented in Table 4.

BCEP was analyzed in four countries, but was only present above the LOQ in >40% of urine samples from Germany and Czech Republic (Table 4). Czech samples had significantly higher concentrations of BCEP than German (Kruskal-Wallis; $p < 0.001$; Fig. 4A), both in unadjusted data and data adjusted for age and sex. BCIPP was analyzed in seven countries, but, as with BCEP, only samples from Germany and Czech Republic had more than 40% >LOQ. In contrast to BCEP, samples from Germany had higher levels of BCIPP than those from the Czech Republic (Kruskal-Wallis; $p = 0.019$, Fig. 4B), both in unadjusted and adjusted data.

BDCIPP was quantified in Belgium, France, Germany, Slovenia, Czech Republic, Norway, Slovakia, and Denmark (Table 4), but was below LOQ in >60% of samples from Slovakia and Norway. A non-parametric Mann-Whitney pairwise comparison revealed three groupings within the countries (Fig. 5). Czech samples had lower concentrations ($p < 0.001$; Mann-Whitney) than all other countries, while France, Germany, and Slovenia had significantly higher concentrations than the other countries (Fig. 5b). The differences between the country groupings

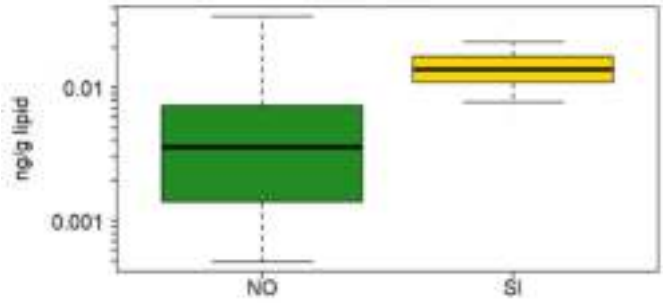

Fig. 3. Box plot of lipid adjusted concentrations of ΣDechlorane Plus (DP) in blood of children from Norway (NO) and Slovenia (SI). The box indicates median, 25% and 75% percentiles. Whiskers indicate 5% and 95% percentiles.

Table 4
Median concentrations and 95th percentiles (in parentheses) of OPFR metabolites in children's urine (μg/g creatinine).

Country	BCEP	BCIPP	BDCIPP	DPHP
Belgium	-[a]	14% DF (1.02)[b]	0.38 (3.11)	2.41 (7.63)
Czech Republic	0.22 (5.60)	0.10 (0.96)	0.26 (1.95)	2.06 (5.97)
Germany	0.14 (0.86)	0.12 (0.61)	0.64 (2.49)	1.66 (6.21)
Denmark	–	7% DF (1.35)	0.49 (3.71)	1.43 (5.16)
France	–	31% DF (5.04)	0.58 (4.55)	1.92 (9.70)
Slovenia	20% DF (1.04)	18% DF (0.25)	0.64 (1.59)	2.43 (5.47)
Slovakia	20% DF (0.98)	29% DF (0.28)	37% DF (1.16)	2.21 (6.48)
Norway	–	–	17% DF (0.56)	1.78 (7.29)
All countries	[c]**0.23 (1.32)**	**0.19 (1.59)**	**0.38 (2.49)**	**1.91 (6.87)**

[a] A dash (-) indicates that the compound was not analyzed in urine from that country.
[b] Where a compound had <40% detection frequency (DF), the DF and 95th percentiles are reported.
[c] Values in bold indicate the median and 95th percentile of all data.

were significant both in the unadjusted data and data adjusted for age and sex. When grouping countries by geographic region (e.g., north/south/east/west according to the UN Geoscheme for Europe) we did not see significant regional differences, suggesting that differences between countries are not due to broad geographic trends (e.g., east-west differences within Europe).

BCEP, BCIPP, and BDCIPP are direct metabolites of chlorinated OPFRs (Liu et al., 2021). The primary compounds (TCEP, TCIPP, and TDCIPP) are usually present in consumer products such as furniture, products containing PUF foam, and electronics (Hoffman et al., 2017). These compounds have been frequently reported in matrices of direct relevance for human exposure, particularly indoor settled dust (Liu and Folk, 2021), indoor air (Yadav et al., 2017), and food products (Blum et al., 2019). While regional differences have been identified in OPFR levels in other matrices, the differences are typically pronounced between Europe, Asia, and North America (He et al., 2015). All countries involved in our study are members of the European Economic Area, which has a common market system enabling the free movement of goods and generally harmonized chemical regulations. Thus, only small differences between countries are to be expected, and these are more likely to be due to small differences in the country's study populations and lifestyle differences between European regions (Section 3.3). The ubiquity of OPFR metabolite contamination can be ascribed to the broad use of chlorinated OPFRs, particularly after the ban on legacy FRs (Percy et al., 2020).

DPHP was the most widely detected FR, present in 99% of urine samples. Mann-Whitney pairwise comparisons revealed five overlapping groups of countries (Fig. 6b). The country groups were identical in both unadjusted data and adjusted data. Denmark had lower DPHP concentrations than all other countries, while Belgium and Slovenia had the highest (Fig. 6a and b). DPHP was also the OPFR metabolite found at the highest concentration in all countries. In some cases, DPHP concentration in children's urine was an order of magnitude higher than other OPFR metabolites (Table 4). DPHP from Slovenian samples had the highest median concentration of all the OPFR metabolites (2.43 μg/g creatinine). Other studies have also reported DPHP to be ubiquitous in human populations (Butt et al., 2014; Dodson et al., 2014; Hoffman et al., 2017). Unlike the other three quantified OPFR metabolites, DPHP is a non-specific metabolite. It is a metabolite of TPHP, a compound used as a flame retardant and plasticizer, and that is present in a large variety of products including PUF, hydraulic fluid, polyvinyl chloride (PVC), and cosmetic products such as nail polish (Preston et al., 2017). DPHP is also a metabolite of, among others, 2-ethylhexyldiphenyl phosphate

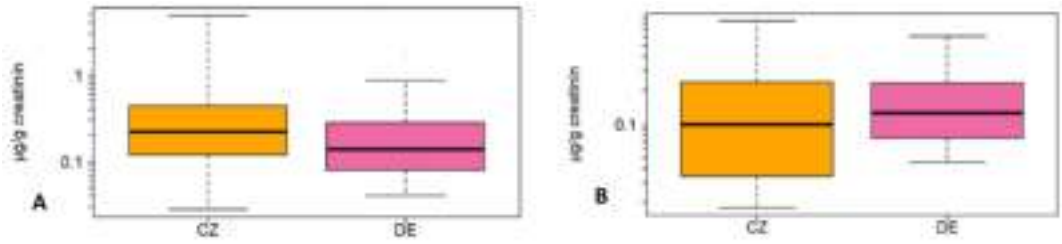

Fig. 4. Box plot of creatinine-adjusted concentrations (µg/g creatinine) of (A) BCEP and (B) BCIPP for Czech Republic (CZ) and Germany (DE). The box indicates median, 25% and 75% percentiles. Whiskers indicate 5% and 95% percentiles.

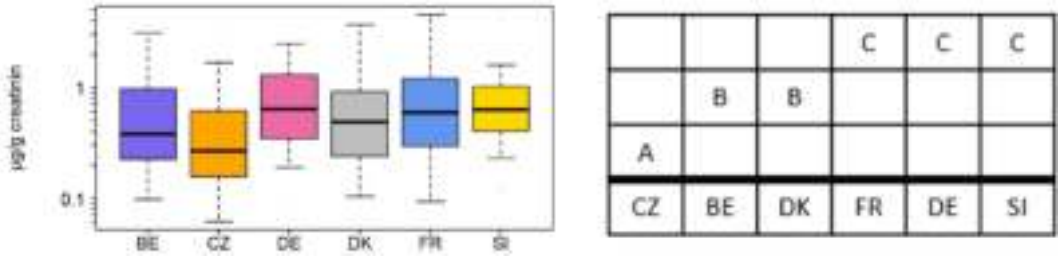

Fig. 5. a. Box plot of creatinine adjusted concentrations (µg/g creatinine) of BDCIPP for Belgium (BE), Czech Republic (CZ), Germany (DE), Denmark (DK), France (FR), and Slovenia (SI). The box indicates median, 25% and 75% percentiles. Whiskers indicate 5% and 95% percentiles.
5b. Mann-Whitney pairwise comparisons between countries. A, B, and C indicate groups of countries with different BDCIPP levels.

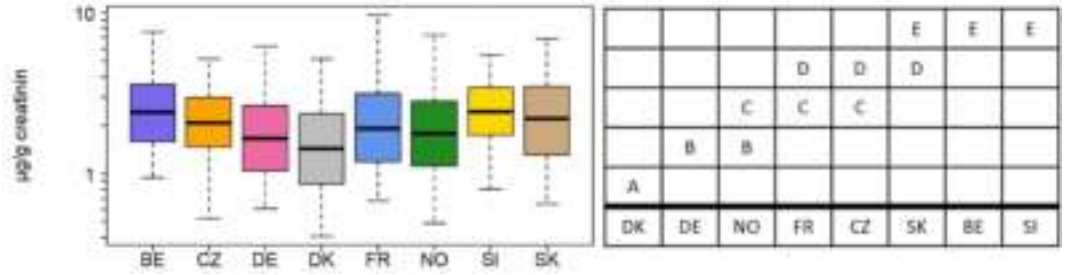

Fig. 6. a. Box plot of creatinine-adjusted concentrations of DPHP for Belgium (BE), Czech Republic (CZ), Germany (DE), Denmark, France (FR), Norway (NO), Slovenia (SI), and Slovakia (SK). The box indicates median, 25% and 75% percentiles. Whiskers indicate 5% and 95% percentiles.
6b. Mann-Whitney pairwise comparisons between countries. Letters groupings indicate groups of countries with different DPHP levels. Countries that do not overlap can be considered to be different from each other.

(EHDPHP) and resorcinol bis(diphenyl) phosphate (RDP) (Hou et al., 2016). Beyond being a FR, DPHP itself is also produced as a plasticizer, and used in certain chemical reagents and intermediate of pesticides, medicines, and organic material, is a polymerization catalyst and is an additive in paints and coatings (Liu et al., 2021). Considering the multiple exposure sources, it is not surprising that DPHP was the compound found most frequently at the highest detection frequencies in most countries.

3.3. Comparisons with other studies

Only a limited number of studies have investigated FRs in children between the ages of 6 and 12 years old. Many studies have quantified FRs in infants or toddlers (often in conjunction with samples from the mother) (Caspersen et al., 2016; He et al., 2018a; Morello-Frosch et al., 2016) or in adults (Brasseur et al., 2014; Lenters et al., 2013; Sochorová et al., 2017; Wang et al., 2019). However, since Moya et al. (2004) noted significant differences in both behavior and physiology between different age groups, we do not expect similar concentrations of FRs across age groups. Typically, when a study includes both adults and children, children have higher concentrations of BFRs than adults (Fischer et al., 2006; Toms et al., 2009; van den Eede et al., 2015). One study that quantified PBDEs in four members of the same household found that the children had 2- to 5-fold higher concentrations than the adults despite identical living conditions and diets (Fischer et al., 2006).

BDE-47 and 153 in children's blood from our study were both comparable, if slightly lower than studies from China (Table S8). The HELIX study analyzed chemicals, including BDE-47 and 153, in children and mothers from various European countries, providing much overlap with our study (Haug et al., 2018). The concentrations found in the HELIX study were comparable to our study. Only BDE-47 from France (0.36 ng/g lipid) was slightly higher in our study than in the HELIX study (0.27 ng/g lipid); the other concentrations from Greece and Norway were slightly higher in the HELIX study (Haug et al., 2018). Studies from the US and Canada reported concentrations of BDE-47 and -153 that were two orders of magnitude higher than what was found in our study (Table S8). PBDEs are typically significantly higher in studies from the US, and Canada to a lesser extent, when compared with other global regions (Frederiksen et al., 2009; Siddiqi et al., 2003; Sjödin et al., 2008) due to higher past use of PBDEs. Serum from Norway (Thomsen et al., 2007) had higher concentrations than what was found in Norway by the NEB II study. However, it should be kept in mind that the samples were collected in 1998, and it is known that the use of, and consequently exposure to, PBDEs has decreased since the 1990s. It was unexpected to

see that BDE-209 from France in our study (8.73 ng/g lipid) was the second-highest concentration of this comparison, after one study from China (95 ng/g lipid (Guo et al., 2016)).

Previous studies have analyzed BFRs in adults from European countries. In 2015, BFRs were quantified in serum from 300 Czech adults. PBDEs were only detected in 9% of the samples above detection limits (Sochorová et al., 2017). Other studies quantified PBDEs in serum from France (Brasseur et al., 2014), Poland, Ukraine (Lenters et al., 2013), and other European countries (Garí and Grimalt, 2013). Almost all concentrations from prior studies in adults were higher than the concentrations found in our study, which we attribute to the more recent sample collection. All children that were involved in our study were born between 2001 and 2015. The European Union restricted the use of Penta- and Octa-BDE commercial products from 2004, and the lower brominated congeners of PBDEs (tetra-, penta-, hexa-, and heptabromodiphenyl ethers) were listed for elimination under Annex A of the Stockholm Convention on Persistent Organic Pollutants in 2009 (Stockholm Convention, 2019). Though these literature-based comparisons should be interpreted with caution, the fact that PBDE concentrations in children's blood from our study are at comparable and lower concentrations than adults from studies in 1990s and early 2000s can be an indication that regulation restricting PBDE production and use are effective at reducing exposure.

While BFRs are reported in lipid-standardized units (e.g., ng/g lipid mass) enabling comparison across studies, studies that quantified OPFRs in urine do not have a standardized unit of quantification; data are presented in unadjusted units (e.g, μg/L), specific gravity (SG) normalized, or creatinine-standardized (μg/g crt), as is the case for our data. Given the limited data on OPFR metabolites in children, we have included studies using unadjusted of SG normalized urine concentrations in our comparison, and done an average-based recalculation based on ratios of creatinine to specific gravity for this age group of children (Table S8). Given the use of this recalculation, the comparisons with other studies should be interpreted with caution.

All BCEP and BCIPP concentrations from our study and previous studies were within the same order of magnitude. BDCIPP concentrations in urine were an order of magnitude higher in US compared with China; and European values from our study fell between these two levels (Table S8). DPHP concentrations were similar in Europe and the US, but China and Japan had lower concentrations of the compound in children's urine (Table S8).

3.4. Lifestyle factors associated with flame retardant exposure

Forty lifestyle factors were examined and the responses recorded by country, presented in Table S9.

A significant inverse correlation between age and FR exposure was observed for both BDCIPP and DPHP in France ($p < 0.001$ and $p = 0.005$) and Germany ($p = 0.002$ and $p = 0.006$) (Table S9). This agrees with the findings from several other studies, where OPFR concentrations decreased with children's age (Chen et al., 2018; He et al., 2018a; Sun et al., 2018; van den Eede et al., 2015; Zhang et al., 2018), and is likely related to more hand-to-mouth activity in younger children, resulting in increased exposure to dust-containing FRs (Cequier et al., 2015; Ionas et al., 2016; Moya et al., 2004).

In addition to age, our study found associations between FR concentrations and the physical structure of the indoor environment, cleaning habits, socioeconomic determinants, and diet (Table S9). Cequier et al. (2015) noted that the residential environment might be a more important exposure pathway of FRs than food, which agrees with the findings of our study. Renovation status, PVC floor, and vacuum and cleaning frequency per week were associated with FR concentrations in children from several countries (Table S9). In the Czech Republic, BCIPP ($p = 0.012$) was higher in houses that have been renovated within two years before the study, compared with unrenovated homes (Table S9). BCIPP was also higher in newer homes in France ($p = 0.007$), where home age correlated negatively with BCIPP concentrations. BDCIPP was lower in Czech children from homes with PVC floors ($p = 0.013$). Similarly, lower BDE-153 was quantified in children's blood from Slovenian households with PVC walls than in the blood from homes without ($p = 0.036$). The reason for this is unknown.

BDCIPP and DPHP were both higher in Czech households that "never" or "rarely" clean ($p = 0.003$ and $p < 0.001$). Similarly, BDE-47 was higher in Greek households that "rarely" vacuum ($p = 0.009$) and BDE-153 in Slovenian households that "never" or "rarely" clean ($p = 0.027$) (Table S9). Since FRs are present in indoor house dust (Ali et al., 2012; Fromme et al., 2014; Jílková et al., 2018), removing dust from living spaces is a good way to limit inhalation and dermal exposure, and has been linked to lower levels of FRs in indoor dust (Sugeng et al., 2018). However, BDE-47 was higher in French households that "often" vacuum ($p = 0.009$). The reason for this disparity is unknown.

The only significant association between measured FR levels and consumption of food in our study was observed for DPHP in seafood from Belgium ($p = 0.021$). Dietary exposure to OPFRs has been indicated in prior studies (He et al., 2018b; Xu et al., 2017), but was not very prominent in our study Similarly, the effect of passive smoking was almost negligible in this study; only BDCIPP in Slovenia was significantly higher in children from smoking households ($p = 0.047$).

In the French and Belgium studies, BDCIPP concentrations were positively correlated with time spent in a car ($\rho = 0.189$, $p = 0.001$ for France and Belgium $\rho = 0.195$, $p = 0.024$). TDCIPP, the parent compound of BDCIPP, is known to be present in car upholstery, where elevated temperatures can lead to an increase in volatilization (Phillips et al., 2018). There is evidence from the USA that longer commutes lead to increased TDCIPP exposure (Reddam et al., 2020).

An interesting feature was observed when studying socioeconomic factors. BCIPP ($p = 0.006$), and DPHP ($p = 0.002$) were higher in German households where the father was employed, versus households where the father was unemployed. Another socioeconomic association was found with household education in Germany, where households with the lowest levels of education had the lowest levels of DPHP in children's urine ($p = 0.005$). Conversely, the legacy FR BDE-47, was prominent in households where the father was unemployed ($p = 0.016$), as seen in blood from Norway (Table S9). This suggests a link between socioeconomic status and furniture or product replacement rates. We hypothesize that the higher levels of BDE-47 in houses where the father is unemployed are linked with lower income and related to the older furnishing or appliances containing legacy FRs (e.g., purchased before restrictions on PBDEs). In contrast, employment is linked to higher-income households that have higher purchasing power and are more likely to replace products and furnishings, leading to more products containing OPFRs instead of the now-banned PBDEs. Previous studies have linked BCIPP exposure with the number of electrical appliances and electronics in a home (He et al., 2018a; Sun et al., 2018), which can also be an indication of socioeconomic position.

Gender appeared to impact concentrations in a few instances. Slovenian boys had significantly higher BDCIPP and BDE-153 than girls ($p = 0.021$ and $p = 0.01$). Similarly, boys had significantly higher concentrations of BCIPP in Czechia ($p = 0.019$) and BDE-47 in Greece ($p = 0.024$). However, since this trend was not consistent throughout the study, the effect of gender on FR exposures should be interpreted with caution.

Most associations between FR concentrations and lifestyle factors were found for BDCIPP and DPHP. These compounds had the highest detection frequencies of the studied compounds. The information gained should be interpreted with caution. A more in-depth study of the lifestyle factors correlating with FR and other chemical compounds is recommended.

4. Conclusions

Halogenated FRs were quantified in children's blood from four

countries and OPFRs in urine from children from eight countries. This was the largest aligned study across multiple European countries to quantify FRs in children. This was also the first large-scale comparative study of OPFRs between different countries, providing valuable data to both researchers and policymakers. OPFR metabolites, particularly BDCIPP and DPHP, have ubiquitous distribution in Europe, with limited differences between countries, perhaps due to the open market conditions. OPFR concentrations should be critically evaluated by regulatory institutions due to their high prevalence and indications of endocrine-disrupting effects. The concentrations of BDE-47 in children's blood collected recently were comparable and lower than BDE-47 in adult samples from several years ago, suggesting that the regulation of PBDEs does mitigate the exposure of the compounds to humans. This study has highlighted the need to further build capacity to enable more laboratories to analyze OPFRs, BDE-209 and other halogenated alternative FRs, such as DP, DBDPE, TBBPA, and DBDPE. While it was difficult to ascribe specific lifestyle factors to flame retardant concentrations, factors concerning cleaning, socioeconomic status, and physical properties of the residence had the most significant correlations. It is recommended that future studies further investigate these and other lifestyle factors to better understand FR exposure to children.

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Declaration of competing interest

The authors declare that they have 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.2022.114070.

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From science to policy: How European HBM indicators help to answer policy questions related to phthalates and DINCH exposure

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ABSTRACT

Within the European Human Biomonitoring (HBM) Initiative HBM4EU we derived HBM indicators that were designed to help answering key policy questions and support chemical policies. The result indicators convey information on chemicals exposure of different age groups, sexes, geographical regions and time points by comparing median exposure values. If differences are observed for one group or the other, policy measures or risk management options can be implemented. Impact indicators support health risk assessment by comparing exposure values with health-based guidance values, such as human biomonitoring guidance values (HBM-GVs). In general, the indicators should be designed to translate complex scientific information into short and clear messages and make it accessible to policy makers but also to a broader audience such as stakeholders (e.g. NGO's), other scientists and the general public. Based on harmonized data from the HBM4EU Aligned Studies (2014–2021), the usefulness of our indicators was demonstrated for the age group children (6–11 years), using two case examples: one phthalate (Diisobutyl phthalate: DiBP) and one non-phthalate substitute (Di-isononyl cyclohexane-1,2- dicarboxylate: DINCH). For the comparison of age groups, these were compared to data for teenagers (12–18 years), and time periods were compared using data from the DEMOCOPHES project (2011–2012). Our result indicators proved to be suitable for demonstrating the effectiveness of policy measures for DiBP and the need of continuous monitoring for DINCH. They showed similar exposure for boys and girls, indicating that there is no need for gender focused interventions and/or no indication of sex-specific exposure patterns. They created a basis for a targeted approach by highlighting relevant geographical differences in internal exposure. An adequate data basis is essential for revealing differences for all indicators. This was particularly evident in our studies on the indicators on age differences. The impact indicator revealed that health risks based on exposure to DiBP cannot be excluded. This is an indication or flag for risk managers and policy makers that exposure to DiBP still is a relevant health issue. HBM indicators derived within HBM4EU are a valuable and important complement to existing indicator lists in the context of environment and health. Their applicability, current shortcomings and solution strategies are outlined.

1. Introduction

The „European Human Biomonitoring (HBM4EU) Initiative“ is a joint effort of 30 European countries, and the European Environment Agency (EEA), co-funded by the European Commission under the framework of Horizon 2020 aiming to improve and inform chemical safety. Using HBM methods the internal exposure of a chemical of interest is determined by measuring this substance in human samples such as urine, blood or hair. Since the internal exposure results from multiple sources, it represents the aggregated exposure from all routes (dermal, inhalation and oral). HBM has been identified as an important tool to support policy making (Ganzleben et al., 2017) but an improved science to policy transfer is urgently needed. Therefore, the focus of this publication is on how HBM indicators may answer policy-related questions and help identifying urgent needs for chemical regulation or management.

In the context of knowledge transfer and information processing, indicators are coming to the fore as they are known to be a valuable tool to illustrate rather complex scientific information in a concise and clear manner. Indicators can contribute to an effective science-policy translation for decision makers and direct communication to stakeholders, scientists and the general public (Buekers et al., 2018).

Indicators in the context of environment and health have been derived by the World Health Organization (WHO) (WHO, 1999; WHO, 2022) and the EEA (EEA, 2014; EEA, 2018). HBM indicators at the European level are scarce – to our knowledge only a few HBM indicators are publicly available in the WHO European Health Information Gateway (WHO, 2022). These include "Mean blood lead levels of children measured in areas without significant local sources of lead exposure" and "dioxin levels in human milk in selected countries" and some other persistent organic pollutants (POPs) in human milk. At the same time the progressive development and usage of chemicals demonstrate the need for implementing further European-wide HBM indicators. This is a relevant step towards prioritization of emerging chemicals and may highlight human health risks regarding chemical exposure. Including indicators for presenting HBM data in established indicator lists, such as the ones mentioned above, may provide further information on strategy and policy development in chemicals monitoring, while at the same time, checking progress towards policy targets that have already been set (Buekers et al., 2018).

For this purpose, within HBM4EU a concept has been elaborated to derive HBM indicators (Buekers et al., 2018). Based on Eurostat (2014), we decided to differentiate between two types of indicators, which we defined as follows:

A) result indicators (formerly described by Buekers et al., 2018 as 'HBM indicator for internal exposure'), which compare internal exposure levels between selected population groups, sexes or between regions, and.

B) impact indicators (formerly described by Buekers et al., 2018 as 'HBM indicator for health risk'), which compare exposure levels with health-based guidance values, such as the human biomonitoring guidance values (HBM-GVs) derived within HBM4EU (Apel et al., 2020a). These values allow a health risk assessment (RA) of available HBM data based on currently available scientific knowledge. They can be directly compared with measured internal values. „*The HBM-GVs derived for the general population represent the concentration of a substance or its specific metabolite(s) in human biological media (e.g., urine, blood, hair) at and below which, according to current knowledge, there is no risk of health impairment anticipated, and consequently no need for action*“ (Apel et al., 2020a).

To produce robust and scientifically sound answers to the policy questions, the indicators need to be based on harmonized and quality assured data (Buekers et al., 2018). Under HBM4EU, comparable HBM data with European wide exposure coverage from different countries have been aligned and collated under the HBM4EU Aligned Studies (Gilles et al., 2021 and Gilles et al., 2022). This harmonized data set (Esteban López et al., 2021; Mol et al., 2022) has been used for further development of the indicators. Depending on which information they should provide final visualization of the indicators can be either in a number format, or as an infographic (Buekers et al., 2018).

In this publication, we present the derived European HBM indicators for one selected phthalate Diisobutyl phthalate (DiBP) in children as a case study and the non-phthalate substitute Di-isononyl cyclohexane-

Abbreviations

3xG Health – Municipalities – Births study (Belgium, BE)
5cx-MEPP Mono(2-ethyl-5-carboxypentyl) phthalate
5OH-MEHP Mono(2-ethyl-5-hydroxyhexyl) phthalate
5oxo-MEHP Mono(2-ethyl-5-oxohexyl) phthalate
BBzP Butyl benzyl phthalate
BE Belgium
BEA Biomonitoring in Adolescents study (Spain, ES)
BPA Bisphenol A
BPF Bisphenol F
BPS Bisphenol S
CELSPAC: TE Central European Longitudinal Studies of Parents and Children: Teenagers (Czech Republic, CZ)
CLP Classification, Labelling and Packaging
CROME Cross-Mediterranean Environment and Health Network study (Greece, GR)
CZ Czech Republic
cx-MiDP Mono(2,7-methyl-7carboxy-heptyl) phthalate
cx-MINCH Cyclohexane-1,2- dicarboxylate-mono-(7- carboxylate-4-methyl)heptyl ester
cx-MiNP Mono(4-methyl-7-carboxyheptyl) phthalat
DE Germany
DEHP Diethylhexyl phthalate
DEMOCOPHES DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale project
DEP Diethyl phthalate
DiBP Diisobutyl phthalate
DINCH Di-isononyl cyclohexane-1,2- dicarboxylate
DiNP Diisononyl phthalate
DK Denmark
DnBP Di-n-butyl phthalate
ED Endocrine disruptor
EE Extent of exceedance
EEA European Environment Agency
EFSA European Food Safety Authority
ES Spain
ESB German Environmental Specimen Bank
ESTEBAN Health study on environment, biomonitoring, physical activity and nutrition study (France, FR)
EU European Union
FLEHS IV 4th cycle of the Flemish Environment and Health Survey (Belgium, BE)
FR France
GerES V-sub (unweighted) 5th cycle of the German Environmental Survey (subsample, unweighted data, DE)
GM Geometric mean
GR Greece
HBM4EU The European Human Biomonitoring Initiative
HBM Human Biomonitoring
HBM-GV Human Biomonitoring guidance values
HI Hazard index
HU Hungary
InAirQ Transnational Adaption Actions for Integrated Indoor Air Quality Management study (Hungary, HU)
IT Italy
LoC Level of confidence
LOD Limit of detection
LOQ Limit of quantification
LU Luxembourg
MBzP Mono-benzyl phthalate
MEP Mono-ethyl phthalate
MiBP Mono-isobutyl phthalate
MnBP Mono-n-butyl phthalate
MRA Mixture risk assessment
NAC II Northern Adriatic cohort II (Italy, IT)
NEB II Norwegian Environmental Biobank II (Norway, NO)
n Number of samples/participants exceeding the HBM-GV
N Total number of samples/participants
NEP N-Ethyl-2 pyrrolidone
NGO Non-Governmental Organisation
NIPH Norwegian Institute of Public Health
NL The Netherlands
NMP N-Methyl-2-pyrrolidone
NO Norway
OCC Odense child cohort (Denmark, DK)
OH-MiDP Mono-hydroxy-isodecyl phthalate
OH-MINCH Cyclohexane-1,2- dicarboxylate-mono-(7- hydroxy-4-methyl)octyl ester
OH-MiNP Mono(4-methyl-7-hydroxyoctyl) phthalate
P50 50th percentile; median
P95 95th percentile
PCB Polychlorinated biphenyls
PCB cohort follow-up Endocrine disruptors and health in children and teenagers in Slovakia study (follow-up study; Slovakia, SK)
PE Percentage exceedance
PFAS Per- and polyfluoroalkyl substances
POLAES Polish Aligned Environmental Study (Poland, PL)
QA/QC quality assurance/quality control
RA Risk assessment
REACH Registration, Evaluation, Authorisation and Restriction of Chemicals
Riksmaten Adolescents Riksmaten Adolescents 2016-17 (Sweden, SE)
SE Sweden
SES Socioeconomic status
SIN-list Substitute it now-list
SK Slovakia
SL Slovenia
SLO CRP Exposure of children and adolescents to selected chemicals through their habitat environment study (Slovenia, SL)
SPECIMEn-NL Survey on Pesticide Mixtures in Europe (The Netherlands, NL)
SVHC Substances of very high concern
TWI Tolerable weekly intake
UBA German Environment Agency
US EPA United States Environmental Protection Agency
WHO World Health Organization

1,2-dicarboxylate (DINCH). Phthalates are a group of industrial chemicals that are extensively used as plasticisers. They are used in a wide range of consumer products including vinyl flooring, food contact materials, personal care products and children's toys (German HBM Commission, 2011; Silano et al., 2019). In animal studies certain phthalates were found to affect fertility and reproduction of both sexes. Developmental effects in the offspring were also observed. The prenatal exposure to some phthalates during a critical time window (i.e. late 1st to early 2nd trimester in humans) induces adverse effects summarized as "phthalate syndrome" which comprises irreversible developmental and reproductive disorders mainly in male offspring (Main et al., 2009; German HBM Commission, 2011). Children are a sensitive group with

regards to the adverse effects of phthalate exposure because of their development, their surface area per body weight and their specific behavior (like hand-to mouth behavior for younger children).

Phthalates are of great societal concern as revealed under the substance prioritization carried out by HBM4EU (Ougier et al., 2021). They are widely used, toxic and present in all humans. Greenpeace has highlighted the presence of phthalates in consumer products and the potential health effects of phthalate exposure due to their endocrine disrupting effects (Greenpeace International et al., 2006). All phthalates for which indicators have been derived are included in the SIN list (i.e. Substitute It Now[1]) for which they were nominated from an advisory committee of NGO's working in the fields of environment, health and consumer.

Of the phthalates considered for the derivation of indicators Butyl benzyl phthalate (BBzP), Di-n-butyl phthalate (DnBP), DiBP and Diethylhexyl phthalate (DEHP) are officially recognized in the EU as endocrine disrupting chemicals and as toxic to reproduction (ECHA, 2022a). Also, Diethyl phthalate (DEP) is under assessment as an endocrine disrupting chemical (ECHA, 2022b). HBM4EU has therefore developed the health-based guidance values HBM-GV for phthalates and DINCH (Lange et al., 2021).

DINCH was put on the market in 2002 as a substitute for high molecular weight phthalates such as DEHP (German HBM Commission, 2014). It has no toxic effects to reproduction and is not an endocrine disruptor, but nephrotoxic effects have been observed in rat studies at high doses (EFSA Panel on Food Additives, 2006; German HBM Commission, 2014). For the substitute DINCH, no hazards in CLP notifications have been classified.

In the following the methodology for the HBM indicator derivation defined by Buekers et al., (2018) is summarized and the question as to whether policy–related questions can be addressed with these HBM indicators (for DiBP and DINCH) is examined.

2. Methods

During a HBM4EU workshop in Copenhagen in June 2017, a selection of substances was performed to identify substances that are relevant for the translation into HBM indicators. This was done by the application of criteria concerning relevance with regard to various aspects and data quality (see below). Consequently, the following substances were selected for indicator development: bisphenols A, F, S (BPA, BPF, BPS), some per- and polyfluoroalkyl substances (PFAS), cadmium, a number of pesticides, several phthalates and the non-phthalate substitute DINCH. Additionally, for showing the applicability of result indicators for investigating time trends data from the German Environment Specimen Bank (ESB) has been used from the HBM4EU dashboard for two aprotic solvents (N-Methyl-2-pyrrolidone; NMP and N-Ethyl-2-pyrrolidone; NEP). The above-mentioned selection criteria include a) **relevance** for EU policy, society and health and b) **data quality**, i. e. availability and comparability of data as described in the paper from Buekers et al. (2018).

Examples of EU policy relevance are the existence of a clear policy question or that the exposure to the selected substance may be a public health issue and there is a clear possibility for prevention and risk management options. Societal relevance was identified when public demand from newspapers or other lists, like the SIN list, was confirmed. Health relevance was confirmed for a substance when there was evidence of exposure for humans and an association with adverse health effects.

Concerning the criterium data quality, only substances were selected for which a) HBM data were available from European countries (at least 120 persons per study population), b) the comparability of the data was ensured due to harmonized procedures in, e.g., analytical methods. As an additional criterium it was decided to c) avoid overlaps to other existing indicator lists. Since all these criteria were met for the group of phthalates, we derived indicators for seven phthalates and a non-phthalate substitute. For the group of phthalates and DINCH, specific metabolites are measured in urine during human biomonitoring. For DiBP the measured metabolite is Mono-iso-butyl phthalate (MiBP), whereas for DINCH two metabolites were analysed, namely Cyclohexane-1,2- dicarboxylate-mono-(7-hydroxy-4-methyl)octyl ester (OH-MINCH) and Cyclohexane-1,2- dicarboxylate-mono-(7-carboxylate-4-methyl)heptyl ester (cx-MINCH). In Table 1, the two parent compounds are given together with their CAS numbers and their metabolites. For the other phthalates not presented in this publication, but for which indicators have been derived within HBM4EU, this information is given in the Supplementary Materials in Table S1.

Table 1
Overview of the parent compounds for phthalates and DINCH and their respective metabolites investigated in this publication.

Phthalate/ DINCH compounds (acronym)	Full name	CAS-Number	Metabolite(s) investigated acronym	Full name
DiBP	Diisobutyl phthalate	84-69-5	MiBP	Mono-isobutyl phthalate
DINCH	Di-isononyl cyclohexane-1,2-dicarboxylate	166412-78-8	OH-MINCH	Cyclohexane-1,2-dicarboxylate-mono-(7-hydroxy-4-methyl)octyl ester
			cx-MINCH	Cyclohexane-1,2-dicarboxylate-mono-(7-carboxylate-4-methyl)heptyl ester

To show how suitable our indicators are for answering policy-related questions gathered within HBM4EU, two illustrative examples were selected. DiBP was selected to represent the indicators for those phthalates belonging to the highly regulated group. DINCH was selected to represent indicators for a non-phthalate substitute.

2.1. Available datasets for the derivation of HBM indicators

For the derivation of HBM indicators there are several requirements with regards to harmonized and quality approved data, and only a few studies have been conducted to date harmonizing HBM data at the European level.

The **DEMOCOPHES project** was a first HBM feasibility study in 17 European countries (Den Hond et al., 2015). DEMOCOPHES data comprises urine samples from children (6–11 years) in a selected study area (see Table S2), collected between 2011 and 2012 and are currently available in the HBM4EU data repository.[2] This contains DEMOCOPHES data for DiBP from 8 countries and data for DINCH from 6 countries. DEMOCOPHES data for phthalates were quality controlled by a QA/QC scheme (Schindler et al., 2014). For the result indicators on "time patterns" (i.e. the comparison of data from two periods of time (DEMOCOPHES (2011–2012) versus HBM4EU Aligned Studies (2014–2021)) the median (50th percentile; P50) of metabolites of DiBP and DINCH were gathered from urine samples obtained from a sample of children (6–11 years) from the general population from different countries (see Table S3).

To get an overview of recent chemical exposure (sampling years

[1] https://sinlist.chemsec.org/.

[2] https://www.hbm4eu.eu/what-we-do/european-hbm-platform/eu-hbm-dashboard/.

2014–2021) of European citizens, one aim under HBM4EU was to perform so called **HBM4EU Aligned Studies** (Gilles et al., 2021; Gilles et al., 2022). The ethics related to data and sample handling in studies included in HBM4EU was compliant with national and EU regulation as described in "Implementation and coordination of an ethics framework in HBM4EU – experiences and reflections" (Knudsen et al., 2022) (this issue). Both for DiBP and DINCH metabolites in children (6–11 years) data are available from 11 studies from the HBM4EU Aligned Studies (see Table S3). For teenagers (12–18 years) data are available from 9 studies both for DiBP and DINCH. These data were used for the comparison of age groups (see Table S4).

Chemical analysis of the biomarkers in the HBM4EU Aligned Studies was quality controlled (Esteban López et al., 2021; Mol et al., 2022) and was done for phthalates and DINCH by 7 different analytical laboratories (Esteban López et al., 2021).

Urine samples were either first morning urine samples or spot urine samples collected in the HBM4EU Aligned Studies. Urine samples taken in the frame of the DEMOCOPHES project in children were all first morning urine samples. All concentrations are given in μg/L and are not creatinine-adjusted. Since creatinine excretion is age dependent in children (German Human Biomonitoring German HBM Commission, 2005) we decided not to present the study results on a creatinine-adjusted basis. Data for specific gravity was only available for teenagers within the HBM4EU Aligned Studies (Gilles et al., 2022), and no adjustments were made to enable a better comparability of the data sets for children and teenagers. In addition, for the impact indicators a volume-based indication in μg/L of the results was necessary, since HBM-GVs are given in a volume basis in μg/L.

An overview of the corresponding studies used for the derivation of indicators including their study names, sampling years, number of participants, age range and matrix type (spot or first morning urine) for determination of the phthalates and DINCH is given in the Supplementary Materials Tables S2, S3 and S4.

2.2. Derivation of result indicators

Our result indicators compare internal exposure levels between selected population groups, sexes and regions. For illustrating exposure at the population level, the use of different percentiles is possible and we selected P50 (median) values for this comparison. Different types of result indicators have been derived for showing: 1) differences in exposure between age groups, 2) differences between geographical regions, 3) differences in exposure between boys and girls and 4) differences in exposure of different time points.

The policy-related questions to be answered by the result indicators are:

1. What is the extent of the current exposure of the EU population, especially children, to DiBP and the non-phthalate substitute DINCH?
2. Do the exposure levels differ between the studies from different countries?
3. Is there a difference in internal exposure between boys and girls?
4. Is there a difference in internal exposure between different age groups?
5. Are there indications for an increase or decrease in internal exposure for DiBP and DINCH?

2.2.1. Calculation of result indicators 1) to 3) for geographical differences, sex differences and age differences

To show differences in internal exposure in the format of our result indicators, P50 values have been calculated from the data from the HBM4EU Aligned Studies (2014–2021). A difference in exposure has been defined when no overlap of the 95% confidence intervals between the P50 values of the different geographical regions/sexes/age groups was found. When an overlap was observed, no significance could be stated as further statistical tests should be performed. The P50 values and 95% confidence intervals for the calculated result indicators are given in the Supplementary Materials in Tables S6–S13. Meanwhile statistical analyses of the individual data of the HBM4EU Aligned Studies have been performed (Vogel et al., 2022a (this issue), Martinsone et al. (2022 in preparation)). Therefore, we decided to refer in the results sections to the outcomes from these statistical analyses.

For the indicator on sex differences, only 10 studies were available since for one study the number of participants was low, and no stratification could be made for sex (i.e., no P50 or P95 data for this study are available in the data repository).

2.2.2. Calculation of result indicators 4) for "time patterns"

For this indicator, P50 values of phthalate metabolites for children (6–11 years) from the HBM4EU Aligned Studies (2014–2021) were compared to P50 values from the previous conducted DEMOCOPHES project (2011–2012). A difference in exposure was defined when no overlap of the 95% confidence intervals between P50 internal exposure values of the different studies was seen. An analogous indicator was also derived for the non-phthalate substitute DINCH. To give an overview on P50 values for the two periods of time, all the available data from the HBM4EU repository are shown and this resulted in 8 studies with data for DiBP and 6 studies with data for DINCH. Since only data from countries with studies available for both time periods have been compared, the number of studies for this comparison was 4 for DiBP and 3 for DINCH.

2.3. Derivation of impact indicators

As impact indicators directly evaluate the internal exposure to a substance within a health risk context, the importance of having HBM-GVs is coming to the fore. HBM-GVs have been derived for selected substances under HBM4EU, and specifically for phthalates HBM-GVs have been derived for 5 phthalates and DINCH for the general population for the following two population groups: 1) children (6–13 years) and 2) adolescents, adults (from 14 years onwards) (Lange et al., 2021). The HBM-GVs for children for DiBP and DINCH, the sensitive endpoint on which the HBM-GV derivation was based and the level of confidence (LoC) evaluating the data that has been used for the derivation are presented in Table S5 in the Supplementary Materials. The P95 values (and 95% confidence intervals) for the impact indicator calculation are given in the Supplementary Materials for all graphs (Tables S14 and S15).

The policy-related questions to be answered by the impact indicators are:

1. Is the exposure to phthalates, like DiBP and their substitutes of health-relevance for the general population and vulnerable groups e. g. children?
2. In which countries does exposure exceed the HBM-GV for children?

2.3.1. Calculation of impact indicators

Two types of HBM impact indicators have been derived. The first one is the „percentage of sample (i.e., individuals) exceeding the HBM-GV (called PE), and the second is the „extent of exceedance" on a sample level (called EE). As impact indicators evaluate exposure within a health risk context, it was decided to compare the HBM-GVs with the P95 values of the measured concentrations. A P95 can be interpreted as 95% of the study participants having an internal concentration equal or below this value. All values are given in μg/L. The P95 have been calculated with RStudio Team (2022).

1) The **percentage of population exceeding the HBM-GV (PE)** can be calculated as follows:

PE = n/N x 100%

Where n is the number of samples/participants with HBM exposure levels above the HBM-GV (n > HBM-GV) and N is the total number of samples/participants participating in the study. Thus, the PE describes the percentage in a given population that exceeds the HBM-GV.

2) The **extent of exceedance (EE)** describes the extent by which the HBM-GV is exceeded by the 95th percentile (P95). It is calculated by dividing the P95 percentile by the HBM-GV:

EE = P95/HBM-GV

A value > 1 means that the P95 exceeds the HBM-GV. Below 1 there is no exceedance by the P95. Thus, the EE describes the factor of exceedance or non-exceedance of the P95.

2.4. Visualization of indicators

For the visualization of the result and impact indicators, the data have been graphed using the R statistical analysis software package (R Core Team, 2018). These graphs show P50 and P95 as bars and the HBM-GVs as dotted lines.

For the result indicators, the indicators were stratified according to the selected parameters (e.g. sex and age). For the geographical comparison the studies from the respective countries have been assigned to one of the four regions according to the United Nations geoscheme in the HBM4EU Aligned Studies (United Nations, 1999,) (, i.e. North, South, East and West.

The graphs showing the impact indicator with the focus on illustrating the percentage of participants exceeding the HBM-GV have been plotted using Microsoft Excel (version 2019).

2.5. Workshop on policy uptake of HBM4EU results – a practical reality check

The workshop policy uptake of HBM4EU results was held in virtual format on 30–31 May 2022. Participants were experts from the HBM4EU project as well as policymakers from the European Commission. The purpose of this workshop was to present the highlights and main messages based on HBM4EU findings in response to policy questions and needs, in which the results for phthalates and DINCH were discussed. Topics that were discussed included: determination of main highlights, reflection of policy needs by results presented, addressees for further action in regulation and policy making, expectations towards the practices in risk assessment, management, regulation, policy, awareness raising and communication initiatives.

In Fig. 1, the process of indicator development within HBM4EU is presented.

3. Results

Within the HBM4EU Aligned Studies most of the phthalates including DiBP could be detected in the vast majority of samples with the percentage of values above the limit of quantification (LOQ) ranging from 90 up to 100% in children for the corresponding metabolites of the phthalates where indicators have been derived. This demonstrates the ubiquitous exposure of children in Europe to phthalates. Furthermore, the substitute DINCH could be detected in almost all samples (with the percentage of values for the corresponding metabolites that were above LOQ ranging from 96 up to 99% in children).

In the following sections, our HBM indicators are presented using DiBP as a case example for a regulated phthalate and DINCH as a non-phthalate substitute. The indicators will be further analysed in the Discussion.

Under HBM4EU indicators for other selected substances (including other phthalates) have been developed. They will be presented on the HBM4EU website.[3]

3.1. Result indicators

Result indicators illustrate differences in the internal exposure between geographical regions, sex, age, and time.

3.1.1. Result indicator for geographical differences

In Fig. 2 the result indicator for DiBP in children is shown for the different geographical regions. In Fig. 3 this indicator is shown for DINCH in children.

The result indicator for geographical differences (Fig. 2) provides an overview of internal exposure to DiBP in European children (6–11 years) from studies in 11 European countries between 2014 and 2021 by plotting the P50 values of MiBP.

Regarding geographical differences, the exposure of children towards DiBP metabolites was highest in an eastern European study (Slovakia, PCB cohort study, 2014–2017) and lowest in a northern European study (Denmark, OCC study, 2018–2019). The P50 values differed more between the sampling sites than between regions. Also Vogel et al. (2022a) (this issue) found no differences between the regions in urinary metabolite concentrations of DiBP in multivariate analyses. The result indicator for geographical differences of DiBP metabolite concentrations in children showed that P50 values varied by a factor of almost 5 (4.7) between the sampling sites. It must be noted, that the MiBP measurements in PCB-cohort study from Slovakia were not quality assured within HBM4EU (Govarts et al., 2022) (this issue), therefore comparability cannot be guaranteed (see Esteban López et al., 2021).

The result indicator for geographical differences (Fig. 3) provides an overview of the internal exposure to DINCH of European children (6–11 years) from studies in 11 European countries between 2014 and 2021 by plotting the P50 values of the sum of OH-MINCH and cx-MINCH.

Regarding geographical differences, the exposure of children towards the sum of the two DINCH metabolites was highest in one southern European study (Italy, NAC II study, 2014–2016) and lowest in one eastern European study (Hungary, InAirQ study, 2017–2018). The P50 values for DINCH metabolites were also higher in the south compared to the east. The P50 values for DINCH metabolites were also higher in the northern region (but represented by only one country) than in the eastern region. This was statistically confirmed by Vogel et al. (2022a) (this issue) who also found further differences between the regions in multivariate analyses.

The P50 values varied by a factor >2 (2.76) between the sampling sites.

3.1.2. Result indicator regarding sex differences

Result indicators on sex differences for children are shown in Fig. 4 for DiBP and in Fig. 5 for DINCH.

The result indicator regarding sex differences (Fig. 4) provides an overview of the internal exposure to DiBP of European boys and girls (6–11 years) from studies in 10 European countries between 2014 and 2021 by plotting the P50 values of MiBP.

The result indicator for sex differences showed that boys and girls were similarly exposed to DiBP. This was statistically confirmed by Martinsone et al. (2022, in preparation).

This result indicator regarding sex differences (Fig. 5) provides an overview of the internal exposure to DINCH of European boys and girls (6–11 years) from studies in 10 European countries between 2014 and 2021 by plotting the P50 values of the sum of OH-MINCH and cx-MINCH.

The result indicator for sex differences showed that boys and girls were similarly exposed to DINCH. This was statistically confirmed by

[3] HBM4EU – science and policy for a healthy future.

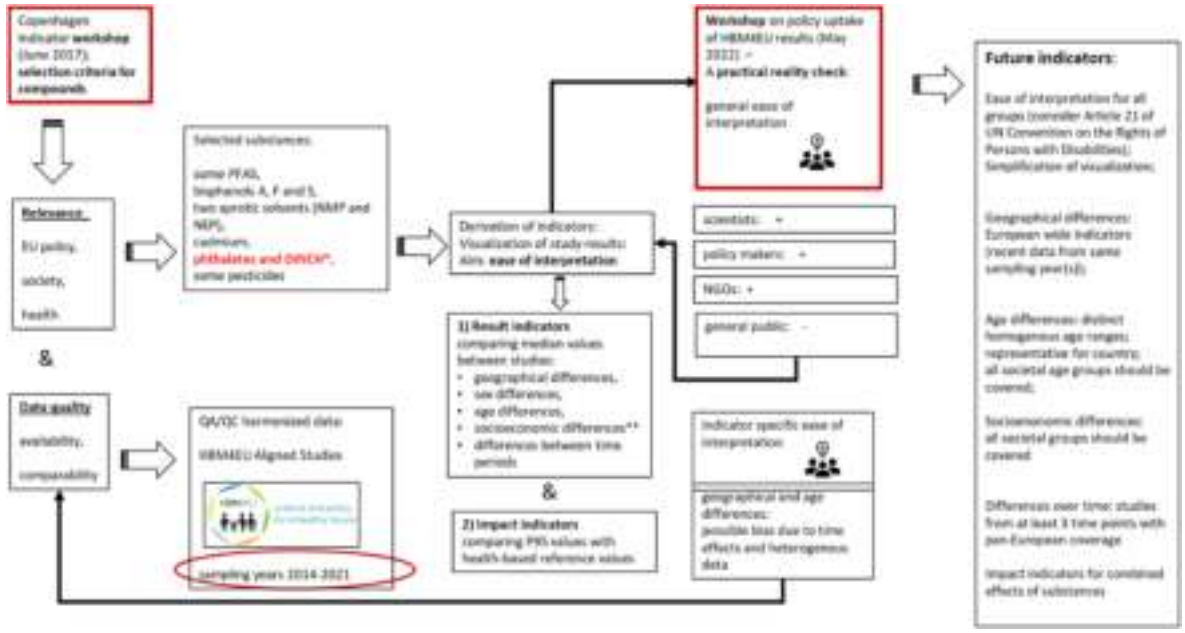

Fig. 1. *Development of HBM indicators within HBM4EU. *phthalates and DINCH were used to exemplify the process of indicator development. **there were not sufficient data to derive this indicator for phthalates and DINCH.*

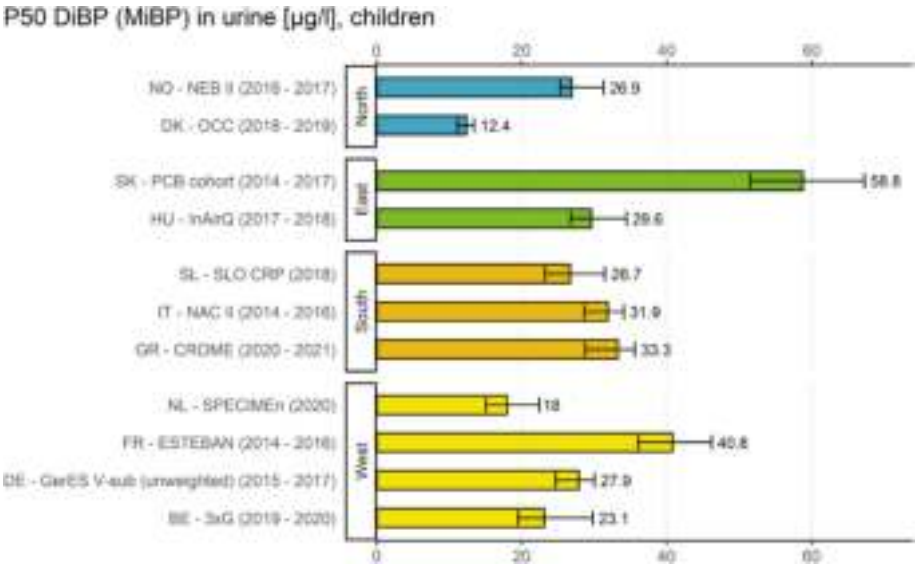

Fig. 2. Result indicator for geographical differences of P50 values (and 95% confidence intervals) in DiBP exposure (MiBP in µg/L) in children (6–11 years) in the HBM4EU Aligned Studies. Country names, study names and sampling years (in brackets) are given. DiBP metabolite levels were either measured in first morning or random spot urine samples.

Martinsone et al., (2022 in preparation).

3.1.3. Result indicator regarding age differences

Result indicators on age differences in children are shown for DiBP in Fig. 6 and for DINCH in Fig. 7.

The result indicator on age differences (Fig. 6) provides an overview of the internal exposure to DiBP of European children (6–11 years) and teenagers (12–18 years). These were based on 11 studies in children and 9 studies in teenagers between 2014 and 2021 by plotting P50 values of MiBP.

Regarding the data from the HBM4EU Aligned Studies, the exposure towards DiBP metabolites was in a similar range in children (6–11 years) and teenagers (12–18 years) in most studies. This was statistically confirmed by Vogel et al. (2022a) (this issue). However, when the authors investigated the effect of age in years, decreasing levels of DiBP metabolites were found with increasing age.

Higher exposure in children was also observed compared to teenagers for studies from Slovakia and Greece, based on no overlap of 95% confidence intervals (Table S9). The highest exposure values for DiBP were found for children in the PCB cohort study from Slovakia. In Slovakia however, the samples in children were taken in 2014–2017 whereas samples in teenagers were taken in 2019–2020.

The result indicator regarding age differences (Fig. 7) provides an overview of the internal exposure to DINCH of European children (6–11 years) and teenagers (12–18 years) from 11 studies in children and 9 studies in teenagers between 2014 and 2021 by plotting P50 values of the sum of OH-MINCH and cx-MINCH.

The result indicator for age differences revealed that exposure

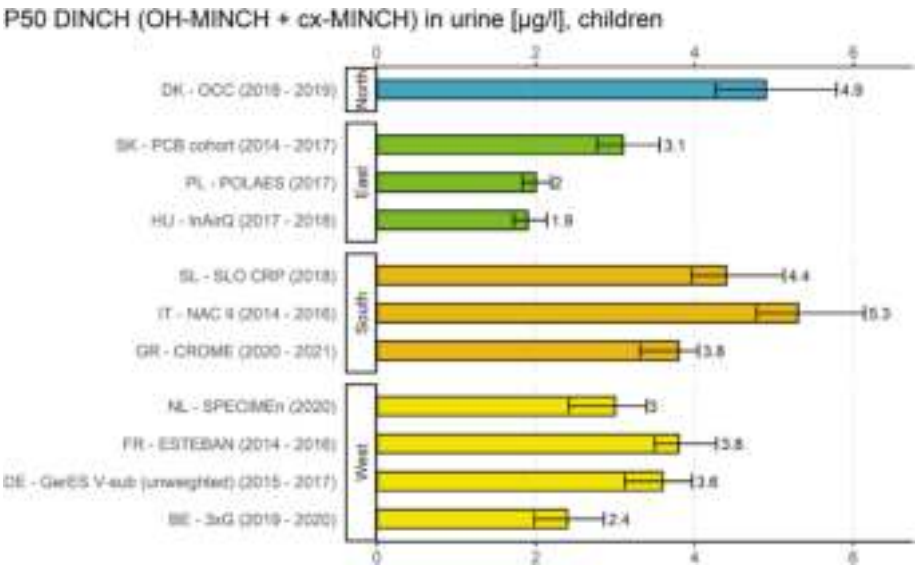

Fig. 3. Result indicator for geographical differences of P50 values (and 95% confidence intervals) for DINCH exposure (∑(OH-MINCH + cx-MINCH) in µg/L) in children (6–11 years) in the HBM4EU Aligned Studies (collected in the years 2014–2021). Country names, study names and sampling years (in brackets) are given. DINCH metabolite levels were either measured in first morning or random spot urine samples.

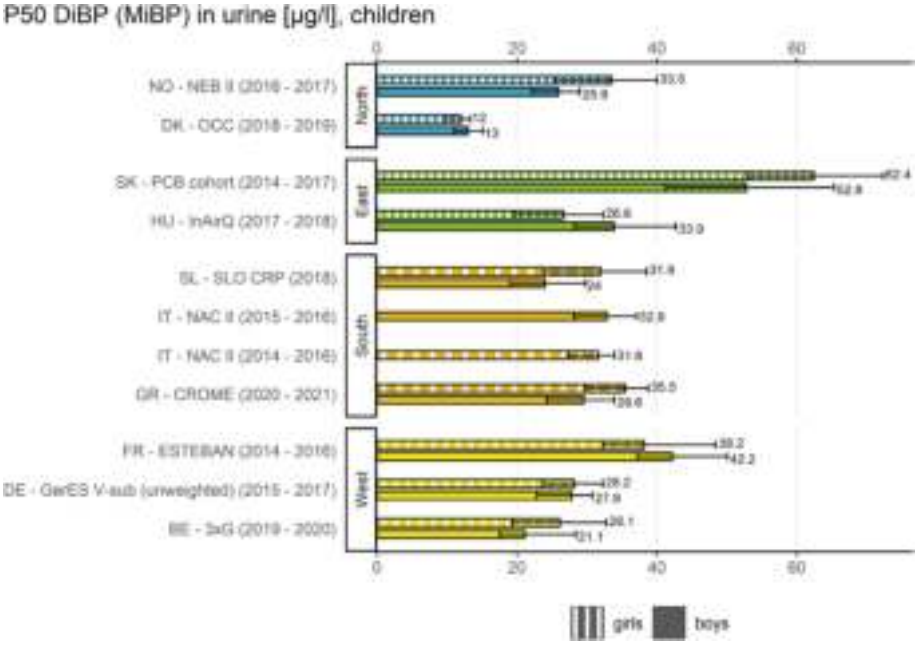

Fig. 4. Result indicator regarding sex differences of P50 values (and 95% confidence intervals) of DiBP exposure (MiBP in µg/L) in children (6–11 years) in the HBM4EU Aligned Studies (collected in the years 2014–2021). Country names, study names and sampling years (in brackets) are given. DiBP metabolite (MiBP) levels were either measured in first morning or random spot urine samples.

towards ΣDINCH metabolites was higher in children than in teenagers in most studies, therefore an age difference can be confirmed, resulting in higher exposure for this sub-group of children. This age difference for DINCH exposure was statistically confirmed by Vogel et al. (2022a) (this issue).

3.1.4. Result indicator for "time pattern" regarding different periods of time

Result indicators on differences in phthalate and DINCH exposure for different periods of time have been developed (the so-called "time pattern" indicator). These indicators are shown in Fig. 8 for DiBP and in Fig. 9 for DINCH from children samples of the HBM4EU Aligned Studies.

The result indicator regarding different periods in time (Fig. 8) provides an overview of the internal exposure to DiBP in European children (6–11 years) for two specific periods in time by plotting P50 MiBP values.

For the first time period, differences in exposure to DiBP metabolites in children from 8 countries based on data of the DEMOCOPHES project and collected in the years 2011–2012 are presented. For the second time period, the exposure of children from 11 European countries conducted under the HBM4EU Aligned Studies between 2014 and 2021 are shown.

Differences in exposure between the studies can be observed with P50 values ranging from 34.5 up to 104 µg/L in the DEMOCOPHES project (values varied by a factor of 3) and P50 values ranging from 12.4

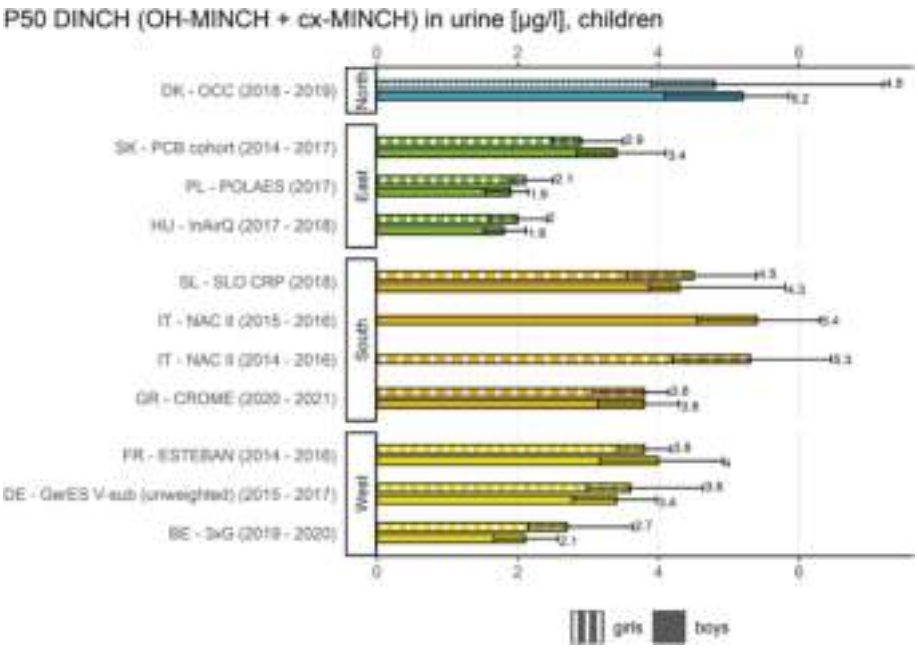

Fig. 5. Result indicator regarding sex differences of P50 values (and 95% confidence intervals) of DINCH exposure ((∑(OH-MINCH + cx-MINCH) in µg/L) in children (6–11 years) in the HBM4EU Aligned Studies (collected in the years 2014–2021). Country names, study names and sampling years (in brackets) are given. DINCH metabolite levels were either measured in first morning or random spot urine samples.

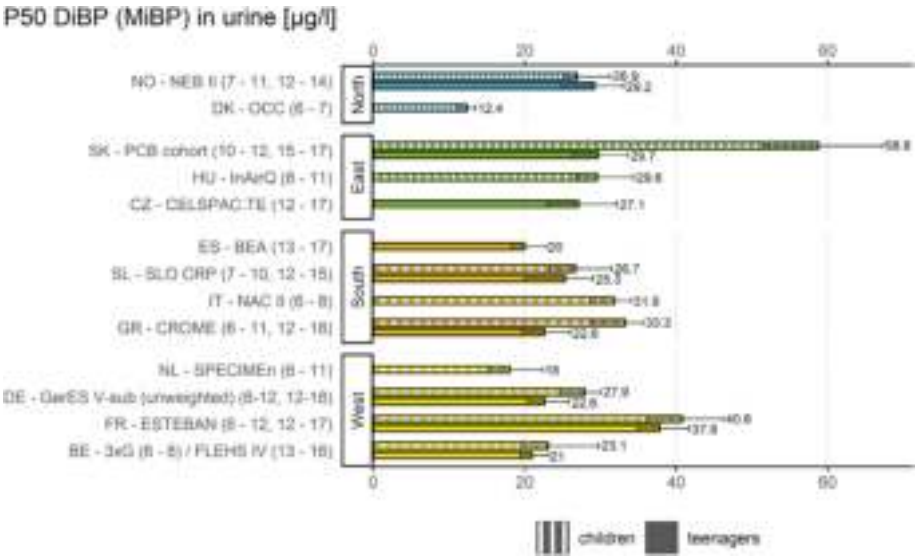

Fig. 6. Result indicator regarding age differences of P50 values (and 95% confidence intervals) of DiBP exposure (MiBP in µg/L) in children (6–11 years) and teenagers (12–18 years) in the HBM4EU Aligned Studies (collected in the years 2014–2021) measured in 11 countries in children and in 9 countries in teenagers. Country names, study names and age range of participants (in brackets) are given. DiBP metabolite levels were either measured in first morning or random spot urine samples.

up to 58.8 µg/L in the HBM4EU Aligned Studies (values varied by a factor of 4.7).

Data from 4 countries are available for both periods of time (i.e., DK, SL, BE and DE). The exposure of children to DiBP metabolites in these studies was lower in the more recent data from 2014 to 2021 compared to earlier data from 2011 to 2012, indicating a decrease in exposure over time These findings are based on visual comparison of an overlap or non-overlap of the confidence intervals (Table S11). Decreasing concentrations for DiBP in young adults were statistically confirmed by (Vogel et al., 2022b) (this issue) in a time trend study assessing data from Denmark and Germany.

The result indicator regarding different periods in times (Fig. 9) provides an overview of the internal exposure based on the sum of the DINCH metabolites OH-MINCH and cx-MINCH in children (6–11 years) for two specific periods in time periods by plotting P50 values. It compares the summed DINCH metabolites levels in children from 6 European countries collected under the DEMOCOPHES project (2011–2012) with data from children in 11 countries from the HBM4EU Aligned Studies (2014–2021).

For the sum of DINCH metabolites data for both periods of time are available from 3 countries (i.e., Denmark (DK), Belgium (BE) and Germany (DE)). The levels of DINCH metabolites in children were higher in samples from DK, BE and DE collected between 2014 and 2021 compared to samples collected in 2011–2012. These findings are based

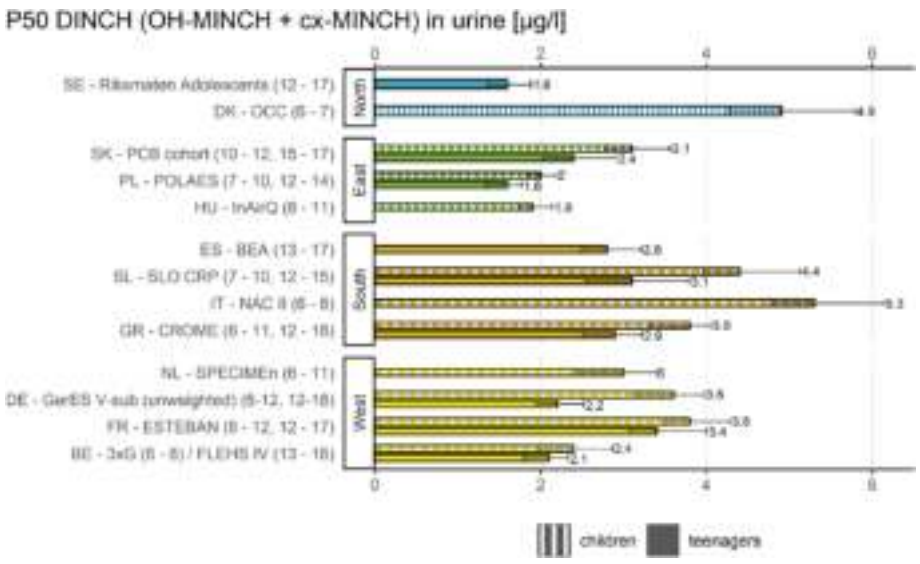

Fig. 7. Result indicator regarding age differences of P50 values (and 95% confidence intervals) of DINCH exposure (∑(OH-MINCH + cx-MINCH) in µg/L) between children (6–11 years) and teenagers (12–18 years) in the HBM4EU Aligned Studies (collected in the years 2014–2021) measured in 11 countries in children and 9 countries in teenagers. Country names, study names and age range of participants (in brackets) are given. DINCH metabolite levels were either measured in first morning or random spot urine samples.

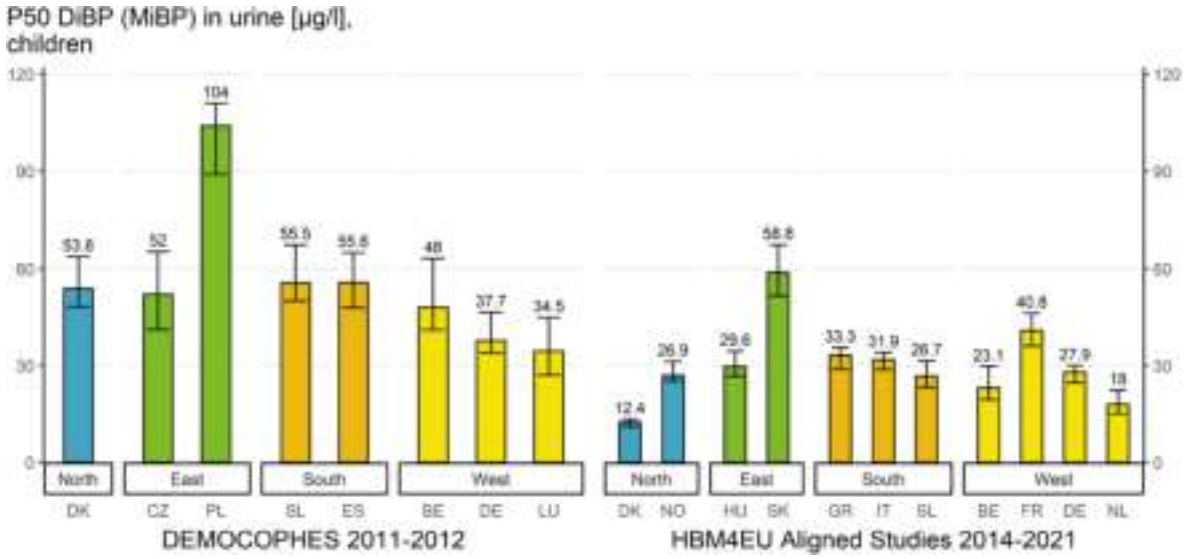

Fig. 8. Result indicator regarding different periods in time showing differences of P50 values of DiBP exposure (MiBP in µg/L). Direct comparison of DiBP exposure from two projects in different time periods in Europe: 1) in children (6–11 years) from 8 studies between 2011 and 2012 (DEMOCOPHES project) and 2) 11 studies in children between 2014 and 2021 (HBM4EU Aligned Studies). DiBP metabolite (MiBP) levels were either measured in first morning or random spot urine samples.

on visual comparison of an overlap or non-overlap of the confidence intervals (Table S12). Vogel et al., (2022b) (this issue) statistically confirmed increasing DINCH levels in young adults in a time trend study assessing data from Denmark and Germany.

3.2. Impact indicators

In the following section two types of impact indicators have been derived, as they are described in section 2.3.1.

1) Percentage of population exceeding the HBM-GV (PE): comparison of P95 with the corresponding HBM-GV; 2) Extent of exceedance (EE): percentage participants exceeding the corresponding HBM-GV.

3.2.1. Impact indicator for DiBP exposure

The impact indicator (Fig. 10) provides an overview of the P95 MiBP (biomarker for DiBP) levels in children (6–11 years) from the 11 European studies and the corresponding HBM-GV of 160 µg/L (Lange et al., 2021) for comparison.

Since this indicator is intended to be used to estimate health risks, the complete set of results are presented in Table 2.

In all but one study, at least some of the children were observed to have DiBP levels larger than the HBM-$GV_{children}$ of 160 µg/L (see Table 2). In these cases, a risk for adverse health effects cannot be excluded.

The extent of exceedance (EE) (i.e., P95/HBM-GV) in the different studies and locations ranges from 0.35 up to 1.54, meaning that the HBM-GV for DiBP was exceeded at the P95 of the population only in the case of Slovakia (54%) and France (13%). The studies with the largest extent of exceedance for DiBP exposure were the PCB cohort study from Slovakia (1.54) and the ESTEBAN study from France (1.13) (see Table S15). This shows that exposure to DiBP in France and Slovakia exceeded established health-based guidance values for DiBP. It has to be noted, that both studies were conducted prior to the other studies which were completed more recently. As regulation might have been effective

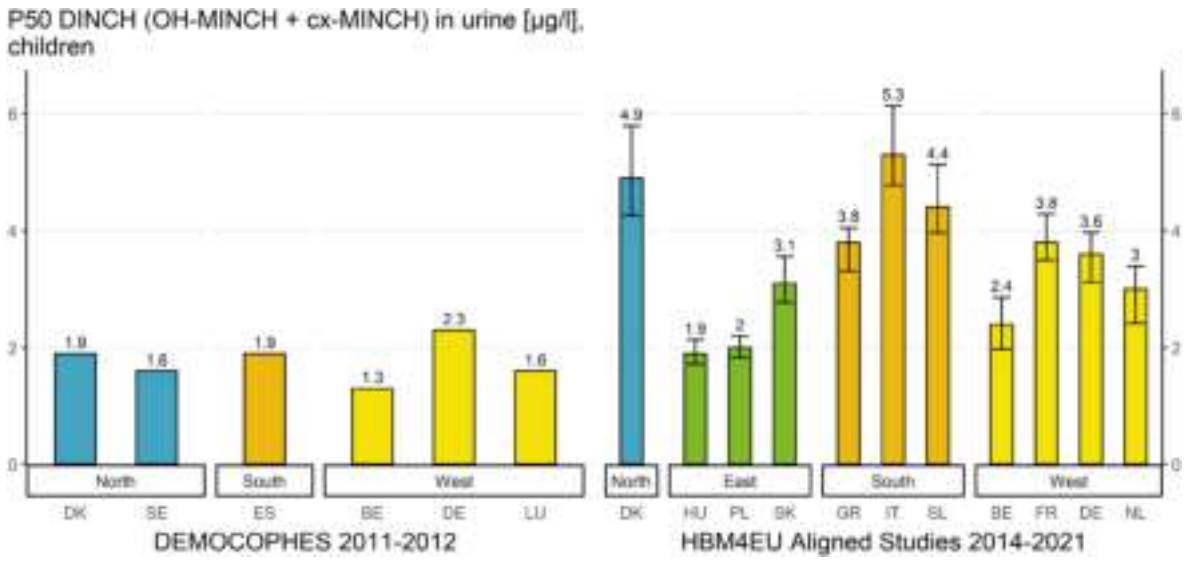

Fig. 9. Result indicator regarding different periods in time in showing differences of P50 values of DINCH exposure (sum OH-MINCH + cx-MINCH in µg/L). Direct comparison of DINCH exposure from two projects in different time periods in Europe: 1) in children (6–11 years) from 6 studies between 2011 and 2012 (DEMOCOPHES project) and 2) 11 studies between 2014 and 2021 (HBM4EU Aligned Studies). DINCH metabolites (OH-MINCH and cx-MINCH) levels were either measured in first morning or random spot urine samples. 95% confidence intervals are only shown for the HBM4EU Aligned Studies as for DEMOCOPHES confidence intervals for the sum of the metabolites were not given for the aggregated data.

over this time, this time difference might have influenced these results.

The "percentage of population exceeding the HBM-GV (PE)" is provided in Fig. 11.

In cases where the HBM-GVs were exceeded, based on current knowledge a risk for adverse health effects cannot be excluded for part of the population.

3.2.2. *Impact indicator for DINCH exposure*

This impact indicator (Fig. 12) provides an overview of the P95 ΣDINCH metabolite levels in children (6–11 years) from 11 studies in Europe compared to the corresponding HBM-GV of 3000 µg/L (Lange et al., 2021).

The impact indicator for DINCH exposure in children showed P95 values that were considerably lower than the HBM-GV$_{children}$ of 3000 µg/L derived within HBM4EU in all considered countries. This indicates

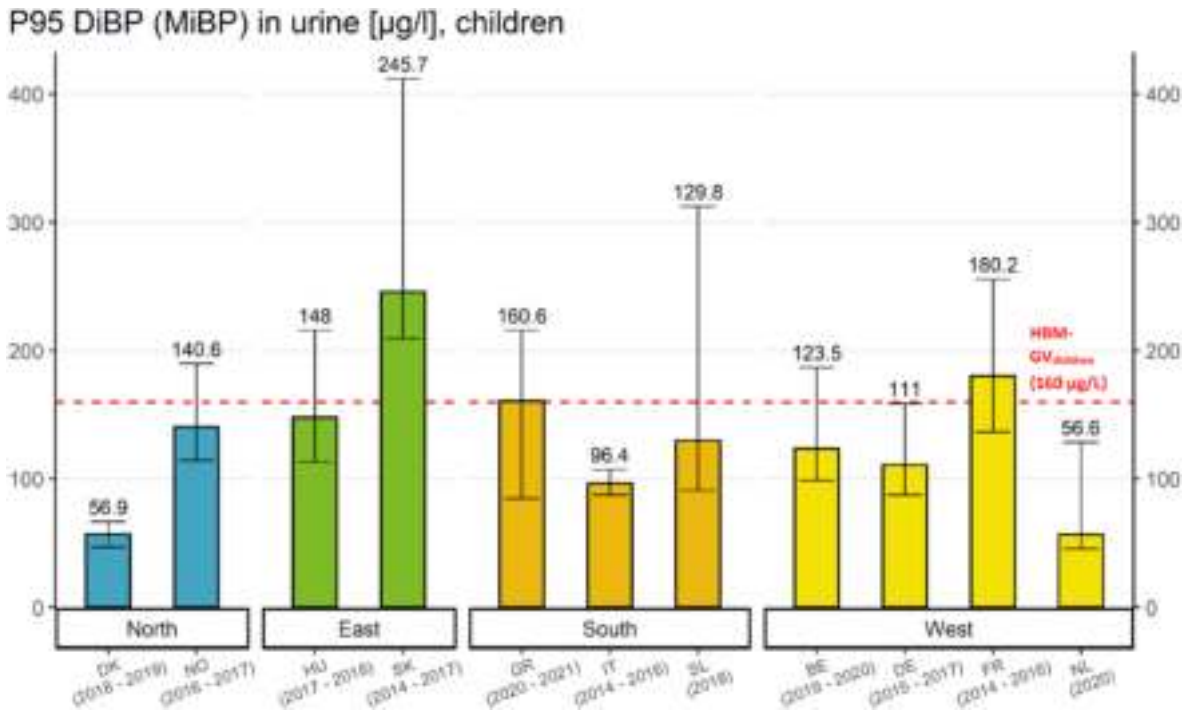

Fig. 10. Impact indicator for DiBP in children (P95 MiBP levels in µg/L with their corresponding 95% confidence intervals) in urine samples from children (age 6–11 years) from different European regions compared with the HBM-GV. The dotted line indicates the HBM-GV$_{children}$ of 160 µg/L. Studies are part of the HBM4EU Aligned Studies in children. Country names and sampling years (in brackets) are given. In three of these studies (i.e. Slovakia, Greece and France), P95 values exceed this guidance value, representing 5% of the most highly exposed children.

Table 2
Impact indicators for DiBP in children (HBM4EU Aligned Study data).

Study name, Country	N	P95 [µg/L]	Number of participants exceeding the HBM-$GV_{children}$	Percentage of participants exceeding the HBM-$GV_{children}$ [%]	Extent of exceedance [P95/HBM-$GV_{children}$]
OCC, Denmark	300	56.9	0	0	0.36
NEB II, Norway	300	140.6	13	4.3	0.88
InAirQ, Hungary	262	148.0	11	4.2	0.92
PCB cohort, Slovakia	296	245.7	36	12.2	1.54
CROME, Greece	161	160.6	9	5.6	1.00
NAC II, Italy	299	96.4	5	1.7	0.60
SLO CRP, Slovenia	149	129.8	4	2.7	0.81
3xG, Belgium	133	123.5	3	2.3	0.77
ESTEBAN, France	286	180.3	17	5.9	1.13
GerES V, Germany	300	111.1	8	2.7	0.69
SPECIMEn-NL, The Netherlands	89	56.6	1	1.1	0.35

N = number of participants.

that there is no health concern regarding DINCH exposure for children based on current data.

The extent of exceedance (EE) in the different studies and locations ranges from 0.003 up to 0.015 (see Table 3).

The impact indicator for DINCH exposure in children showed no exceedance of the HBM-GV in all considered studies. Based on current knowledge, no health effects are expected for DINCH exposure in children from these findings.

3.3. Results from workshop on policy uptake of HBM4EU results

Within the framework of HBM4EU, a workshop on „Policy uptake of HBM4EU results“ took place on 30th and March 31, 2022. During this workshop it became clear that the indicators need to be further simplified to increase general understanding. In the current state, the indicators are well suited for answering policy-related questions, but further improvement and simplification is needed to make them accessible for the general population.

Some of the highlights mentioned at the workshop were related to availability of information on source contribution, such as exposure determinants which would be relevant for policymakers and risk assessors, and to a rapid response mechanism.

4. Discussion

This paper illustrates the usefulness of the result and impact indicators for human biomonitoring in answering policy-related questions regarding DiBP and DINCH exposure and outlines strategies for their future development. Due to the European Chemicals Regulation, decision makers need a transparent and easy interpretable way of setting new research needs or further actions for chemicals. Results from the HBM4EU Aligned Studies and the previously conducted DEMOCOPHES project provided information on the chemicals exposure burden at the European level. By preparing indicators, these study results can be used to provide direct answers to policy-related questions and are thus a valuable tool for decision making. Policy needs can already be identified by applying our indicators.

4.1. Result and impact indicators aspects

The result indicator on **geographical differences** for DiBP and DINCH exposure visualizes the extent of exposure for DiBP and DINCH of children in different European regions. For both DiBP and DINCH differences in exposure burdens were observed between the different studies. For DiBP, the P50 values differed a factor of five between the highest P50 concentrations compared to the lowest values. For ΣDINCH metabolites, the differences between the highest and lowest P50 values

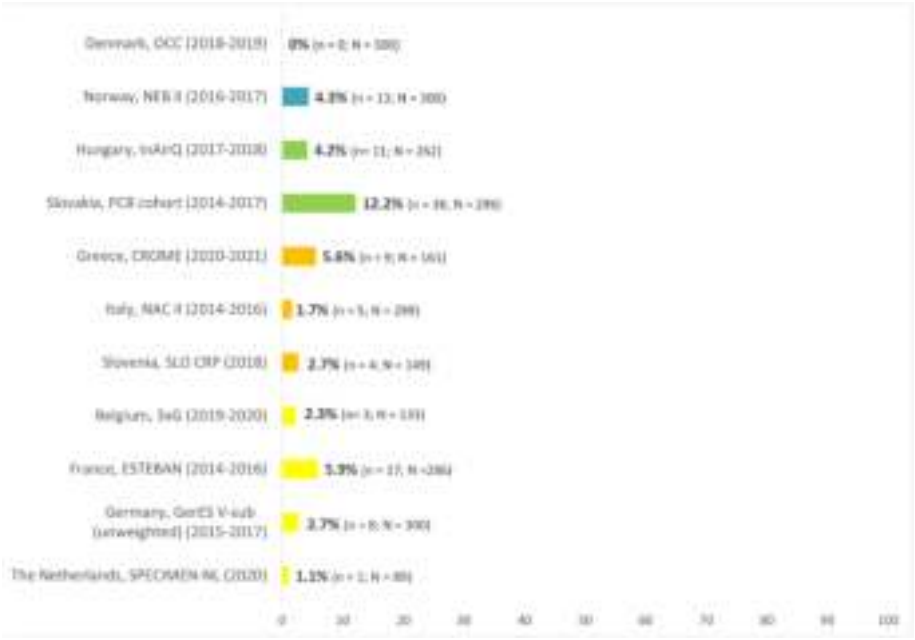

Fig. 11. Impact indicator for DiBP in children showing the percentage of children exceeding the HBM-$GV_{children}$ for DiBP of 160 µg/L. The sampling years are given in brackets after the study names. The different regions are highlighted by specific colours (north: blue, east: green, south: orange, west: yellow). n = number of samples/participants exceeding the HBM-GV, N = total number of samples/study participants. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

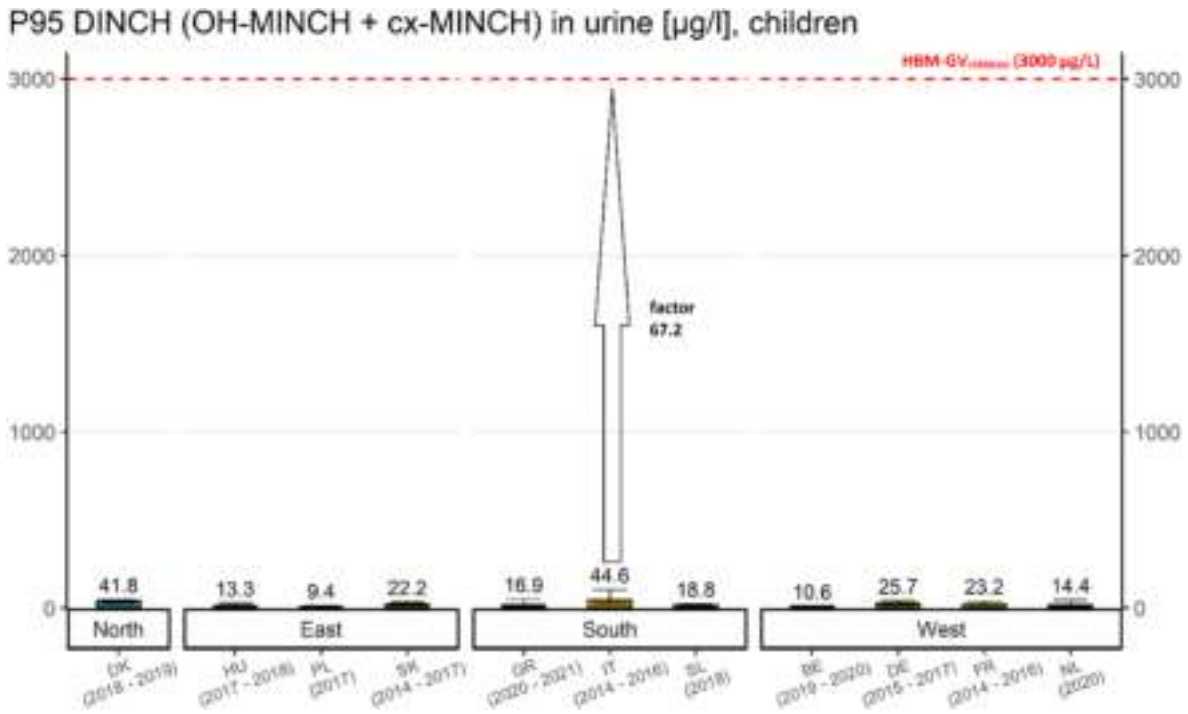

Fig. 12. Impact indicator for DINCH exposure in children (P95 ∑OH-MINCH + cx-MINCH in µg/L and their correspong 95% confidence intervals) in urine samples from children (age 6–11 years) from different European regions compared with HBM-$GV_{children}$. The dotted line indicates the HBM-$GV_{children}$ of 3000 µg/L. Country names and sampling years (in brackets) are given.

Table 3
Impact indicators for DINCH in children (HBM4EU Aligned Study data).

Study name, Country	N	P95 [µg/L]	Number of participants exceeding the HBM-GV	Percentage participants exceeding the HBM-GV [%]	Extent of exceedance [P95/HBM-$GV_{children}$]
OCC, Denmark	300	41.8	0	0	0.014
InAirQ, Hungary	262	13.3	0	0	0.004
POLAES, Poland	300	9.4	0	0	0.003
PCB cohort, Slovakia	300	22.2	0	0	0.007
CROME, Greece	161	16.9	0	0	0.006
NAC II, Italy	300	44.6	0	0	0.015
SLO CRP, Slovenia	149	18.8	0	0	0.006
3xG, Belgium	133	10.6	0	0	0.004
ESTEBAN, France	286	23.2	0	0	0.008
GerES V, Germany	299	25.7	0	0	0.009
SPECIMEn-NL, The Netherlands	89	14.4	0	0	0.005

N = number of participants.

were somewhat smaller at a factor of 2.7. Regional differences were also found within DEMOCOPHES (Den Hond et al., 2015). In the HBM4EU Aligned Studies, the observed differences between the geographical regions are higher than those observed in the DEMOCOPHES project. As the HBM4EU Aligned Studies cover the period between 2014 and 2021, and several studies have shown a decreasing time trend for phthalates and an increasing one for DINCH (e.g., Lemke et al., 2021), the described exposure differences in the geographical regions might be biased by the factor of time. This effect could be confirmed by a recent study from the German Environmental Specimen Bank showing a continuous decrease in phthalate concentrations in the years 2014–2021 (Kasper-Sonnenberg et al., 2022 in preparation). Such a possible "time trend effect" within the Aligned Studies due to the large time span of the sampling years is also discussed by (Gilles et al., 2022) . The authors recommend a shortening of sampling periods to three-year sampling periods. For phthalates and DINCH, since they are short lived chemicals, samples for these compounds should preferably be taken within the same sampling year to increase the robustness of this indicator.

Nevertheless, the result indicator on geographical differences provides information that country or regional differences exist and highlights the need to find the sources of these differences. Reasons for different exposure patterns between countries may be related to country-specific behavioral patterns, differences in the regional presence of a naturally occurring substance or differences in product placement of regional markets. These exposure determinants may help in setting targeted actions for policy makers.

The result indicator on **sex differences** for DiBP and DINCH exposure in children visualizes the extent of exposure for DiBP and DINCH of European boys and girls. The indicator shows that internal exposure for DiBP and DINCH of boys and girls from the respective regions were in a similar concentration range. Since no major differences were observed for the sexes based on current data, no political measures are currently needed to lower the exposure of one sex or the other. Despite these results, our result indicator on sex differences may help in identifying the highly exposed sex for a respective substance. Having identified a sex difference in exposure, reasons for this should then be identified via

exposure determinant studies (e.g. Martinsone et al., 2022 (in preparation)). If in other cases, differences between sexes are shown by the indicators, those differences might be related to variations in lifestyle behavior or physiological differences related to metabolism. If information is available it can help to prioritize further steps in chemical regulation or targeted education of product use for consumers.

The result indicator for **age differences** for DiBP showed that exposure to DiBP does not differ between children (6–11 years) and teenagers (12–18 years) in most studies. Vogel et al. (2022a) (this issue) analysed the individual data from HBM4EU and confirmed these findings for DiBP. However, when the authors used single years of age instead of the selected age groups of children and teenagers, they found lower levels for DiBP with increasing age. In contrast to our findings, differences in phthalate metabolite concentrations for different age groups have been reported in several studies, both when comparing children and adults but also for children and adolescents of different age groups (Bastiaensen et al., 2021; Den Hond et al., 2015; Schwedler et al., 2020a; Silva et al., 2004). In the DEMOCOPHES project, levels of phthalate metabolites were in general higher in children than in their mothers. Furthermore, younger children (5–8 years) had higher levels of phthalate metabolites compared to older children (9–11 years) (Den Hond et al., 2015). In a review paper from Choi et al. (2017), the authors found that children generally have higher body burdens of phthalate metabolites, with the exception of DEP. DEP is often present in cosmetics and a major part of the exposure to DEP is via personal care products (Wormuth et al., 2006), which might explain the higher exposure in the group of teenagers. One reason why an age dependency across all studies could not be shown for DiBP with the HBM4EU Aligned Studies might be due to the wide distribution of ages in the single studies (Gilles et al., 2022). In the HBM4EU children's group not all ages are represented equally. The same is the case for teenagers. Further the age range with children aged 6–11 years and teenagers aged 12–18 years also includes some "overlapping" of 12-year-old in the group of children (i.e. 3.9% of the children were 12 years old (Gilles et al., 2022). This had happened since some 11 year old children turned 12 in the course of the respective studies. Furthermore, very young children (<6 years) were not included. Since aggregated data were used for the derivation of the indicator, other age groups could not be constructed out of the available data. For infants and toddlers, different behavior patterns like crawling on the floor and hand-to-mouth behavior are well known. Also, the food intake in comparison to the body weight is much higher for the very young age groups (US EPA, 2011). Teenagers, on the other hand, are exposed via their usage of specific personal care products (Wormuth et al., 2006). Not all studies within the HBM4EU Aligned Studies provided data for both children and teenagers. Another influencing factor that may lead to an over- or underestimation is the wide range of sampling years (as has already been discussed above for the indicator on geographic differences). Here, time trend studies for both children and teenagers would be needed to adjust for possible time effects.

For DINCH, our result indicators on age differences revealed higher exposure of children compared to teenagers. An age dependency of DINCH exposure was also observed by Schwedler et al. (2020b), who reported HBM data from the German Environmental Survey of Children and Adolescents (GerES V, 2014–2017). The authors reported the highest geometric mean (GM) biomarker concentrations for the youngest children, while the concentrations decreased with increasing age. DINCH biomarker levels in children (3–5 years) were almost 3-fold higher than in teenagers (14–17 years) (Schwedler et al., 2020b). DINCH has been developed for sensitive applications, such as children's toys, as young children likelyhave the closest contact with these. This assumption of increased exposure to toys could be confirmed by our indicator in the group of children.

To summarize, the result indicator for age differences is suitable to highlight potential differences in exposure for different age groups. For DINCH and DiBP (in two studies) a higher exposure of children could be shown. The next step would be to determine the reasons for this higher exposure. Age differences may be related to differences in lifestyle behavior or differences in metabolism. Those reasons are relevant to know for taking further steps in chemical risk management.

Further, with our indicators on age differences, we could demonstrate the necessity of a valid database for the derivation of this indicator. Comparable data are a prerequisite for mapping existing differences.

To inform on **temporal trends**, the HBM4EU Aligned Studies were compared to the earlier DEMOCOPHES project (2011–2012). This so-called "time pattern" indicator revealed lower levels of phthalate biomarkers and higher levels of DINCH biomarkers for children in the HBM4EU Aligned Studies. A decrease in DiBP metabolite levels (and metabolites of other regulated phthalates) in children and adolescents over time was also observed by Schwedler et al. (2020a). This decrease over time might largely be due to regulatory measures such as restrictions and regulations in consumer products being in place and being effective, as was also confirmed in time trends from the German Environmental Specimen Bank (Koch et al., 2017; Apel et al., 2020b) and studies from other European countries (e.g. Frederiksen et al., 2020; Bastiaensen et al., 2021).

DINCH data from the literature also reported an increasing time trend of DINCH biomarkers (e.g. Kasper-Sonnenberg et al. (2019), Lemke et al. (2021) and Schwedler et al. (2020b)). Lemke et al. (2021) reported that substitutes mimic the exposure behavior of REACH regulated phthalates. As a shift to non-regulated phthalates or substitutes (such as DINCH) takes place, an increase in measured concentrations for these substitutes can be followed. Vogel et al., 2022b (this issue) confirmed an increasing time trend for DINCH when analyzing HBM data from Denmark and Germany, including samples collected at different time points. The "time pattern" indicator is suitable for revealing differences in internal exposure over time. If lower concentrations are observed, this might be an indication that political measures were successful and effective, or that consumer use is decreasing or that maybe industry has changed their application. These changes in exposure need to be analysed to set priorities for further actions.

The **impact indicators** on health relevance of DiBP and DINCH exposure in children and teenagers confirmed their relevance for comparing exposure levels to existing guidance values and enable policy makers to set priorities for further actions. The impact indicators showed that even when single substances are assessed, the HBM-GV is exceeded for DiBP in some regions by a considerable number of participants even in the most recently collected data. This is a flag for risk managers and policy makers that exposure to DiBP still is a relevant health issue.

Despite regulations, bans and restrictions being in force for several phthalates, the impact indicator showed that there are still children with urinary phthalate levels exceeding the HBM-GV for DiBP. This is of particular concern since phthalates can act in a dose additive manner.

It should also be kept in mind that health-based guidance values, like the HBM-GVs may be revised when new scientific findings on dose-related health effects are available. Currently, the level of confidence (loC) for the derivation of HBM-GV for DiBP is low, whereas for DINCH the loC is medium. This is because the derivation of both is based solely on animal studies. A good example for this is the lowering of the tolerable weekly intake (TWI) for the combined exposure to some perfluoroalkyl substances (PFAS) due to new toxicological findings (Schrenk et al., 2020).

A continuous monitoring of phthalate exposure and their substitutes should be implemented to allow for a continuous monitoring of exposure levels of the population in Europe and to assess the effectiveness of new regulatory measures and to avoid regrettable substitution.

4.2. Limitations

Limitations regarding our indicators are mainly focused on data quality aspects and the availability of data. When using indicators for decision-making, the demand for data quality such as

representativeness, reliability and comparability of data comes to the fore.

It has to be noted, that in a few exceptional cases, data were used for the derivation of indicators that were not quality assured within HBM4EU (Gilles et al., 2021;Govarts et al., 2022). Theses include 1) MiBP measurements for children in the PCB-cohort study (SK) and 2) MiBP measurements for teenagers from CELSPAC:TE (CZ) and the PCB cohort follow-up (SK). All data were included in the analysis of exposure determinants and risk assessment.

It was not possible to derive indicators for socioeconomic status (SES), as data on these were not available in a sufficiently large number of studies. However, many scientific studies (Den Hond et al., 2015; Bastiaensen et al., 2021; Schwedler et al., 2020a) have shown that socioeconomic status (indicated as educational level in some studies) can be a determinant of exposure to phthalates.

Another important aspect is the collection of urine samples for phthalate exposure. Phthalates are rapidly excreted via urine and do not circulate for a long time in the human body. After a single oral dose in experimental studies DEHP and DiNP (Diisononyl phthalate) metabolites indicated that around 50% were excreted in urine after 24 and 48 h, respectively (Koch et al., 2005; Koch and Angerer, 2007;). Based on this relatively short half-life and rapid urinary excretion, it has to be kept in mind, that a single urine sample represents only the more recent exposure. Therefore, the assessment of spot and morning urine samples may lead to an over- or underestimation of internal exposure due to intra-individual variation over the day (Mok et al., 2022). Nevertheless, it has been shown for some phthalates that compared to the best case of a 24h-urine sample, the best comparability comes from morning urine samples (Frederiksen et al., 2013). Spot urine is less comparable as it can be collected at different time points during a day. However, sometimes the only available matrix in some studies is spot urine. In the HBM4EU Aligned Studies, urine samples were either first morning urine samples or spot urine samples. Urine samples taken in the frame of the DEMOCOPHES project in children were all first morning urine samples. Uncertainties regarding the determination of phthalate exposure of first morning versus spot urine samples needs to be considered.

Due to the wide use of phthalates, humans are exposed to a variety of these compounds simultaneously (Husøy et al., 2019). Mixtures of some of the selected phthalates can have direct combined effects (Howdeshell et al., 2017; Kortenkamp and Koch, 2020) as well as combined effects with other endocrine disrupting chemicals (Howdeshell et al., 2017; Kortenkamp 2020; Runkel et al., 2022). Therefore, mixture risk assessments (MRAs) have been performed for the cumulative risk of these compounds (e.g. Apel et al., 2020b). The importance of mixture risk assessment (MRA) is also addressed in several communication papers[4] from the European Commission, and is also part of the European Chemicals Strategy for Sustainability[5] (Bopp et al., 2018; Socianu et al., 2022). Within HBM4EU, chemical mixture risk assessment was also carried out for prioritized groups of chemicals, among them the phthalates. (Lange et al., 2022) (this issue) performed a mixture risk assessment of five selected phthalates (i.e., BBzP, DEHP, DiBP, DnBP and DiNP) and found that approximately one sixth of the European children and teenagers is at risk from adverse effects of combined exposure to these 5 phthalates. The mixture risk for the majority of children and teenagers would have gone unnoticed in single substance risk assessment. But in this study only single substance assessments for the derivation of indicators have been used and combination effects are not considered within our indicators at current state.

4.3. Future activities identified from workshop

During the workshop, two important topics were identified that are to be further processed within the future work on the indicators.

a) Wish for simplification to increase general understanding

When using a simplified document targeted to the citizens, valuable information may be lost. Therefore, all information required for an explanation of the indicators has to be added in text boxes and adequate visuals have to be provided to summarize the statistical results. Further efforts are needed to convey a more informed message.

b) Availability of information on source contribution influencing exposure

Information on relevant sources of phthalates is currently not integrated in our indicator concept. It is planned that also main sources of exposure to chemicals will be identified and shown in the form of indicators. This information may help to create targeted information materials that may help to minimize exposure.

5. General conclusions

The first set of pan-European HBM indicators for phthalates and DINCH provide a valuable tool to highlight differences in exposure between geographical regions, age groups, sexes and periods of time, but also to assess health impacts by comparing exposure values with HBM-GVs. The corresponding policy-related questions developed under HBM4EU could be answered by our indicators.

The HBM indicators can be compared with existing indicators based on environmental data, consumption data and food datasets. They are aimed at complementing other sets of indicators in the field of environment and health (EEA) and on human health (WHO).

With these indicators, a first step has been made for processing HBM findings in a clear and comprehensible manner. Further steps are needed to further simplify these messages, possibly in an interactive graphic design, as it has been done for the indicators which present blood lead levels of children by the U.S. EPA (US EPA, 2022).

Therefore, the HBM4EU indicators demonstrate that they are useful tools for a direct and simple interpretation of HBM data that can help policy makers to answer policy-related questions for the respective compounds and thus enable them to set priorities for further actions on their way to a toxic-free environment under the EU's chemicals strategy for sustainability (Ganzleben et al., 2017).

Lessons learned for deriving HBM indicators comprise aspects mainly related to data quality, namely:

a) analytical methods should be quality assured and comparable between the studies/countries
b) sampling should take place within a narrow time frame, especially for substances with a short half-life
c) homogenous population groups should be compared to enable the mapping of existing differences for regions, sex or age groups
d) samples should be representative for the parameter investigated: to map country differences a representativeness at national level should be the aim, to map differences between age groups these should be representative for the country
e) exposure should be continuously monitored to enable time trend observations for the whole of Europe and thus create a database for deriving real "time trend indicators" at the European level
f) the latest scientific findings and policy demands have to be considered, which also includes consideration of combination effects

[4] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52012DC0252.

[5] https://ec.europa.eu/environment/pdf/chemicals/2020/10/Strategy.pdf.

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Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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HBM4EU from the Coordinator's perspective: lessons learnt from managing a large-scale EU project

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ABSTRACT

We discuss some important management issues of the Human Biomonitoring Initiative (HBM4EU) from the perspective of the Coordinator that may be valuable for the design and management of similar projects. As a large-scale international collaborative project, HBM4EU comprised 118 institutions from 30 countries and the European Environment Agency and had a budget of about €74 million. It has set up an innovative cooperative network of national and EU authorities and scientific institutions at the science-policy interface. A project of this scale raises major management challenges and requires transparent, efficient, and well-organized administrative and scientific steering structures. We present four major points: *First*, prior to the beginning of the project, the Consortium Agreement needs to be well elaborated to prevent conflicts during the project lifetime. *Second*, a strong role for national and EU policy-making authorities in the administrative governance structure enhances the interest of recipients of project results. *Third*, large-scale international collaborative projects need an elaborate and well-financed scientific governance structure. *Fourth*, a differentiation of funding rates among project activities threatens to create conflicts. HBM4EU provides a prototype for EU funded large-scale projects targeting future policies for realizing the Green Deal and Zero Pollution Ambition in the field of chemicals, health, and environment.

1. Introduction

The European Human Biomonitoring Initiative (HBM4EU) started in 2017 and ended mid-2022. As a large-scale multi-national project, it focused on science-to-policy cooperation, broad data sharing and the systematic establishment of networks in the field of Human Biomonitoring (HBM) at national, European, and international levels (Ganzleben et al., 2017). Over a period of five and a half years, HBM4EU has produced new methods and findings that provide a scientific basis for policy making in the sector of environmental health and chemical policy. HBM4EU is unique in its form. It was the first HBM project located directly at the science-policy interface and had developed an ambitious research programme targeted to answer open policy relevant questions concerning prioritised chemicals, which had been identified by EU institutions and partner countries. The project was organized as a co-funded European Joint Programme (European Commission, 2022), designed to support coordinated national research and innovation projects and allowing for the implementation of joint activities, e.g. research and innovation, networking and training. HBM4EU provides a blueprint for the even larger Partnership for the Assessment of Risk from Chemicals (PARC) and other EU funded large-scale projects targeting future policies for realizing the Green Deal and Zero Pollution Ambition in the field of chemicals, health, and environment.

A project of this scale raises major management challenges. HBM4EU had an overall budget of nearly €74 million, of which roughly €50 million were funded by the European Union's research and innovation funding programme Horizon 2020. EU funding was complemented by roughly €24 million matching funds from participating countries. The project started in 2017 with 106 partners from 26 countries and the European Environment Agency (EEA). By the end of its lifetime, it had grown to 116 partners from 30 countries plus the EEA. It included more than 600 collaborators, mostly scientists from public authorities, research institutions and universities. The German Environment Agency was appointed as Coordinator of HBM4EU.

In this article, we discuss some management issues of this large-scale, international collaborative project from the perspective of the Coordinator that may be valuable for the design and management of similar projects. While numerous substantial insights produced by HBM4EU and

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other projects are reported elsewhere, there is sparse information concerning experience made in the management of such large-scale co-funded EU initiatives. The project has produced many valuable lessons that may be useful for future initiatives. In the field of HBM, literature on previous projects mostly describes scientific results or the management of data and work content (Den Hond et al., 2015; Fiddicke et al., 2015). Thus, this paper differs from many reports of EU projects. It discusses some "lessons learnt" from the management of HBM4EU from the Coordinator's perspective. It focuses on four management issues, which might be of particular interest for future initiatives, namely the preparatory phase, the administrative governance structure, the scientific governance structure, and financial matters.

We develop four major points: *First*, prior to the beginning of the project, the Consortium Agreement needs to be well elaborated to prevent conflicts during the project lifetime. *Second*, a strong role for national and EU policy-making authorities in the administrative governance structure enhances the interest of recipients of project results. *Third*, large-scale international collaborative projects need an elaborate and well-financed scientific governance structure. *Fourth*, differentiation of funding rates among project activities threatens to create conflicts among project partners.

2. Preparatory phase: negotiating the Grant Agreement and the Consortium Agreement

Transparent and fair decision-making was essential for joint work in HBM4EU, even before the project started in 2017. Based on the Horizon 2020 guidelines, HBM4EU was contractually anchored in two agreements, namely the Grant Agreement, signed by the EU and the project Coordinator, and the Consortium Agreement signed in 2017 by then 38 Grant Signatories. The number of Grant Signatories increased over time to 41, to which another 77 organizations were associated as Linked Third Parties.

Both the European Commission and the Coordinator encouraged the participating countries to name only one agency each as Grant Signatory, while other national institutes could be included as Linked Third Parties. This organizational structure was intended, on the one hand, to promote cooperation among different agencies and research institutions within a country, rather than advocating their own organizational needs in the consortium. On the other hand, it reduced management complexity by limiting direct interaction between the Coordinator and the Management Board on the one hand and the Grant Signatories on the other hand. The Grant Signatories would assume responsibility as lead agencies for all Linked Third Parties of their country involved in HBM4EU. This three-tier organizational structure (see Fig. 1) proved to be successful and did not create any specific problems.

The content of the Grant Agreement was largely pre-determined by, or negotiated with, the European Commission as the funding authority. The Grant Agreement defined the legal rights and obligations of the funding authority and the Grant Signatories. HBM4EU used a model Grant Agreement provided by the European Commission (European Commission, 2014). Options in the model contract itself were selected according to the envisaged actions, which limited opportunities for proposing modifications. It its annexes, the Grant Agreement (HBM4EU, 2016b) outlined further structures (including budget and work package descriptions for the entire project runtime as well as descriptions of all partners, milestones and deliverables) for the work of the initiative.

In contrast, the Consortium Agreement (HBM4EU, 2016a), an agreement between the Coordinator and all Grant Signatories defining roles, rules and responsibilities, provided ample room for negotiations within the project. The Consortium Agreement relied on the DESCA Horizon 2020 Model Consortium Agreement (DESCA Core Group, 2016), which provided suggestions for numerous formal provisions that were mostly kept. However, the provisions of the template were considerably expanded to tailor the Agreement to the specifics and needs of the HBM4EU consortium. The set-up of the Consortium Agreement was an integral tool to establish basic conditions for the cooperation within the consortium, especially for preventing misunderstandings and conflicts that potentially could have arisen over time between the numerous partners in such a large-scale initiative.

From the Coordinator's point of view, two aspects were essential when elaborating the Consortium Agreement: *First*, with the Consortium Agreement we endeavored to avoid postponing many issues that had to be addressed during the project's lifetime and sought to regulate them already in advance. Hence, the Agreement became a lengthy document that regulated in some detail, inter alia, the responsibility of partners, liability and financial provisions, the administrative governance structure of the project, the scientific governance structure, governance bodies and their decision-making procedures (including agenda setting and deadlines for availability of documents), as well as issues of data protection and the exchange of (partly sensitive) HBM data, data dissemination and access rights, etc. We will discuss some of these topics below. The preparatory phase proved to be time-consuming, but the negotiation of project rules and obligations en bloc supported agreement, because all sides had to compromise. The detailed Agreement facilitated the day-to-day management of the project, and helped avoid conflicts and cumbersome debates at later stages of the project. Moreover, it provided the Grant Signatories with certainty on how the project would be conducted. The Consortium Agreement had to be signed by all Grant Signatories as a precondition for participation and also became binding for the Linked Third Parties. It did not have to be significantly amended during the lifetime of the project, thus reflecting the low level of conflict on project governance.

Second, negotiation of the Consortium Agreement was conducted in a highly transparent and fair manner. Agreement drafts were widely circulated and comments and proposals for revisions were communicated openly and accompanied by indicating the response action, i.e. whether the draft was amended or not, and in the latter case, the respective reasoning. In case of disagreement, meetings were held on short notice to facilitate finding solutions suitable for all involved parties.

Fig. 1. HBM4EU organizational structure.

3. The administrative governance structure

The Consortium Agreement defined the administrative governance structure and established the following bodies for HBM4EU (see Fig. 2):

- The **Governing Board** as the ultimate decision-making body of HBM4EU;
- The **Management Board** as the operative body for the execution of HBM4EU, which reported and was accountable to the Governing Board;
- The **Stakeholder Forum** providing opportunities for stakeholders to feed in their knowledge and perspectives on priority setting and implementation of HBM4EU;
- The **Advisory Board** providing scientific and policy advice;
- The **Ethics Board** providing advice on the ethically correct conduct of HBM4EU.

The *Governing Board* was the project's supreme decision-making body. It comprised the programme owners of the national programmes engaged in the HBM4EU Initiative as well as two EU agencies with particular interest in HBM, namely the European Chemicals Agency (ECHA) and the European Food Security Agency (EFSA) – but not the Grant Signatories. The national programme owners were superior public authorities (often national ministries) from the participating countries, which steer and finance national HBM studies and research programmes of their countries. Bringing in these programmes and respective data as background was a prerequisite for participation in HBM4EU. The Governing Board made the most important decisions on an annual basis, including adoption of the Annual Work Plan, project budget, intellectual property rights, and the evolution of the consortium. There were two main reasons for assigning these decisions to the Governing Board. *First*, this body comprised those member state authorities that were responsible for financing the matching funds of 30 percent of the whole project budget. *Second*, these national institutions and the two European Agencies were national and European regulators that would make use of HBM4EU results and data. The alternative had been to involve these actors in HBM4EU via an advisory body. By giving them the opportunity to decide on critical matters as member so of the Governing Board, they gained more interest in the project and were directly involved in setting project priorities, as reflected in the Annual Work Plans and progress reports to the European Commission. This arrangement proved to strengthen the science-policy interface of HBM4EU tremendously, as indicated by the relatively high level of representatives.

To establish the Governing Board as the supreme decision-making body, some institutional arrangements had to be made.

The members of the Governing Board were not those agencies receiving research money and doing the actual project work, and they were not signatories of either of the two project agreements. The Grant Agreement was signed by the Coordinator (German Environment Agency) and the funding agency (European Commission), while the Consortium Agreement was signed by the Grant Signatories. This raised the question of how the Governing Board could be authorized to make major project decisions. As a solution, the Consortium Agreement stipulated to assign this authority to the Governing Board. By this arrangement, the Grant Signatories as the contracting parties to the Consortium Agreement delegated some decision-making authority to the Governing Board and accepted decisions of the Board as binding. To commit the Governing Board to the Grant Agreement and the Consortium Agreement, acceptance of Governing Board decisions as binding for the project was limited to decisions being made in accordance with the two founding agreements. In practice, this arrangement was successfully implemented and did not create any specific problems for the project.

Furthermore, five countries did not manage to designate a single national authority as national programme owner and member of the Governing Board. Three countries designated two authorities and two countries even three. The main reason was that several ministries, e.g. health, environment, and research, were involved in national activities related to HBM4EU and did not manage to agree on a lead authority. In these cases, countries were represented by more than one member, but did only have one joint vote and had to indicate, which of their members would cast that vote. It made voting procedures more complex, but did not pose any serious problems.

As a corollary of establishing the Governing Board as the supreme decision-making body, HBM4EU did not have a body comprising the Grant Signatories. Given that major decisions were made by the

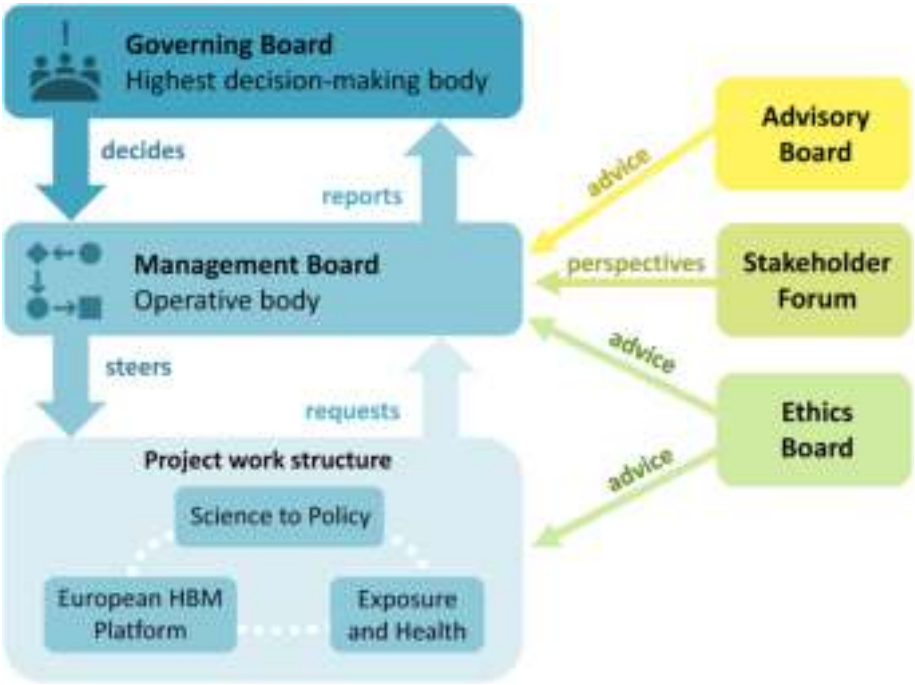

Fig. 2. HBM4EU governance structure.

Governing Board and day-to-day decisions by the Management Board, a body of Grant Signatories was deemed to have no function within the administrative structure of HBM4EU. However, the Grant Signatories played a major role in the scientific governance structure of the project. At some points, concerns were raised that Grant Signatories carried financial responsibility for their institutions and their Linked Third Parties without having a voice in administrative decision-making. Rather, they had to voice specific interests or needs through their country's members in the Governing Board. However, during the lifetime of the project, the lack of direct representation of Grant Signatories did not create any single problem brought to the attention of the Coordinator.

The *Management Board* was the main operational body of HBM4EU. It comprised the leaders of the fifteen work packages, as well as pillar leaders and the Coordinator and Co-coordinator, all of which were also Work Package Leaders. Since some persons led two work packages, the Management Board consisted of thirteen members and met roughly six times per year. Until the Covid-19 crisis forced a switch to virtual or hybrid meetings, these meetings were usually held in person. The main task of the Management Board was the preparation of the Annual Work Plan and the Annual Summary Progress reports as well as amendments to the Grant Agreement, all of which were submitted to the Governing Board for adoption. The Management Board also adopted decisions on many other issues, such as publications and the allocation of the reserve budget. It also discussed proposals for new activities and proposed the candidates for various boards, to be appointed by the Governing Board. Some of its decisions, especially those with implications for the use and allocation of resources, bore the potential for tension in the consortium. A transparent and structured decision process was therefore required.

To structure the decision process and enable the Management Board to take rapid and informed decisions, decision proposals submitted by the Coordinator or any other board member were prepared by standardized "Decision Memos", which provided a simple description of the decision asked for by the petitioner (see Table 1). Decision Memos were primarily used to facilitate proposals to decide on changes of the five and a half year work plan (Description of the Action) and the Annual Work Plans, which had implications for project activities and resources, as well on project publication initiatives. They required petitioners to justify changes to proposed activities and related resources, thus making the implications transparent. The use of Decision Memos available in time before a meeting as a preparatory tool for Management Board decision-making proved to be highly successful, as it ensured well-prepared and transparent decisions. Thus, it allowed participants to review proposals in advance and coordinate with partner institutions; and it precluded ill-prepared ad hoc decisions. The preparatory tool of Decision Memos was adopted later by the Governing Board. It is highly recommendable, especially for larger-scale projects with multi-player boards in charge of decision-making. To avoid repeated discussion on its usefulness, Decision Memos should be provided for in the Consortium Agreement.

Table 1
Structure of a Decision Memo as used in HBM4EU Management Board and Governing Board.

Section No.	Section	Question to be answered in this section
1	Cause and purpose	• Why does this decision need to be taken by the board? • What exactly is the issue the board is asked to decide upon?
2	Current status and background information	• What is important background knowledge required for the board's informed decision? • What is the current status of the issue?
3	Proposal for solutions	• Which solutions can be proposed for the aforementioned issue?
4	Consequences	• What are the consequences of the proposed solutions (e.g. financial impact)?
5	Vote/recommendation	• What is recommended for the board to agree upon?

The *Advisory Board* played an important role in the project. It comprised members from international organizations and EU agencies, key players of international HBM studies from the US, Canada and Japan, and experts from various research areas related to HBM4EU. The Advisory Board was actively used to obtain input in and feedback on project activities. Repeatedly, Pillar Leaders asked the board for advice on the appraisal of progress achieved, remaining gaps, and perspectives for subsequent activities. The advice given has been implemented in HBM4EU activities and responses were reported back to the Advisory Board with requests to discuss their appropriateness. The interdisciplinary input of the Advisory Board has considerably improved HBM4EU activities. Moreover, the board supported the international outreach of the project to numerous institutions involved in related activities.

The *Ethics Board* consisted of several ethics experts and was consulted when advice was needed. It supported the task lead in making all partners aware of national ethics requirements and the EU General Data Protection Regulation (GDPR), which entered into force during the project's lifetime. It had an advisory and oversight role, while national contributions for studies still had to obtain separate ethical agreements prior to the respective activity.

The *Stakeholder Forum* was used to inform various stakeholders about, and enable them to comment, on HBM4EU activities. The forum comprised a broad range of members of different backgrounds, such as non-governmental organizations and industry associations. It enabled the HBM4EU project to realize the perspectives and needs of the represented stakeholder groups and design communication and dissemination strategies accordingly.

An important component of communication within HBM4EU was the so called "meeting week". All project bodies, with the exception of the Management Board, met generally once a year back-to-back during the same week. One important part of this meeting week was the meeting of the Consortium, in which all project partners discussed content-related issues and held work package meetings. The format of a meeting week was highly successful because it provided the prime opportunity for project partners and members of administrative bodies to meet and interact. Scheduling meetings in a single week also saved travel time and expenses.

4. The scientific governance structure

A science-to-policy project of this size and complexity cannot be effectively steered by a single person; it needs a scientific governance structure in addition to the administrative governance structure. HBM4EU included a wide range of scientific activities and required expertise from various disciplines. Many activities were based on highly specialized knowledge, such as conduction and interpretation of epidemiological studies, targeted and non-targeted chemical analysis of exposure in human blood and urine, computational modelling of exposure and intake of chemicals by human beings, investigation of effects by systematic derivation of effect markers and adverse outcome pathways, assessment of mixtures of chemicals and their adverse effects, science-to-policy transfer of results, and communication with policy makers and stakeholders. Guidance and supervision of these activities, as well as the control of the quality of products were key scientific tasks with a strong relevance for reaching the overall project goals. They determined the extent to which project results would be useful for policy-making and regulation.

HBM4EU had a pyramidal scientific governance structure (see Fig. 3). It was organized in the form of three pillars, which dealt with science-to-policy issues, HBM studies, and exposure and health studies. Every pillar comprised four to six work packages, and every work

Fig. 3. HBM4EU scientific governance structure (schematic).

package was composed of several tasks. This structure reflected the idea of a clear hierarchical responsibilities. Pillar Leaders were responsible for all activities within their respective pillar. Under their authority, Work Package Leaders were responsible for the sub-set of activities within their work package. Under their authority, Task Leaders in turn were responsible for the subset of activities within their respective tasks. Hence, Task Leaders reported to their respective Work Package Leaders, which reported to their respective Pillar Leaders, which reported to the Coordinator. This applied also to quality control and approval of products and deliverables, as a precondition for the release of funding.

Outside of this hierarchical structure, Chemical Substance Group Leaders were responsible for integrating diverse insights on specific groups of chemicals elaborated in different pillars, work packages, and tasks. HBM4EU focused on answering open questions on priority substances relevant for policy-making and regulation identified by EU institutions and HBM4EU partner countries. These questions did not fall into a single task or work package. The task of the Chemical Substance Group Leaders was to encourage interaction across all work packages and to integrate results. It was highly important for the project as a whole and relied heavily on the output of relevant tasks and work packages.

Likewise, outside the hierarchical structure, National Hubs contributed to building a long-term HBM European Programme and infrastructure by bringing together national HBM activities. They were coordinated by a National Hub Coordinator and fed their domestic needs into the European process. Thus, they contributed to the objectives of HBM4EU and learned from the work carried out in the project. This approach enhanced coordination in countries, in which no systematic coordination for activities in the field of HBM had existed before.

Altogether, this scientific governance structure worked quite well. Most Pillar Leaders, Work Package Leaders and Task Leaders, as well as Chemical Substance Group Leaders took their responsibility serious. They coordinated and integrated the activities in their respective areas actively and supervised the timely submission and quality of deliverables effectively, with a view to realizing the overall goals of the project. They heavily contributed to the success of the project. As a result, HBM4EU delivered quality assured HBM data from across Europe, which establishes the baseline for the assessment of the EU chemical policy strategy and its success. HBM4EU also established a broad dialogue with policy-makers, stakeholders, and the wider public, including dissemination of project results through a broad variety of communication channels. However, the activities of the Pillar Leaders, Work Package Leaders and Task Leaders relied heavily of the commitments of individuals to the overall goals of HBM4EU and to fulfilling their steering responsibilities within the project.

In retrospect, HBM4EU had allocated far too few resources to scientific steering activities. This was an unfortunate result of the widely shared endeavour to limit the administration and coordination costs of the project to a minimum. However, the scientific governance of an international and interdisciplinary project of the size of HBM4EU is in itself a scientific, not an administrative task, and it influences the overall project success heavily. After all, its pillars and work packages in themselves are comparable in size to many other EU projects as a whole. Well-structured scientific governance helps to use allocated resources effectively and in accordance with the project objectives. In practice, Pillar Leaders and Work Package Leaders were funded in an amount of few person months per year. This meant that they could not invest all or most or their work time in project steering activities, which then proved to be insufficient. Chemical Substance Group Leaders were also underfinanced, which proved to be particularly difficult for those responsible for big substance groups comprising numerous substances, such as pesticides or plasticisers.

To strengthen the scientific leadership tasks structurally, incentives for individuals to fulfil their responsibilities properly should be reinforced. This might be realized in a number of ways. *First*, Pillar Leaders and Work Package Leaders could be assigned separate tasks of producing deliverables that integrate findings from more detailed activities within their areas of responsibility. For HBM4EU, it could have meant producing deliverables that compare findings across national studies or compare HBM data with results of exposure modelling. Integrated tasks should be carefully defined in the Description of Action and in Annual Work Plans. This would create a scientific interest, rather than mainly an administrative one, in steering and integrating activities. *Second*, a larger amount of funding should be allocated to the expanded scientific leadership functions (in contrast to purely administrative coordination). Pillar Leaders, and possibly Work Package Leaders, should invest most of their working time in the project, rather than in activities beyond the project. Depending on the extent of their integrative tasks, this might imply funding of senior scientists as assistants or small working groups, depending on the extent of the task. *Third*, scientific leadership tasks should be carefully defined in the Consortium Agreement to avoid conflicts about roles and responsibilities later on.

5. Financial matters

Budgetary issues are always of utmost concern for all project participants and bear high potential for conflicts within a consortium, unless clearly regulated from the beginning. The general allocation of funding for the project was defined in the Description of Action as part of the Grant Agreement, agreed upon with the European Commission. It was further specified in the respective Annual Work Plans. However, the need for resources could not always be exactly pinpointed in advance. Moreover, some tasks were not realized for different reasons and others were added later on, so that some resources were reallocated through the Annual Work Plans. Accordingly, many budgetary details remained open and needed to be decided on during the project lifetime. Detailed procedures and criteria for preparing and deciding on such changes to resource allocations can facilitate agreement and at the same time avoid conflicts and competition over these resources. Such procedures should be defined in the Consortium Agreement and comprise detailed

guidance for the continuous and transparent monitoring of budgetary issues and regular budget reviews provided by the Coordinator.

Two specific issues should be mentioned. First, the HBM4EU budget was determined as a total sum in Euros, while the allocation of funding of project staff to participating institutions occurred in the so called "Person Months" as predetermined by the European Commission. Of course, there are good reasons for this system. Person Months provide a simplified solution when planning activities in order to reach project objectives, because they are directly related to the work done by a given person in a month. Moreover, Person Months account for the differences in salary-levels of the participating countries. However, we did not translate the amount of Person Months assigned to a given institution for their project activities into a fixed amount of funding in Euros. This meant that any amount of costs per Person Month could potentially be eligible as long as the respective partner could provide proof of the actual costs. In some cases, this created claims beyond estimated costs. In other cases, actual cost claims were lower the approximated sum allocated to them at the beginning of the project, but project partners expected that the whole sum would be available for them during the entire runtime of HBM4EU. Even more important, the final budget per partner could only be estimated at the end of each reporting period when costs had been claimed. To avoid these budgetary issues, consortiums should find ways for translating calculations made in terms of Person Months into fixed budgets in Euros, which will remain set for the participating institutions throughout the project runtime.

Second, project internal funding rates assigned to different activities were a particular source of budgetary conflict. HBM4EU had an overall funding rate of 70% from the EU research programme Horizon 2020 and 30% matching funds from the member states. However, there was agreement that some coordinating activities should have a funding rate of 100%. To compensate for a selective 100% funding rate and still stay within the overall project budget, all other activities had to receive somewhat less than 70% of EU funding. Therefore, the consortium agreed to and defined in the Consortium Agreement several categories of differing internal funding rates. For example, concept development was funded at 70%, while the conduct of HBM studies received only 50 percent of EU funding, as these studies were typically also for the benefit of the respective member states. However, the boundaries of categories were subject to interpretation and, accordingly, led to some conflict. To prevent such conflict, categorization of activities with different internal funding rates to compensate for 100% funding of coordination should be avoided. A single funding rate for all other activities would have precluded these conflicts.

6. Conclusions

HBM4EU has created an innovative type of project focusing on the science-to-policy interface. It was conducted to support policy-makers and regulators with targeted research results and the data needed at EU and national levels for setting priorities and regulation for the management of chemicals. As a major innovation, it heavily involved policy-makers in all project phases, starting early on with the preparation phase and involving them until the project closure. It aimed to identify open policy-relevant questions and to develop a demanding research plan to answer these questions. This process was accompanied by a continuous dialogue between scientists, regulators and policy makers which established and strengthened cooperation among these groups. Another innovative step was the creation of an EU-wide network of national and EU agencies, research institutions, universities, and stakeholders. This network was interlinked with national networks coordinated via the National Hubs and resulted in a new level of shared agreement on the meaning and interpretation of data and results as well as on the health relevance of the exposure of the European population to chemical substances.

As the first initiative of its size and nature in the field of exposure and health, HBM4EU generated new challenges for project coordination. Its size in terms of funding, its number of collaborating partners, and its ambitious scientific goals exceeded preceding projects in this area by far. As the spectrum of activities was enormously broad, steering required new ways of administrative and scientific governance. The effort invested in the elaboration of the extremely detailed Consortium Agreement ensured procedural transparency and clear expectations for all actors involved and thus precluded many conflicts during the lifetime of the project. Another major innovative element was the specific construction of the Governing Board, which bound policy-makers as addressees of results exceptionally tightly to the project. Altogether, the carefully prepared governance arrangements worked well. However, one major conclusion with a view to future projects is that in large-scale projects of this nature, scientific governance would benefit from further development of structure, tasks, and incentives for leaders. This would lead to safeguarding the most effective use of resources to realize overall project goals. Moreover, differentiation of funding rates among project activities threatens to create conflicts.

Major innovative elements of HBM4EU were taken up by the subsequent Partnership for the Assessment of Risk from Chemicals (PARC), which receives funding from the EU's Horizon Europe research and innovation programme under Grant Agreement No 101057014. These elements include the established network of national and EU authorities and scientific institutions at the science-policy interface, the National Hub structure, the inclusion of EU agencies as partners and Governing Board members, and the interlinkage of national and EU agencies. HBM4EU provides a blueprint for EU funded large-scale projects targeting future policies for realizing the Green Deal and Zero Pollution Ambition in the field of chemicals, health, and environment. It offers a best practice example. Therefore, its experiences should be considered for similar, future endeavours and may contribute to the success of large-scale EU projects in other sectors.

Declaration of competing interest

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

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Impact of air pollutants on influenza-like illness outpatient visits under urbanization process in the sub-center of Beijing, China

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ABSTRACT

Air pollutants can cause serious harm to human health and a variety of respiratory diseases. This study aimed to explore the associations between air pollutants and outpatient visits for influenza-like illness (ILI) under urbanization process in the sub-center of Beijing. The data of ILI in sub-center of Beijing from April 1, 2014 to December 31, 2020 were obtained from Beijing Influenza Surveillance Network. A generalized additive Poisson model was applied to examine the associations between the concentrations of air pollutants and daily outpatient visits for ILI when controlling meteorological factors and holidays. A total of 322,559 patients with ILI were included. The results showed that in the early urbanization period, the effects of $PM_{2.5}$, PM_{10}, SO_2, O_3, and CO on lag0 day, and $PM_{2.5}$, PM_{10}, O_3, and CO on lag1 day were not significant. In the later urbanization period, AQI and the concentrations of $PM_{2.5}$, PM_{10}, SO_2, NO_2 and CO on lag1 day were all significantly associated with an increased risk of outpatient visits for ILI, which increased by 0.34% (95%CI 0.23%, 0.45%), 0.42% (95%CI 0.29%, 0.56%), 0.44% (95%CI 0.33%, 0.55%), 0.36% (95%CI 0.24%, 0.49%), 0.91% (95%CI 0.62%, 1.21%) and 0.38% (95%CI 0.26%, 0.49%). The concentration of O_3 on lag1 day was significantly associated with a decreased risk of outpatient visits for ILI, which decreased by 0.21% (95%CI 0.04%, 0.39%). We found that the urbanization process had significantly aggravated the impact of air pollutants on ILI outpatient visits. These findings expand the current knowledge of ILI outpatient visits correlated with air pollutants under urbanization process.

1. Introduction

Influenza-like illness (ILI) is a group of illnesses defined by influenza-like clinical manifestations like fever, cough and sore throat. Many respiratory illnesses, including seasonal influenza and human infection with other respiratory pathogens, are characterized as ILI, and approximately half of patients with ILI seek healthcare (Ma et al., 2018). Studies have showed that influenza had an important contribution to the disease burden of influenza-like illness (Feng et al., 2020; Fowlkes et al., 2013). About 291–646 thousand seasonal influenza-associated respiratory deaths occur annually worldwide (Iuliano et al., 2018; Paget et al., 2019). The people aged ≥65 years and <5 years contributed greatly to mortality and morbidity burden in China due to influenza (Li et al., 2021a). Many factors can affect the influenza epidemic, such as individual and environmental factors (Lai et al., 2013; Mertz et al., 2013). The urbanization leads to many challenges for the epidemiology of infectious diseases and the cities are becoming important centers for the transmission of infectious diseases (Alirol et al., 2011; Neiderud, 2015).

The urbanization is mainly characterized by urban population growth and expansion of construction land (Liu et al., 2021). With the continuous advancement of urbanization, more carbon emissions may be generated (Aslan et al., 2021), making the air pollution problem more

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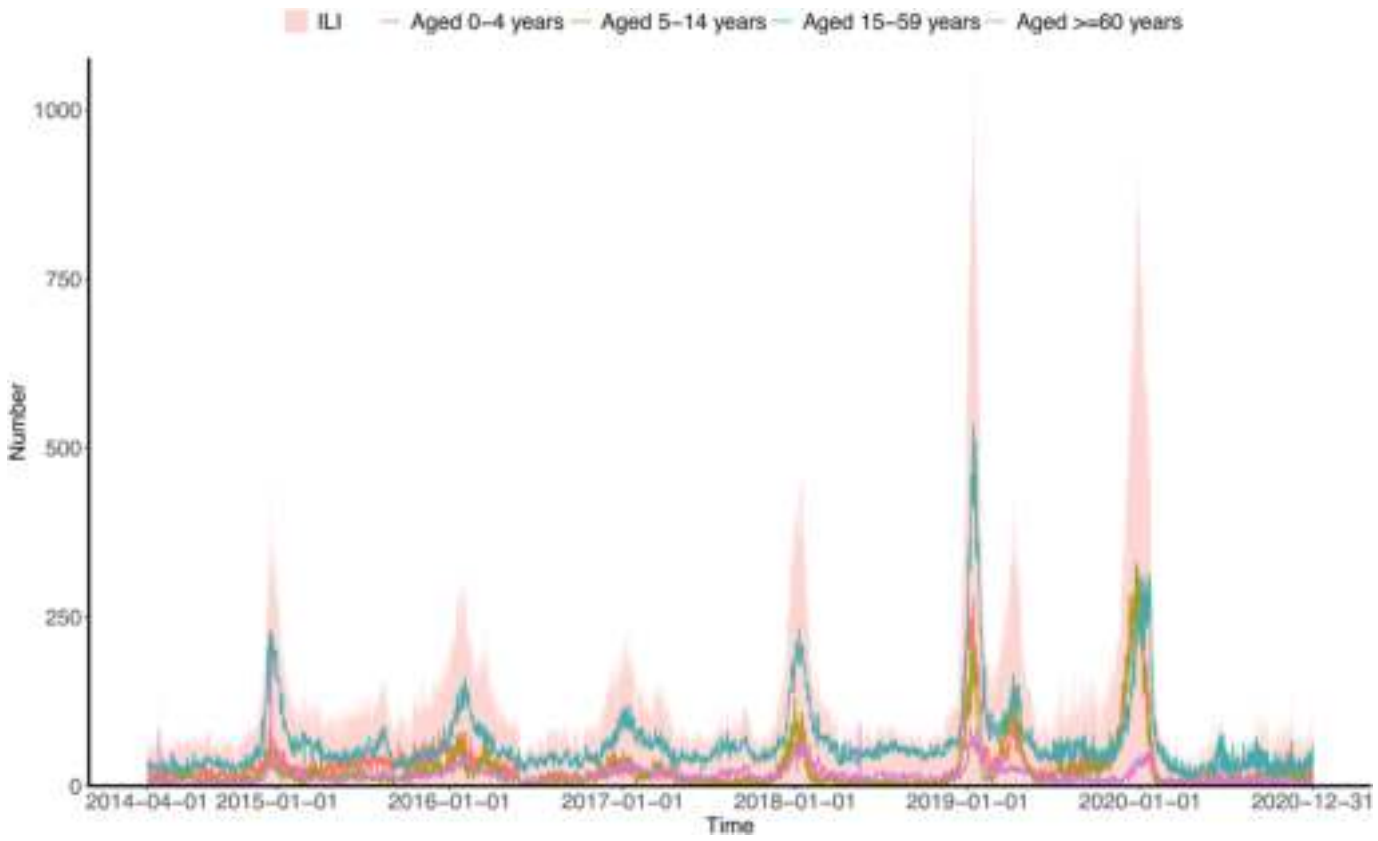

Fig. 1. Time series of influenza-like illness cases (number of daily cases) during the study period in Tongzhou.

serious. Air pollution can cause serious harm to the human health and a variety of respiratory diseases (Manisalidis et al., 2020). The urbanization has a complex and vital impact on influenza epidemic (Dalziel et al., 2018; Zachreson et al., 2018), and the speed of urbanization may be closely associated with the intensity and the peak of influenza epidemic, and may be influencing influenza epidemic along with climatic factors.

Tongzhou District of Beijing is the municipal administrative center of Beijing. In recent years, the urbanization process has developed rapidly. We aimed to explore the impact of air pollution on the risk of ILI outpatient visits under urbanization process in the sub-center of Beijing, which may help the government to formulate the strategies of influenza prevention and air control policies in the process of urbanization in the similar regions.

2. Methods

2.1. Study site

Tongzhou District of Beijing locates in the southeast of Beijing, which is the municipal administrative center and the subcenter of Beijing. In terms of transportation, it has important railways and highways. The levels of economy, industry and medical treatment in Tongzhou District have developed rapidly. Under the process of urbanization, the gross domestic product (GDP) of Tongzhou District grew rapidly especially from 2017 to 2018 and the population growth in Tongzhou District also increased from 1.25 million in 2013 to 1.65 million in 2020 (Beijing Statistics Bureau, 2021). According to the population change (the proportion of urban residents, total population number etc.) and the economy growth, the urbanization process of Tongzhou District was divided into two periods with January 1, 2018 as the critical point: the period from April 1, 2014 to December 31, 2017 as the early period of urbanization and the period from January 1, 2018 to December 31, 2020 as the later period of urbanization.

2.2. Data source

ILI epidemiological surveillance data of Tongzhou district were obtained from Beijing Influenza Surveillance Network (BISN), administrated by Beijing Center for Disease Control and Prevention (CDC). All the municipal hospitals were involved into the network. The fever clinic, internal medicine clinic, internal emergency department, pediatric clinic, and pediatric emergency department of sentinel hospitals were set as sentinel spots. Epidemiological surveillance data were required to be registered and reported every 24 h by sentinel hospitals and verified by local CDCs.

The concentrations of air pollutants, including particulate matter with an aerodynamic diameter less than 2.5 μm ($PM_{2.5}$), particulate matter with an aerodynamic diameter less than 10 μm (PM_{10}), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), ozone (O_3), carbon monoxide (CO) and air quality index (AQI), were collected from April 1, 2014 to 31 December 31, 2020 from Beijing Municipal Ecological and Environmental Monitoring Center. The meteorological data of Tongzhou District came from the National Meteorological Information Center, including the daily data of temperature, relative humidity, wind speed, and precipitation in Tongzhou District from April 1, 2014 to December 31, 2020.

2.3. Statistical analysis

Descriptive statistics were performed for categorical variables as frequencies and proportions or rates, as well as for continuous variables were expressed as medians and interquartile ranges (IQR).

Daily ILI visits usually follow a Poisson distribution, so this study adopted a generalized additive Poisson model. As the association between meteorology and health effects was generally nonlinear, the smoothing spline function was used to control the meteorological parameters, and the time smoothing function was set to control the in-

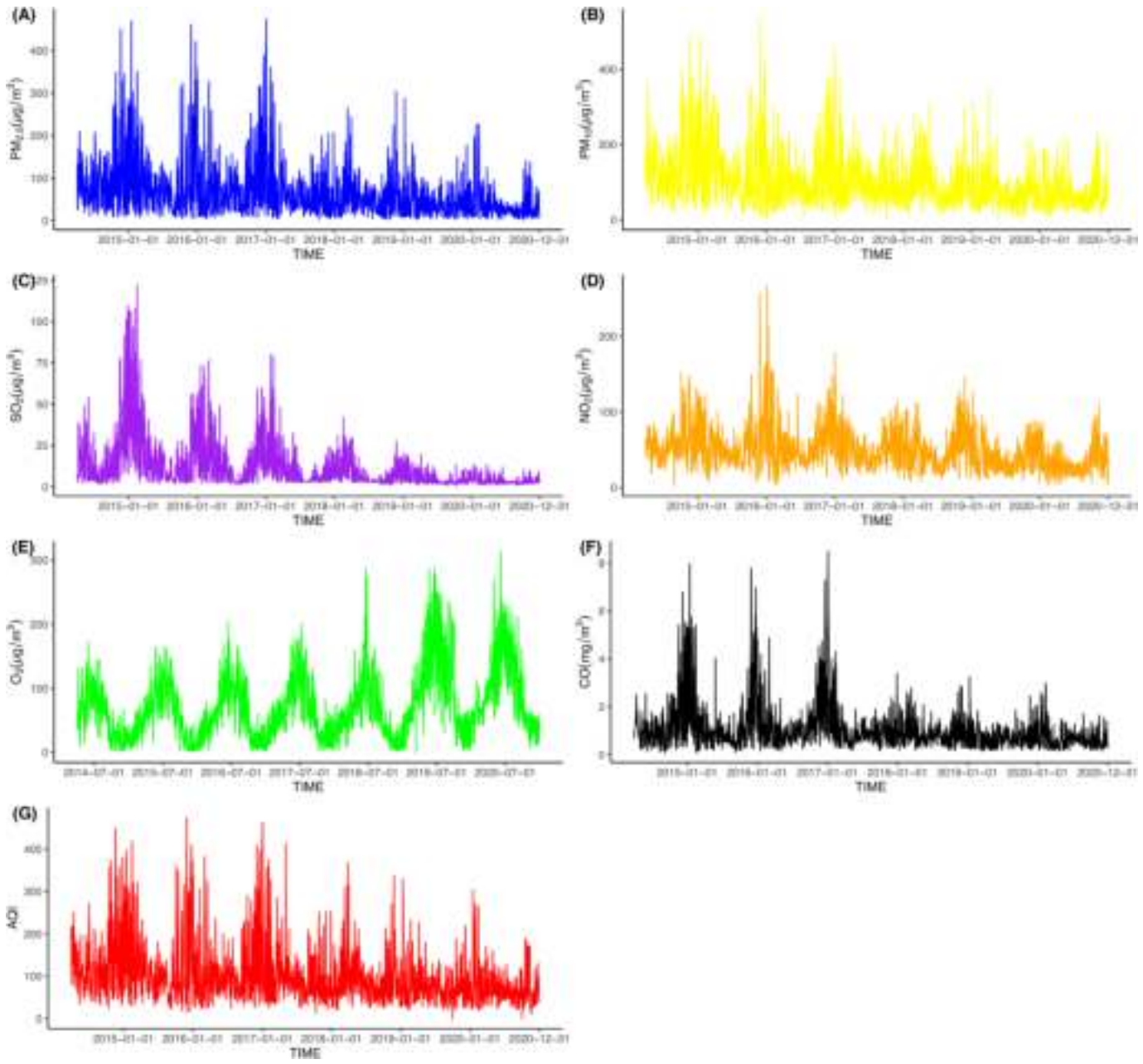

Fig. 2. Time series of air pollutants in Tongzhou District, Beijing during the study period (A–G). Air pollutants involved were (A) $PM_{2.5}$, (B) PM_{10}, (C) SO_2, (D) NO_2, (E) O_3, (F) CO, (G) AQI.

fluence of the long-term trend. The day of the week effect was also considered to control the short-term fluctuation. Holiday effect was introduced into the model as a dummy variable. The single pollution model was as follows:

$$Y_t \sim Poisson(\mu_t)$$

$$Log(\mu_t) = \beta X_i + s(temperature, df) + s(humidity, df) + s(wind\ speed, df) + s(precipitation, df) + s(time, df_t) + as.factor(dow) + holiday + intercept$$

Among them, μ_t is the expected value of the daily number of patients with ILI, β is the regression coefficient of each air pollutant, X_i represents $PM_{2.5}$, PM_{10}, SO_2, NO_2, O_3, CO, AQI, *intercept* is the intercept item. According to the principle of residual independence, the degrees of freedom per year of the time smoothing function for each period were selected. According to previous studies, the degree of freedom of the smoothing function of meteorological factors was set to 3 (Tian et al., 2014). Besides, a multi-pollutant model was used to investigate the independent effect of individual air pollutant.

This study investigated the associations between the concentrations of air pollutants and the number of daily visits for ILI over different periods. According to previous studies, the associations between air pollutants concentrations and the cases of ILI and respiratory tract infection were normally considered within a week (Li et al., 2018, 2021b; Liu et al., 2019; Su et al., 2019). In our study, to control the lag effect, the air pollutants concentrations in this day (lag0) and the next 7 days (lag1-lag7) were included in the model. Four age groups were classified, including 0–4 years, 5–14 years, 15–59 years, and ≥60 years.

The results were expressed as 95% confidence intervals (CI) for the percentage change in daily visits to ILI for a 10 μg/m^3 increase in air pollutant concentrations (1.0 μg/m^3 for SO_2 and 0.1 mg/m^3 for CO) or

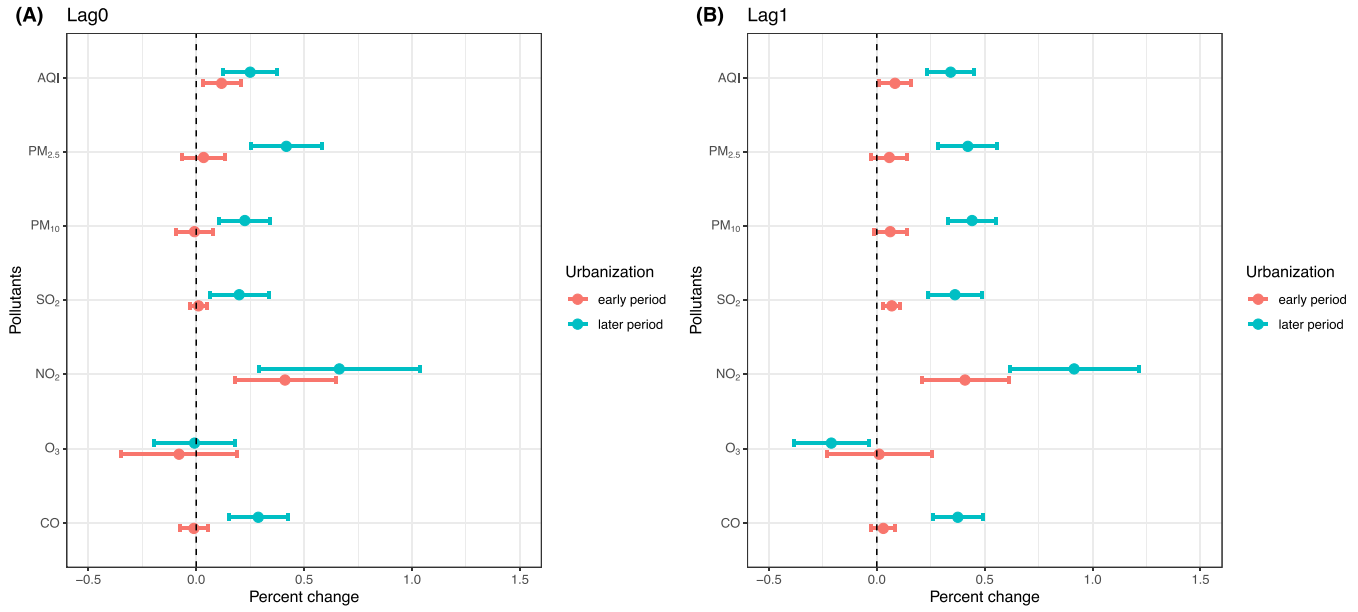

Fig. 3. Percent change of outpatient visits for ILI on lag0 and lag1 day for a 10 μg/m^3 (SO_2 1.0 μg/m^3 and CO 0.1 mg/m^3) increase in air pollutants during the early period and later period of urbanization process
(A) percent change of outpatient visits for ILI on lag0 day (B) percent change of outpatient visits for ILI on lag1 day.

for a 10-unit increase in AQI. Exposure-response analyses were performed using R V.4.1.2 with the "mgcv" package. A two-sided P value of less than 0.05 was considered statistically significant.

The following information was supplied relating to ethical approvals: The Peking University Institutional Review Board Office granted Ethical approval to carry out the study within its facilities (IRB00001052−19005).

3. Results

3.1. Outpatient visits

There were 322,559 ILI cases from April 1, 2014 to December 31, 2020 (Table S1). Of all ILI cases, 39.8% aged between 0 and 14 years and 11.7% aged ≥60 years. The median of the number of outpatient visits attributed to daily ILI was 91 (IQR 67–137). The peak of ILI occurred from December to January next year. The highest peak was observed from December 2018 to January 2019 with 1077 cases, followed by the peak from December 2019 to January 2020 (Fig. 1).

3.2. Description of exposure and meteorological parameters

The AQI value from 2018 to 2020 was lower than that in previous years (Fig. 2). The overall concentrations of $PM_{2.5}$, PM_{10}, SO_2, CO and NO_2 were higher in cold period (from December to January of next year). The peaks of these air pollutants were not evident in the cold period from 2018 to 2020. The concentration of O_3 was lower in cold period. The concentration of O_3 from 2018 to 2020 was higher than that in previous years. The correlations were detected among air pollutants (Table S2). The meteorological parameters of relative humidity and precipitation were lower in cold period. The meteorological parameters of temperature, relative humidity, wind speed and precipitation showed no significant difference between different years.

3.3. Exposure-response analysis

Air pollutants had the greatest impact on the incidence of ILI on lag1 day (Fig. S1). Thus, the impacts of air pollutants on the ILI outpatient visits on lag0 and lag1 day were selected (Fig. 3). In the early urbanization period, the effects of $PM_{2.5}$, PM_{10}, SO_2, O_3, and CO on lag0 day, and $PM_{2.5}$, PM_{10}, O_3, and CO on lag1 day were not significant. The concentrations of SO_2 and NO_2 on lag1 day were significantly associated with an increased risk of outpatient visits for ILI, which increased by 0.07% (95%CI 0.03%, 0.11%), and 0.41% (95%CI 0.21%, 0.61%) (Fig. 3).

In the later urbanization period, AQI and the concentrations of $PM_{2.5}$, PM_{10}, SO_2, NO_2 and CO on lag0 day were significantly associated with an increased risk of outpatient visits for ILI, which increased by 0.25% (95%CI 0.13%, 0.37%), 0.42% (95%CI 0.25%, 0.58%), 0.23% (95%CI 0.11%, 0.34%), 0.20% (95%CI 0.06%, 0.34%), 0.66% (95%CI 0.29%, 1.04%), 0.29% (95%CI 0.15%, 0.42%), respectively (Fig. 3). AQI and the concentrations of $PM_{2.5}$, PM_{10}, SO_2, NO_2 and CO on lag1 day were all significantly associated with an increased risk of outpatient visits for ILI, which increased by 0.34% (95%CI 0.23%, 0.45%), 0.42% (95%CI 0.29%, 0.56%), 0.44% (95%CI 0.33%, 0.55%), 0.36% (95%CI 0.24%, 0.49%), 0.91% (95%CI 0.62%, 1.21%) and 0.38% (95%CI 0.26%, 0.49%), respectively (Fig. 3). The concentration of O_3 on lag1 day was significantly associated with a decreased risk of outpatient visits for ILI, which decreased by 0.21% (95%CI 0.04%, 0.39%) (Fig. 3). The effects of NO_2 and SO_2 on lag1 day significantly increased compared with the early period of urbanization (Fig. 3).

The subgroup analysis by age group showed that air pollutants had the greatest impact on the incidence of ILI on lag1 day for the age groups of 0–4 years, 5–14 years, and on lag2 day for the age group of 15–59 years and ≥60 years (Figs. S2–S5). In the later period of urbanization, the impact of various air pollutants on the ILI incidence had become significant (Fig. 4). Among them, the impact of SO_2 and NO_2 became particularly obvious on lag1 day for age groups of 0–4 years, 5–14 years and lag2 day for age groups of 15–59 years and ≥60 years in the later period of urbanization (Fig. 4). For children aged 0–4 years and 5–14 years, AQI and the concentrations of $PM_{2.5}$, PM_{10}, SO_2, NO_2, and CO were all associated with an increase in the risk of ILI outpatient visits in the later period of urbanization (Fig. 4).

We applied a multi-pollutant model to investigate the independent effect of individual air pollutant (Table 1). In the early urbanization period, the effects of $PM_{2.5}$, PM_{10}, O_3, and CO on lag1 day were not significant. AQI and the concentrations of SO_2 and NO_2 on lag1 day were all significantly associated with an increased risk of outpatient visits for ILI, which increased by 0.18% (95%CI 0.04%, 0.32%), 0.05% (95%CI

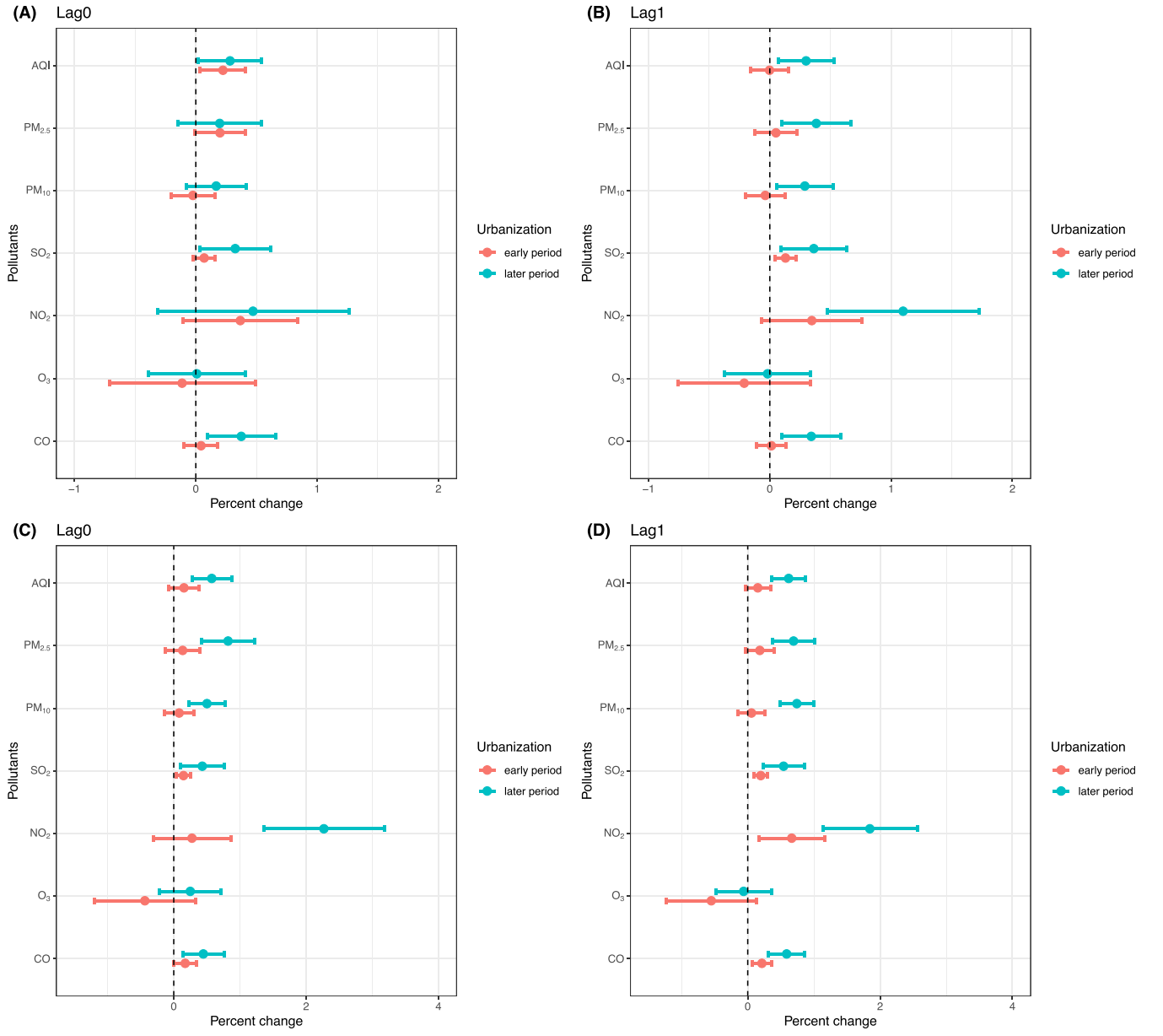

Fig. 4. Percent change of outpatient visits for ILI within different age groups on lag0 and lag1 day (lag2 day for 15–59 years and ≥60 years) for a 10 μg/m^3 (SO_2 1.0 μg/m^3 and CO 0.1 mg/m^3) increase in air pollutants during the early period and later period of urbanization process
(A) age group of 0–4 years on lag0 day (B) age group of 0–4 years on lag1 day (C) age group of 5–14 years on lag0 day (D) age group of 5–14 years on lag1 day (E) age group of 15–59 years on lag0 day (F) age group of 15–59 years on lag1 day (G) age group of ≥60 years on lag0 day (H) age group of ≥60 years on lag2 day.

0%, 0.11%) and 0.45% (95%CI 0.16%, 0.74%), respectively. In the later urbanization period, the concentration of PM_{10} on lag1 day was significantly associated with an increased risk of outpatient visits for ILI, which increased by 0.52% (95%CI 0.28%, 0.77%). AQI and the concentration of SO_2 on lag2 day were all significantly associated with an increased risk of outpatient visits for ILI, which increased by 0.79% (95%CI 0.41%, 1.16%) and 0.35% (95%CI 0.17%, 0.53%). The concentration of O_3 on lag2 day was significantly associated with a decreased risk of outpatient visits for ILI, which decreased by 0.18% (95%CI 0%, 0.36%).

In the sensitivity analyses, we excluded the year of 2020 in the later period of urbanization to remove the impact of the COVID-19 (Fig. S6). The results indicated that the effects were not substantially changed.

4. Discussion

Air pollutants are a major source of an increased risk of disease, hospitalization, morbidity, and mortality globally (Rebuli et al., 2021), which have also been shown to increase the risk of influenza-like illness (Su et al., 2019). Because of rapid industrial development and a great amount of solid fuel combustion, China is encountering serious air pollution challenges. In Beijing and surrounding areas, in addition to transportation, power sectors and industry emissions, residential resources contribute a high percentage to primary $PM_{2.5}$ emissions each year (Liu et al., 2016). It is of vital importance to assess the impact of air pollutants on outpatient visits for ILI. In the sub-center of Beijing, the effects of air pollutants were different on the incidence of ILI in different periods of the urbanization. Our study focused on ILI as the monitoring and analysis of ILI could more comprehensively reflect the transmission

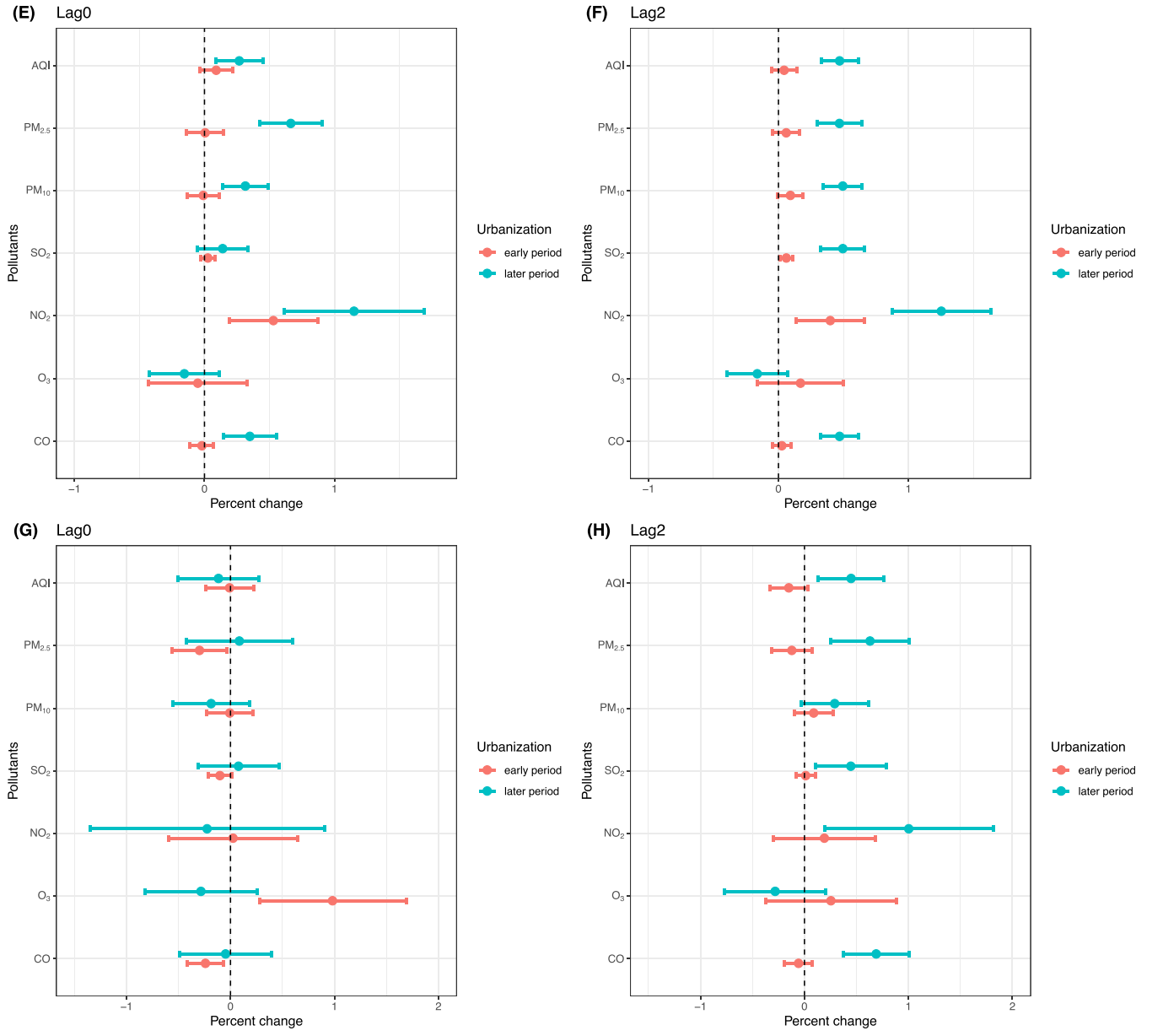

Fig. 4. (*continued*).

of respiratory infectious diseases such as influenza and help more timely control such epidemic.

In our study, urbanization process had significantly aggravated the impact of air pollutants on ILI. In the early urbanization period, the effects of most air pollutants were not significant. But in the later urbanization period, except O_3, all the air pollutants were significantly associated with an increased risk of outpatient visits for ILI. The urbanization process had increased population density and population mobility (Liu et al., 2021), and could be a major cause of air pollution (Shi et al., 2020), which make people more vulnerable to ambient air pollutants, thereby aggravating the impact of air pollutants on ILI.

In the later period of urbanization, we observed a significant increase in the concentrations of SO_2 and NO_2 on the risk of ILI outpatient visits compared with the early period of urbanization. Besides, the multi-pollutant model showed the independent effect of SO_2 in the later urbanization period in our study. NO_2 exposure may increase the risk of respiratory tract infections, and SO_2 contributes to respiratory symptoms in both healthy patients and those with underlying pulmonary disease (Chen et al., 2007). A clinical study suggested that NO_2 alone might play a role in increasing the susceptibility of adults to respiratory virus infections (Goings et al., 1989). Animal experiments in mice showed that the increase in pneumonia was associated with SO_2 concentration which induced low-grade, inflammatory lesions in the lung (Fairchild et al., 1972). Another study showed that exposure to NO_2 caused alveolar macrophages to tend to inactivate influenza virus less effectively (Frampton et al., 1989).

In the later period of urbanization, O_3 on lag1-lag3 day was significantly associated with a decreased risk of outpatient visits for ILI. In the sensitivity analysis excluding the effect of COVID-19, the effect of O_3 on lag1-lag2 day turned to be not significant. Li et al. suggested that O_3 on lag1 day during the non-outbreak period was associated with a decreased risk of outpatient visits for ILI, but the result was not significant (Li et al., 2021b). Su et al. suggested that O_3 were significantly associated with a decreased risk of ILI for people aged ≥60 years, 5–14 years and 0–4 years (Su et al., 2019). Studies have shown that the increase of ambient ozone reduced influenza transmissibility, which may be associated with ozone's virucidal activity and the effect of ozone on the host defense (Ali et al., 2018). It may be one of the reasons why the

Table 1
Percent change of outpatient visits for ILI for the increase in air pollutants by the multi-pollutant model.

Air pollutants	Early period		Later period	
	Lag1	Lag2	Lag1	Lag2
$PM_{2.5}$	−0.23 (-0.47,0.01)	−0.06 (-0.29,0.18)	0.13 (-0.29,0.55)	−0.77(-1.18,-0.35)
PM_{10}	0.02 (-0.13,0.16)	0.07 (-0.08,0.21)	0.52 (0.28,0.77)	−0.07 (-0.31,0.18)
AQI	0.18 (0.04,0.32)	−0.05 (-0.19,0.09)	−0.28 (-0.65,0.09)	0.79 (0.41,1.16)
SO_2	0.05(0,0.11)	0.02 (-0.04,0.07)	0.14 (-0.03,0.32)	0.35 (0.17,0.53)
NO_2	0.45 (0.16,0.74)	0.49(0.20,0.77)	0.06 (-0.40,0.52)	0.10 (-0.36,0.55)
O_3	0.20 (-0.07,0.47)	0 (−0.27,0.26)	−0.13 (-0.31,0.05)	−0.18 (-0.36,0)
CO	−0.07 (-0.19,0.05)	−0.05 (-0.16,0.07)	0.03 (-0.23,0.29)	0.04 (-0.21,0.30)

Note: The results were expressed as 95% confidence intervals (CI) for the percentage change in daily visits to ILI for a 10 μg/m^3 increase in air pollutant concentrations (1.0 μg/m^3 for SO_2 and 0.1 mg/m^3 for CO) or for a 10-unit increase in AQI.

concentration of O_3 was associated with the decline in ILI visits. In animal experiments, continuous exposure to 0.5 ppm ozone during influenza virus infection reduced the severity of the disease with less widespread infection (Jakab and Hmieleski, 1988). The inhalation of ozone inhibited influenza virus growth in the nose of mice (Fairchild, 1977).

For children aged 0–4 years and 5–14 years, AQI and the concentrations of $PM_{2.5}$, PM_{10}, SO_2, NO_2, and CO were all associated with an increase in the risk of ILI outpatient visits in the later period of urbanization, indicating that air pollution may have a more negative impact on children. The infants and children are particularly sensitive to air pollution (Brumberg and Karr, 2021). The impact on the children aged 5–14 years was more significant than the children aged 0–4 years. The difference may be that the children aged 0–4 years do not have to go out to school regularly and are more likely to take protective measures at home in times of serious air pollution, while the children aged 5–14 years are more vulnerable to air pollution because they need to go to school regularly. In China, the increased urbanization is associated with the increased population age and the decreased proportion of school-aged people (Lei et al., 2021). The health impact of air pollution on this group needs to be paid close attention.

For the group of 15–59 years, they are more often exposed to air pollutants due to commuting activities and other reasons (Nazelle et al., 2011). In the context of COVID-19 pandemic, online distance education and work at home have become a normal state (Galanti et al., 2021; Schneider and Council, 2021). Distance education and online work not only prevent the spread of diseases, but also reduce the mobility of the population and reduce the impact of air pollution on people's health. Therefore, it is necessary to actively develop relevant technologies of online work and teaching, improve relevant policies, and reduce the impact of air pollution on the health of commuters.

This study had some limitations. Firstly, the ILI data of this study was limited to the sub-center of Beijing, and there may be a selection bias. Secondly, considering that not all ILI patients chose to seek healthcare, this study included only the outpatient visits of ILI, not all ILI patients. Consequently, the underestimation of the number of ILI may influence the representativeness of the sample and cause bias to the results, which might be resolved in future studies. Thirdly, this study used an ecological design, which may ignore some potential confounding factors, such as socio-economic factors, behavioral factors and so on. Fourthly, in the later period of urbanization, the occurrence of COVID-19 may affect the emission of air pollutants and the percentage of ILI patients seeking medical services. In the sensitivity analyses, we excluded the year of 2020 in the later period of urbanization to remove the impact of the COVID-19, and the results indicated that the effects were not substantially changed but the effects of O_3 on lag1 day turned to be not significantly associated with a decreased risk of outpatient visits for ILI.

5. Conclusion

In this study, we found that the urbanization process had significantly aggravated the impact of air pollutants on ILI outpatient visits. Therefore, we recommend that air pollution control and influenza prevention need to be strengthened in the process of urbanization. This study provides a decision-making basis for influenza prevention and air governance in the sub-center of Beijing, and has reference significance for the formulation of public health and environmental governance measures in other regions. Based on this study, the associations between various measures in the urbanization process and the risk of ILI outpatient visits need to be further evaluated.

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Data availability statement

The original dataset presented in this study included in the supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

Qing-Bin Lu, Fuqiang Cui and Yan-Na Yang conceived and designed the experiments, reviewed drafts of the paper, and approved the final draft. Zhong-Song Zhang and Lu Xi and Li–Li Yang collected and analyzed the data, prepared Fig.s and tables, authored drafts of the paper, and approved the final draft. Xi Lu, Yan-Na Yang, Yan Cui, Hong-Jun Li collected the data and performed the investigations. Juan Du, Zhong-Song Zhang, Xin-Yao Lian and Wan-Xue Zhang cleaned the data and prepared Fig.s and tables. All authors have approved the final draft and agreed to the published version of the manuscript.

Human ethics

The following information was supplied relating to ethical approvals: The Peking University Institutional Review Board Office granted Ethical approval to carry out the study within its facilities (IRB00001052-19005).

Declaration of competing interest

The authors declare there are no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.114076.

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Inhalation and dermal absorption as dominant pathways of PCB exposure for residents of contaminated apartment buildings

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ABSTRACT

Applications of polychlorinated biphenyls (PCBs) in buildings and their persistence in indoor environments have led to cases of current and highly elevated exposure in humans, despite the global cease of production decades ago. Personal exposure to PCBs was assessed among residents in a social housing estate in Denmark containing both contaminated (n = 67) and non-contaminated (n = 23) apartments. Samples and estimated daily intakes (EDIs) were assessed for 15 PCB congeners, and body burden, which was limited by the dietary data availability, was compared across 7 indicator PCBs, with its sum (PCB_{sum7}) often applied in European regulation of PCBs. Median PCB_{sum7} EDI across measured pathways for exposed residents was 101 ng· (kg bodyweight)$^{-1}$· day^{-1}, with the majority of exposure (60%) coming from inhalation of contaminated indoor air. Calculated from both PCBs measured in indoor air and on hand wipes, dermal absorption estimates showed comparable results and served as a secondary exposure pathway, accounting for 35% of personal exposure and considering selected assumptions and sources of physical-chemical parameters. Estimates revealed that diet was the primary PCB source among the reference group, accounting for over 75% of the PCB_{sum7} EDI across exposure routes. When evaluating overall EDIs across the two study groups and including dietary estimates, PCB exposure among exposed residents was around 10 times higher than the reference group. Solely within the exposed population, pathway-specific body burdens were calculated to account for exposure across years of residence in contaminated apartments, where lower chlorinated PCBs were dominant in indoor air. Among these dominant congeners, estimated body burdens of PCB-28 and -52 were significantly correlated with measured serum ($r_s = 0.49, 0.45$; $p < 0.001$). This study demonstrates that inhalation and dermal absorption serve as dominant exposure pathways for residents of apartments contaminated with predominantly lower chlorinated PCBs and suggest that predictions of body burden from indoor environment measurements may be comparable to measured serum PCBs.

1. Introduction

Polychlorinated biphenyls (PCBs) were used as flame retardants, plasticizers, and dielectric fluid, among other applications, with production starting in the 1930s (IARC, 2016). Consisting of 209 distinct congeners of varying numbers and positions of chlorine atoms, PCBs were manufactured in mixtures, the most common being the Aroclor and Clophen series, until production largely ceased in the 1980s. They were particularly lauded for their high chemical stability, which inadvertently led to their environmental persistence, also in indoor environments (Audy et al., 2018). When the Stockholm Convention on Persistent Organic Pollutants (POPs) went into effect in 2004, PCBs were listed as one of the initial "Dirty Dozen". They are classified as carcinogenic to humans (Group 1 in IARC framework) and are considered to be endocrine disruptors, neurotoxic, and immunotoxic with effects on cardiovascular and reproductive health (ATSDR, 2000; Heilmann et al., 2010; IARC, 2016).

Within the Stockholm Convention treaty, all use of PCBs is required to be phased out by 2025, with total elimination of PCBs by 2028 (UNEP, 2017). However, historic applications, such as elastic sealants and fluorescent lighting ballasts, are still present in buildings currently in use, which contribute to ongoing contamination of indoor environments and to continued human exposure to PCBs. With these continuing exposures in mind, the Danish Health Authority established two

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recommended action values in 2009 for PCBs in indoor air: an air concentration ≥300 ng PCB_{total}/m^3 is considered a possible health risk and an action plan is needed, and ≥3000 ng PCB_{total}/m^3 requires immediate action (Jensen, 2013). PCB_{total}, as defined by the Danish Health Authority, is the sum of seven indicator congeners (PCB-28, -52, -101, -118, -138, -153, and -180) measured in air multiplied by a corrections factor of 5, based on practice and action levels first established in Germany (Jensen, 2013). Notably, current Danish recommended action values only consider the inhalation pathway of PCB exposure for individuals living in contaminated homes. These recommendations resulted in increased awareness and screening of residential buildings, particularly among social housing organizations and municipalities, throughout Denmark.

In one such social housing estate, Brøndby Strand Parkerne, several high-rise apartment buildings were built using PCB-containing sealants. The indoor environment in some apartment buildings within the estate has been examined thoroughly, through dust, air, and surface wipe samples, and residents' exposure has been characterized using samples from their apartments in addition to hand wipes and serum samples (Andersen et al., 2020; Frederiksen et al., 2020). Both environmental and personal samples have indicated that residents' exposure was high and exceeded Danish Health Authority limits, but relative contributions of exposure pathways to internal dose measurements have not yet been determined. The relationship between PCB exposure to internal dose is supported by the wealth of studies conducted among individuals living or working in environments with high indoor air PCB concentrations, which correlated with similarly elevated serum levels and particularly for lower chlorinated PCBs (Ampleman et al., 2015; Herrick et al., 2011; Kraft et al., 2018, 2021; Liebl et al., 2004; Meyer et al., 2013). Given the measured air concentrations and prevalence of lower chlorinated PCBs measured previously in these apartments, dermal absorption is likely to also be an important pathway for PCB exposure, although few studies have evaluated its contribution to personal exposure. While dermal absorption has long been considered important, the difficulty in its measurement has led to body burden contributions to be largely underestimated for many semi-volatile organic compounds, including PCBs (Weschler and Nazaroff, 2012). For those living and working in non-contaminated buildings, diet and particularly fish consumption are likely to be the primary sources of personal exposure to PCBs (Fromberg et al., 2011).

Herein, we examined which pathway of exposure contributed most to personal exposure to PCBs for residents of contaminated buildings compared to those living in non-contaminated apartments. Samples had been previously collected and analyzed for 15 PCB congeners. Dietary exposure was estimated based on available data for specific congeners (8 out of the 15). Sum of the seven indicator PCBs, PCB_{sum7}, was used for comparisons of relative exposure contributions and body burden measurements.

2. Methods

2.1. Population and recruitment

Located about 15 km southwest of Copenhagen, Denmark, Brøndby Strand Parkerne is a housing estate containing 12 fifteen-story apartment buildings, with 4–5 apartments per floor, and several shorter apartment buildings. Erected between 1969 and 1974, only the first 5 high-rise buildings were constructed with PCB-containing sealants, which were placed around light façade elements indoors and on the enclosed balconies and windows outdoors (Andersen et al., 2020). Based on analysis of sealant materials, congener patterns indicate that they likely contained Clophen A-40 or Aroclor 1248, both of which are dominated by lower (mostly tetra-) chlorinated PCBs (Andersen et al., 2020; Takasuga et al., 2006). The remaining seven high-rises as well as surrounding smaller apartment buildings were constructed without use of PCB-containing sealants. Additional details about the buildings and PCB content have been published previously (Andersen et al., 2020; Frederiksen et al., 2020).

Study participants were recruited from Brøndby Strand Parkerne, with the exposed group formed by residents in the first 5 high-rise apartments and the reference group residing in other apartment buildings on the premises. Additional recruitment details were described previously in Frederiksen et al. (2020). All participants gave informed consent prior to providing any personal information and were informed of their individual results, upon request. Study protocols and related materials were approved by the Regional Ethics Committee (H-16041946) and reported to the Data Protection Agency through University of Copenhagen (SUND-2017-03).

2.2. Sample collection and analysis

Samples from participants' home environment (e.g., air, house dust, surface wipes) and personal samples (e.g., hand wipes, serum) were collected between October and December 2017. Further details regarding sample collections and results were reported for the home environmental samples in Andersen et al. (2020) and for personal samples in Frederiksen et al. (2020). In brief, active air samples were collected from each apartment's living room over a 24-h period, dust samples from participants' vacuum cleaners, and hand wipes by wiping both palms with an isopropyl alcohol wipe. All samples were stored at −20 °C following collection until extraction and analysis, except the dust, which was stored in a refrigerator until handling (i.e., sieving to <75 μm, then analyzed). All samples were analyzed for the 7 indicator PCBs and further PCB-8, -11, -18, -31, -44, -66, -74, -99 and -105, for 15 PCB congeners, total. This set of 15 PCBs, listed in Table S1 with relevant physicochemical properties, were compared across matrices. Dietary information was available for 8 of these congeners. As such, a subset of 7 indicator PCBs and their sum (ΣPCB-28, 52, 101, 118, 138, 153, 180), or PCB_{sum7}, for which all information was available, was used for comparisons of estimated daily intake (EDI) considering possible pathways of exposure.

2.3. Calculations

Potential pathways relevant to human exposure to PCBs include inhalation, dermal absorption, diet, dust ingestion, and hand-to-mouth contact. Given the physical-chemical properties of the PCBs, dust ingestion and hand-to-mouth contacts are anticipated to play comparatively minor roles but are still evaluated here (**SI Section S.3**). For each pathway of exposure, a pathway-specific EDI was calculated using concentrations measured in the corresponding environmental or personal sample. All EDIs [ng·(kg bodyweight)$^{-1}$·day^{-1}] were normalized to participants' body masses, which were self-reported.

2.3.1. Treatment of questionnaire data

Several parameters for EDI calculations were taken from questionnaires administered to all study participants. These data included an array of questions regarding their home environment, behavior, potential past exposures, height, weight, and dietary habits. To determine personal exposure with more specificity to individual behavior, cofactors such as exposure duration were calculated from questionnaire responses, or imputed if missing, based on estimations from the Danish Health Authority or US Environmental Protection Agency Exposure Factors Handbook. Exposure duration was calculated as the fraction of time spent per day in the home, which was determined from questionnaire data. If missing (n = 18), exposure duration was replaced with an estimate of activity factors based on age group (18-<65 years: 15.8 h/day; ≥65 years old: 19.6 h/day) (USEPA, 2011c). If body weight was missing (n = 1), this was imputed to 70 kg, as recommended by the Danish Environmental Protection Agency guidance document (Höglund et al., 2012).

2.3.2. Inhalation pathway

Exposure via inhalation was estimated from indoor air concentration (C_{air}) measured in participants' homes (ng/m^3). Inhalation rate was the recommended mean long-term exposure value for inhalation of combined males and females, based on age (range: 12.9–16 m^3/day) (USEPA, 2011a). The absorption fraction for PCBs from air in the lungs was assumed to be 100%, and measured air concentrations in residences were assumed constant and consistent within the various rooms of a single apartment.

$$EDI_{inhalation} = \frac{C_{air} \times inhalation\ rate \times exposure\ duration \times absorbance\ fraction}{body\ weight} \quad (1)$$

2.3.3. Dermal absorption

Exposure to PCBs through dermal absorption was estimated in two ways – from indoor air concentrations and from a hand wipe, which was taken from both palms. Hand wipes potentially capture dermal uptake via air and surface contact, whereas estimations from air concentrations likely reflect transdermal transport from chemicals in the gas phase in indoor air. Dermal uptake can also vary based on behaviors such as handwashing, which is likely to remove some particles and PCBs on the skin surface; however, this is not considered here, and participants were asked to not wash their hands for at least 30 min prior to study visits.

In calculating an EDI, air and hand wipe concentrations were used to determine a gas-phase concentration of individual PCBs and a concentration in surface skin lipids, respectively, and then calculate the transdermal flux, J, of transport from the boundary layer (air) or skin surface lipids (hand wipe) to the dermal capillaries at steady state (Weschler and Nazaroff, 2012, 2014). J was applied to total body surface area, which was estimated by age and sex of the participant and ranged from 1.69 to 2.15 m^2 (USEPA, 2011b). For calculating dermal exposure, measurements of PCB concentrations in air and on wiped hands are assumed to represent daily (24-h) exposure, where people are exposed on a continual basis via a personal cloud effect for air (e.g., clothing contribution) or via a constant skin surface PCB concentration. As such, the exposure duration is assumed to be 24 h/day.

$$EDI_{dermal} = \frac{J \times total\ body\ surface\ area \times exposure\ duration}{body\ weight} \quad (2)$$

Additional details for calculating the transdermal flux, J, for each method are included in Supplementary Information (**SI Section S1 and S2**).

2.3.4. Diet

Diet is considered a major pathway of exposure for PCBs because of their persistence in the environment and accumulation in food chains. Estimates of dietary intake of PCBs from food was only available for 8 of the 15 PCB congeners measured in the samples (the 7 indicator PCBs and PCB-105) for the adult Danish population. These were estimated from multiple food sources for individuals in Denmark, ages 15–75 years, with food samples taken between 1998 and 2003 (Fromberg et al., 2011). PCB concentrations in food were used to calculate an intake per age group using a typical Danish diet from that time period. PCB exposure from food in Denmark is expected to have decreased slightly since then, whether from changes in PCB levels in food and/or fish consumption, and whole diet adult exposure estimates for summed PCBs were roughly 3 times lower when assessed in 2004–2011 (Duedahl-Olesen et al., 2020); however, Fromberg et al. (2011) was the most recent report of overall dietary exposure to individual PCBs in the Danish population. We assume here that dietary consumption of PCBs in food for the participants during this study period is similar to this report, and thus, the dietary estimates calculated here could be slightly overestimated. Gastrointestinal (GI) uptake is assumed to be 90%, which was estimated from studies assessing dietary absorption of PCBs (Andreas Moser and McLachlan, 2001; Aylward et al., 2014; Ritter et al., 2011).

$$EDI_{diet} = \frac{Dietary\ intake \times GI\ uptake\ fraction_{diet}}{body\ weight} \quad (3)$$

2.3.5. Pathway-specific body burden

For exposed residents, internal dose of PCBs was assessed using EDIs from each exposure pathway to predict body burden concentrations for individual congeners. The body burden concentrations (C_{PCB}(t) [ng/g lipid]) were calculated to reflect presumed body burden (i.e., presence of PCB in the body) from a specific exposure pathway at time of sampling, while considering the number of years participants were exposed in their homes and the half-life ($t_{1/2}$) of the congener. This was intended to serve as a back calculation to determine how much PCB (i.e., dose) could be attributed to a specific pathway of exposure and then compare to the measurement in serum, which incorporates all pathways of exposure. A constant dose by each pathway over time was assumed, even though certain exposures, like diet, are likely to have changed over time. This also includes the assumption that measurements (e.g., PCBs in indoor air, dust and hand wipes) and parameters (e.g., body surface area, food consumption, and lipid mass) are constant over time. Pathway-specific EDIs were converted to a daily dose (i.e., mass of PCB per day), then normalized to body lipid (BL), which was calculated based on BMI and age, as in Aylward et al. (2014) (**SI Section S.4**). Sums of these body burden concentrations were compared then to serum concentrations, as were previously reported in Frederiksen et al. (2020), to assess the validity of these calculations. These were also assessed via Spearman correlations. Serum measurements for specific congeners were considered if detection frequency was >70%, and non-detects in serum were imputed to the limit of quantification (LOQ)/2.

PCB body burdens were assessed in a one-compartment, first-order model, as outlined in Lorber (2008) and Aylward et al. (2014). Since the residents all lived in the homes for different lengths of time, and the samples as well as personal measurements were cross-sectional, time (t) as the exposure period was assessed based on the time residents reported living in the contaminated apartment. These calculations were only conducted among the exposed population, as the body burden at time 0, $C_{PCB}(0)$, which was defined as the baseline body burden of PCBs at the time of move-in to a contaminated apartment, was calculated from pathway-specific median EDIs from the reference population. The first-order dissipation rates of specific congeners in the body, k, was represented by $\ln 2/t_{1/2}$ [day^{-1}]. Half-lives used were intrinsic human elimination half-lives for individual congeners, by Ritter et al. (2011) for the majority of compounds. The half-life for PCB-101 was calculated by Schettgen et al. (2012) (Table S2).

$$Change\ in\ PCB\ over\ time = \frac{\partial C_{PCB}}{\partial t} = \frac{EDI_{pathway}}{BL\ (t)} - kC_{PCB}(t) \quad (4)$$

$$C_{PCB}(t) = C_{PCB}(0)^{*}e^{-kt} + \frac{EDI_{pathway}(t)}{BL\ (t)}{}^{*}\frac{1-e^{-kt}}{k} \quad (5)$$

A simulated example for an average exposed participant was also calculated for PCB-28, comparing exposure via inhalation and dermal uptake across the range of years lived in a contaminated apartment as reported within the study population.

3. Results & discussion

3.1. Study population & PCB measurements

Residents from both non-contaminated (n = 23) and contaminated buildings (n = 71) provided personal and environmental samples from their home environments. Four residents from contaminated buildings were excluded from analyses due to incomplete sample sets (i.e., missing dust, air, or hand wipes). As such, the total sample population was 90, with 23 from non-contaminated residences and 67 from contaminated ones. In general, characteristics of the study population, comparing the

exposed and reference groups, were very similar (Table S3), as previously observed by Frederiksen et al. (2020). These similarities expanded beyond physical characteristics of participants to years of living in their current residence and the time spent daily in their homes. As such, the primary difference between the groups was their exposure to PCBs via contaminant sources within their individual apartments. Notably, individuals in the exposed population had been living in contaminated apartments for an average of 15.5 years (Table S3). Two participants included in the reference population had briefly lived in a PCB-contaminated apartment within Brøndby Strand Parkerne (<5 years), but their move-out occurred >10 years prior to sampling. This also occurred among the exposed population, with some participants (n = 11) having lived previously in one contaminated apartment and moved to another contaminated apartment within the housing estate, hence the difference in years in current residence and exposed time period (Table S3). Questionnaire responses also reported that only one participant had worked directly with PCBs for a short time early in their career (i.e., over 30 years prior to study sampling), and nearly all others had not worked in a PCB-contaminated building to their knowledge, indicating that PCB exposure was likely attributed to the home environment.

Measurements in individual matrices have been previously reported in depth (Andersen et al., 2020; Frederiksen et al., 2020). Briefly, active air samplers, dust, hand wipes, and serum were analyzed for 15 PCB congeners. Among these matrices sampled from the exposed population, detection frequencies were >70% for individual congeners, with the exception of air for PCB-138 and -180, and serum for PCB-101. PCB congeners were detected notably less in reference population samples, with only 8 of 15 congeners detected in >70% of samples in both air and dust (Andersen et al., 2020). Previous reports of PCBs from hand wipes and serum examining home environments are limited, and in general, participants living in the contaminated apartments had lower levels in serum and on hand wipes than those who had been occupationally exposed (e.g., capacitor workers, e-waste recycling workers) and higher

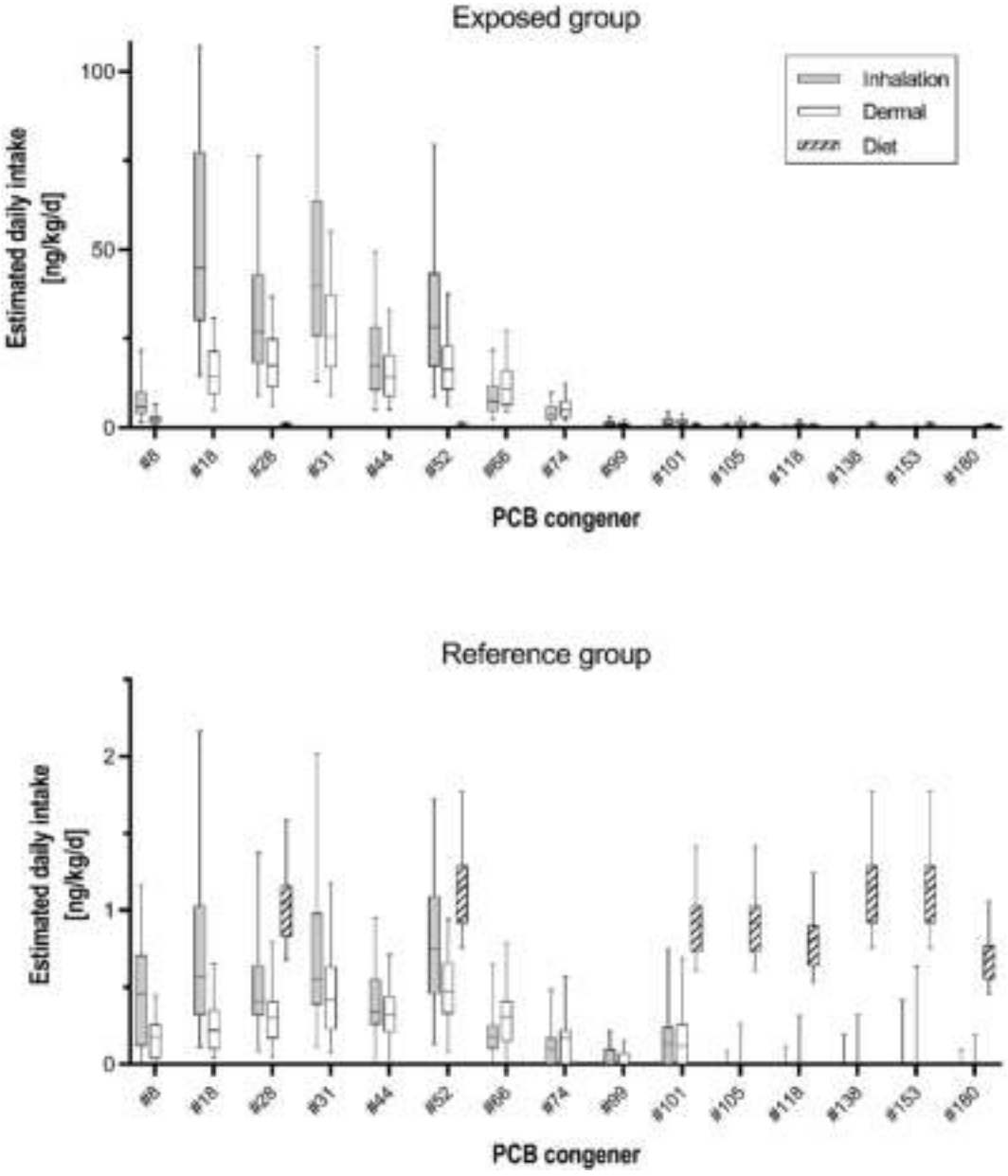

Fig. 1. Estimated daily intakes (interquartile range and 5-95% interval) for specific PCBs from inhalation, dermal absorption, and diet are shown for the exposed (above) and reference (below) populations. Dietary exposure was only available for PCB-28, 52, 101, 118, 138, 153, and 180.

serum levels than most previous evaluations in general populations (Frederiksen et al., 2020).

3.2. Dominant pathways of PCB exposure

3.2.1. Exposed population

Among the exposed population, inhalation was the most prominent pathway of exposure for PCBs, accounting for roughly 60% of the estimated daily intake (EDI) of PCB_{sum7} (Figure S1). For all individual PCBs up to PCB-101, inhalation of contaminated air was the largest contributor to PCB EDI, with a median inhalation EDI for PCB_{sum7} of 57 ng kg bw^{-1}·day^{-1} (Fig. 1). The large variation in EDI observed among the exposed participants, particularly for inhalation (Fig. 1), is due to the differences in both the PCB contamination level of the apartment and individual participant behaviors (e.g., time spent in the home daily). In addition, the median inhalation-specific exposure estimates over the course of a year was 1690 μg yr^{-1} for PCB_{sum7} and 5340 μg yr^{-1} for the 15 PCB congeners measured. When comparing these estimates to those calculated in Ampleman et al. (2015), this study would be among the highest observed exposure estimates in studies of contaminated environments and ambient air. Specifically, estimates from this study were similar in magnitude to residents in other studies of contaminated Danish homes (median = 1100 μg yr^{-1} for 24 measured PCBs) and slightly lower than workers in contaminated schools in Germany (median = 10000-36000 μg yr^{-1} for 6 PCBs, which overlap with PCB_{sum7}).

Dermal uptake was observed to be a significant secondary pathway of exposure, accounting for about 35% of the EDI for PCB_{sum7} (Figure S1). Together with inhalation, the two pathways accounted for about 95% of the EDI for PCB_{sum7}. The role of dermal absorption has often been neglected or underestimated (Weschler and Nazaroff, 2012), and thus, estimates and risk assessment of PCB exposure have relied on inhalation alone (Jensen, 2013). The calculations shown here demonstrate that while inhalation is the primary pathway of exposure, the dermal contributions can be substantial for some congeners and add significantly to overall PCB exposure. This is worth considering in terms of risk assessment and regulatory action as well as remediation, given how elevated the total EDIs were for exposed residents.

For the higher chlorinated PCBs (PCB-138, -153, −180), diet still played a prominent role, contributing about 7% to the PCB_{sum7} EDI and a large share of the calculated intake for these individual congeners. However, estimated total exposure and overall concentrations of these congeners in environmental samples were low, compared to the lower chlorinated PCBs, and thus, these constituted minor components of total PCB exposure overall.

The other two pathways (hand-to-mouth contact and inadvertent dust ingestion) contributed minimally to overall exposure, less than 0.5% each of PCB_{sum7} EDI (Figure S1). For both of these pathways, exposure is expected to be low based on physical-chemical properties of PCBs allowing for increased partitioning of PCBs to air compared to dust or being particle-bound (Andersen and Frederiksen, 2021). Given the high PCB content measured in the indoor dust of the contaminated apartments, these exposure pathways would likely be more important among children, who are closer to the ground, consume more dust inadvertently, and more frequently touch their hands to their mouths. This was observed in other previous evaluations, where non-inhalation sources such as diet were considered to be more prominent exposure pathways to PCBs for toddlers living in presumably non-contaminated residences, as compared to adults (Harrad et al., 2006). Individuals under age 18 were not included here; however, it is worth noting that children in various stages of development could have different relevant exposure pathways for PCBs while living in contaminated homes.

3.2.2. Reference population

Among the reference population, diet was the primary pathway of exposure, as evidenced by having the most prominent EDI among individual congeners and PCB_{sum7} (median PCB_{sum7} for diet = 7 ng kg bw^{-1}·day^{-1} and for all other pathways = 2 ng kg bw^{-1}·day^{-1}). PCB exposure via inhalation was the second most prominent pathway, particularly for lower chlorinated PCBs (Figure S1). Previous studies have estimated that up to 90% of human exposure to persistent organic pollutants (POPs) including PCBs are from dietary sources, and particularly fish (Darnerud et al., 2006; Fromberg et al., 2011). This is reflected in the reference group exposure and relative contributions of individual pathways, with roughly 80% of the EDI of PCB_{sum7} attributed to diet (Figure S1). The estimated intake via diet was also similar to EDIs reported in previous studies evaluating dietary exposure to PCBs across Europe and in Canada in the 1990s and 2000s (Aylward et al., 2014). The smaller contributions of PCB exposure from inhalation and dermal absorption are likely due to background air concentrations in the reference apartments. While notable, these air concentrations are comparable to measurements conducted in other uncontaminated Danish homes (Frederiksen et al., 2012), and adult inhalation exposure estimates are similar to air measurements in other non-contaminated indoor environments (e.g., homes, cars, offices) (Harrad et al., 2006). Again, hand-to-mouth contact and inadvertent dust ingestion contributed minimally, if at all, to PCB exposure among the reference population (Figure S1), which can be attributed to very low levels of PCBs measured on hands and in dust in non-contaminated environments. This demonstrates that there are still sources of PCB exposure outside of diet, and people are likely exposed to them on a daily basis, albeit minimally.

3.2.3. Comparison of study populations and relevant regulations

Among the exposed group, median PCB EDI across the 15 congeners, excluding dietary contributions, was 281 ng kg bw^{-1}·day^{-1}. Compared to similar reference group estimates (median Σ_{15}PCBs = 6 ng kg bw^{-1}·day^{-1}), residents living in contaminated apartments were exposed to PCB levels around 40 times higher than the reference group. Dietary contributions were excluded within this calculation due to the lack of dietary information available for several of these congeners. When including diet for the PCB_{sum7}, which constituted 7 ng kg bw^{-1}·day^{-1} for both groups and thus incorporating all measured pathways of exposure, the median PCB EDI was 101 ng kg bw^{-1}·day^{-1} for the exposed and 9 ng kg bw^{-1}·day^{-1} for the reference group. As such, with the inclusion of diet, the residents of contaminated apartments still experienced roughly 10 times higher exposure or daily intake across the sum of indicator PCBs. This further emphasizes the importance of monitoring indoor environments and underlines how contamination sources within residences can contribute heavily to personal exposure.

Currently in Denmark, there is no set tolerable daily intake (TDI), and as previously mentioned, current recommended action values rely on indoor air levels based on an older German TDI of 1 μg kg bw^{-1}·day^{-1}. This German TDI was established from a toxicological study with long-term exposure of rats to a technical PCB mix and typically compared to air concentrations by multiplying 5 times a sum of 6 indicator PCBs, which overlaps with the seven here (Jensen, 2013). With a median measured EDI across all routes of exposure for PCB_{sum7} of 101 ng kg bw^{-1}·day^{-1} (which is about 505 ng kg bw^{-1}·day^{-1} after accounting for the corrections factor), nearly all of the exposed population falls below the established German TDI, with only individuals above the 95th percentile exceeding the limit. The French Food Safety Authority (AFSSA) TDI recommends that the sum of 6 indicator PCBs should not exceed 10 ng kg bw^{-1}·day^{-1} (AFSSA, 2007; Duedahl-Olesen et al., 2020), which was based on the World Health Organization (WHO) discussion of PCB TDI and does not leave any room for exposure from sources other than food. A similar TDI of 20 ng kg bw^{-1}·day^{-1} for Aroclor 1254 was recommended by the WHO, although the same TDI had been proposed for the sum of all 209 congeners (AFSSA, 2007; Faroon et al., 2003; Jensen, 2013). In this case and with the consideration of multiple pathways of exposure, all of the exposed group would exceed AFSSA values. With the stringency of the AFSSA TDI, a few of the reference group also exceeded the AFSSA limit with background

concentration exposures to PCBs.

3.3. Dermal absorption from air and hand wipes

The EDI for dermal absorption was calculated using both PCB concentrations from indoor air and hand wipes from participants' palms, based on equations described in Weschler and Nazaroff (2012). EDIs calculated using both methods were very similar, and the EDIs from indoor air are presented for comparisons to other exposure pathways. Among the exposed population, the two differently derived EDIs were highly correlated ($r = 0.94$, $p < 0.0001$, Figure S2) for the 15 PCB congeners assessed. Their median values were also very similar, indicating that the two methods could be interchangeable and are strongly related (Table S4). Although less congeners were detected in indoor air of reference apartments (i.e., lacking detection of the higher chlorinated PCBs), the same trend was observed among the reference participants as well (Table S4).

These two methods of calculating dermal absorption estimate the same outcome; however, the hand wipe is expected to yield a higher estimate than indoor air because the wipe, particularly of the palms, would capture surface contact in addition to partitioning from air to skin. A skin wipe without the potential for surface contact, such as a wipe of the backs of hands, would likely better reflect dermal absorption from air concentrations, although the two wipes have been shown to be correlated (Yang et al., 2019). From a small number of exposed residents in this study ($n = 6$), both wipes of palms and backs of hands were collected and analyzed separately (Frederiksen et al., 2020; SI). Palm wipes contained slightly higher concentrations of PCBs than the back-of-hand wipes; however, there was not a clear trend across the measured PCBs. Similarly, median dermal EDIs were not consistently higher among hand wipe estimates compared to indoor air, and an obvious trend was not evident (Table S4). The high correlation between the two separate measures across congeners, which cover a wide range of physicochemical properties, could be because the individuals' skin surfaces are approximately at equilibrium with the air in the contaminated apartments. This is assumed in the calculations in Weschler and Nazaroff (2012). The concurrence between the two methods could indicate that a measurement of PCBs in indoor air or skin wipe would yield similar results for the consideration of personal exposure. Any differences between these two calculation methods are likely due to assumptions made regarding PCB concentrations on the hands (i.e., skin surface lipids on palms are likely to be a thinner layer than average body skin lipid thickness) as well as possible differences in estimating parameters (e.g., mass-transfer coefficient, for transdermal permeation from indoor air, and partitioning coefficients, such as Henry's Law constant). Nonetheless, because the hand wipes could be incorporating surface contact with air-to-skin partitioning, the dermal EDI calculated from indoor air was used here in all comparisons to the other pathways of exposure for PCBs.

Previous estimations of dermal absorption from skin wipes or other samples have been calculated frequently using a dermal absorbance fraction, utilizing a range of factors from 14% (Wester et al., 1993) to near 100%, including congener-specific absorbance fractions (Ertl and Butte, 2012; Garner and Matthews, 1998). However, Kissel (2011) pointed out that fractional absorption misrepresents dermal absorption, due to skin loading conditions and flux considerations. In this case, there is likely an 'infinite supply' of PCBs to the skin (i.e., more PCB than can be absorbed by a person's skin). As such, conditions for dermal absorption would likely be flux-limited, based on Kissel's argument. Fractional absorption would not be appropriate for our assessment, particularly with the conditions of contaminated residences as the exposure is likely continuous; thus, we instead utilized transdermal flux to determine PCB partitioning through the skin and into the dermal capillaries for our calculations of dermal absorption.

3.4. Body burden in exposed residents

Pathway-specific body burdens were calculated for the seven indicator PCBs and their sum for the exposed population, with the reference group exposure medians serving as the baseline measurement, $C_{PCB}(0)$. This allowed for comparison to serum, as in a pathway-specific exposure contribution to PCB presence in serum over time. Similar to results from the EDI calculations, inhalation exposure contributed the most to PCB body burden among exposed participants, and PCB-28 was the most prominent congener of the seven indicators (Fig. 2). Again, dermal absorption accounted for significant exposure as well (Fig. 2). As expected, diet appeared to play a larger role among the higher chlorinated PCBs (PCB-118 and larger); however, this contribution was small compared to the overall PCB sum relative to the body burden of the lower chlorinated PCBs.

Numerous studies have investigated associations between indoor air and serum and have demonstrated that PCBs in indoor air are positively and significantly associated with serum PCB concentrations, particularly for lower chlorinated congeners (Ampleman et al., 2015; Kraft et al., 2018, 2021; Meyer et al., 2013). However, no studies to the authors' knowledge have previously conducted a similar assessment, evaluating pathway-specific body burden including dermal uptake and based on PCB half-lives and cumulative exposure over time. One such evaluation was conducted by Lorber (2008) for the flame retardants polybrominated diphenyl ethers, where dermal contact with dust was considered. This lack of assessments is likely due to the fact that it has been rare to find such high contamination in home environments and elevated serum levels among residents (Frederiksen et al., 2012, 2020; Meyer et al., 2013). Other studies of PCB exposure have largely focused on diet, specific occupational exposures or exposures within contaminated schools or office buildings (Ampleman et al., 2015; Aylward et al., 2014; Herrick et al., 2016; Kraft et al., 2018).

As PCB-28 was the most abundant individual contributor of measured congeners to overall PCB body burden, inhalation and dermal pathways of uptake into the body were modeled based on median indoor air concentrations and compared across a range of residence times (1–45 years) for this congener specifically. Assuming constant air concentrations and consistent daily exposure, body burden of PCB-28 appeared to plateau at roughly 20 years of living in a contaminated apartment (Figure S3). Uptake of PCB-28 from living in a contaminated home is fastest during the first decade of living in that residence. The median years of residence in the exposed group was 15.5 years, and about one-third of the residents had lived in a contaminated apartment for over 20 years (Table S3). This suggests that for roughly one-third of residents within the exposed group, body burdens of PCB-28 would have stabilized by the time of sampling, based on solely inhalation and dermal absorption estimates.

Further, as the two largest contributors to the overall PCB_{sum7} body burden across pathways, PCB-28 and -52 were significantly correlated with their corresponding measured serum concentrations ($r_s = 0.49$, 0.45; $p < 0.001$), while the other individual congeners were, in general, positively correlated (Fig. 3). When compared to PCB-52, the estimated body burden sum for PCB-28 congregated more closely to the 1:1 line, indicating that PCB-28 body burden estimates better matched the actual measured serum values. The calculated PCB_{sum7} body burden was also significantly and positively correlated with the summed PCBs measured in serum ($r_s = 0.45$, $p < 0.001$; Fig. 3); however, this is likely driven by the dominance of the body burden of PCB-28 and more limitedly PCB-52. A lack of correlation was observed for PCB-101 to −138 ($r_s = -0.01$-0.15), and relatively weak ones for PCB-153 and -180 ($r_s = 0.21$, 0.25) (Fig. 3), pointing to a general underestimation of body burden by the model. This may be due to the restriction of having evaluated diet over residence time rather than residents' age, as these congeners have long half-lives and are typically attributed to dietary exposure across a lifetime. Such an underestimation could indicate the need for inclusion of a lifelong dietary exposure estimate within future

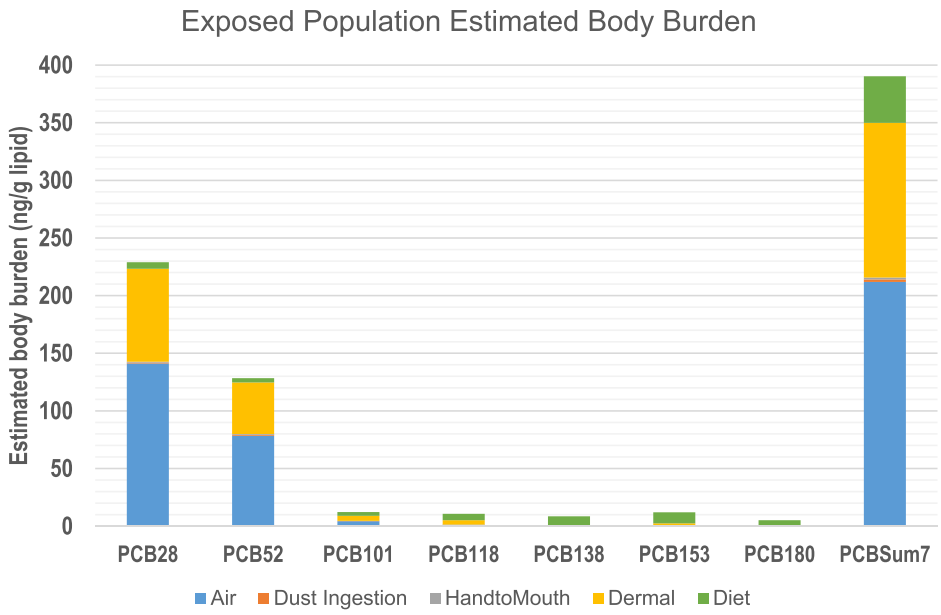

Fig. 2. Median pathway-specific body burdens were calculated for each PCB congener and sum of 7 indicator PCBs for the exposed population. These were normalized to individual estimation of body lipid mass and account for the time participants lived in the exposed apartments, indicating a body burden at the time of sampling while assuming a constant EDI over time

models.

Despite several promising positive correlations, there are evident gaps in the actual numerical body burden values, particularly for PCB-52, which may be caused by a number of factors. The half-life parameter used here could have influenced body burden estimations, as studies have reported a wide range of intrinsic PCB half-lives. We utilized half-life estimations from Ritter et al. (2011) and Schettgen et al. (2012), as they agreed with each other and a number of other studies; however, it is possible that overestimations of the lower chlorinated PCB half-lives and under-estimations of the larger PCB half-lives could have led to the gaps observed for comparing body burden to serum measurements. For instance, a more recent paper by Esser et al. (2021) suggested a half-life of 0.8 y for PCB-52; compared to the half-life of 2.6 y estimated by Ritter et al. (2011), this would yield a much smaller body burden and possibly better estimate serum measurements. For the higher chlorinated PCBs, it is possible that some of the older and longer-term residents may have consumed more PCB in their food (e.g., fish) years before, and thus the dietary estimates presented here were underestimated for the higher chlorinated PCBs. Further, air data was not available for some of the higher chlorinated PCBs, which could have contributed to lesser estimations of exposure to these larger PCBs, since we utilized indoor air concentrations for the calculations of body burden from dermal absorption. However, the magnitude of the dermal estimated doses, when compared between air and hand wipes, suggests that such dermal estimations were likely very small (Table S4). Further explanation for observed differences could include assumptions included in the model, such as assuming constant environmental conditions, equivalent intakes daily, and consistent and maximum flux for dermal uptake throughout the exposure period. In general, the exposure estimates from living in the contaminated homes reflected overall residents' PCB exposure and internal dose measurements, indicating that measurements in home environments could be adequate for future risk assessment.

3.5. Study limitations

Although extensive measurements were conducted among study participants and their respective residences, evaluation of how they have been exposed to PCBs and which route contributed most is not without limitations. First, inherent to calculation of an EDI are a series of behavioral parameters, which were not all measured or queried via questionnaire and thus approximated with the best available information. These variables, such as inhalation rate and total body surface area, were taken from the USEPA Exposure Factors Handbook with estimations based on age and sex of participants.

In addition, recruitment of study participants resulted in a skewed overall study population, which limited our ability to conduct more extensive and sophisticated analyses to compare the two exposure groups. Extensive efforts were made to recruit both exposed and reference participants; following recruitment, all interested participants were included within the study, resulting in the sample sizes here. While this may limit our analyses in comparing the study groups, stratification or limiting analyses to only exposed individuals allowed us to evaluate and compare the relative exposures and estimated intake. As such, we do not expect the sample size inequality to affect the internal validity of our results. We acknowledge, nonetheless, that the sample sizes did not allow us to investigate the impact of other behavioral parameters (e.g., smoking) since very few individuals in both study groups indicated that they were current smokers (Frederiksen et al., 2020).

Estimations of dermal uptake here assumed absorption from the total body skin surface and did not take the role of clothing or other linens which come in contact with the skin (e.g., blankets, sofas coverings, and bedsheets) into consideration. Previous work in the same apartments had assessed nine types of clothing fabrics for absorption of PCB-28 and -52 and indicated that clothing hanging in the apartment can serve as a reservoir for PCBs, thereby potentially contribute to continuous dermal absorption when individuals are not in their contaminated apartment (Morrison et al., 2018). Laundering of clothes and linens could remove a large portion of PCB content following contamination; however, these materials acting as contributors or barriers to exposure depend on time between washings and their exposure to PCB-contaminated air (Kolarik and Morrison, 2022). Based on this assessment, we assumed absorption from the total skin surface as a conservative estimate, while noting that not enough is known currently about the role of clothing as a source or barrier to dermal uptake.

Further, all estimations of body burden assume constant exposure for each pathway as integrated over the exposure period, which is

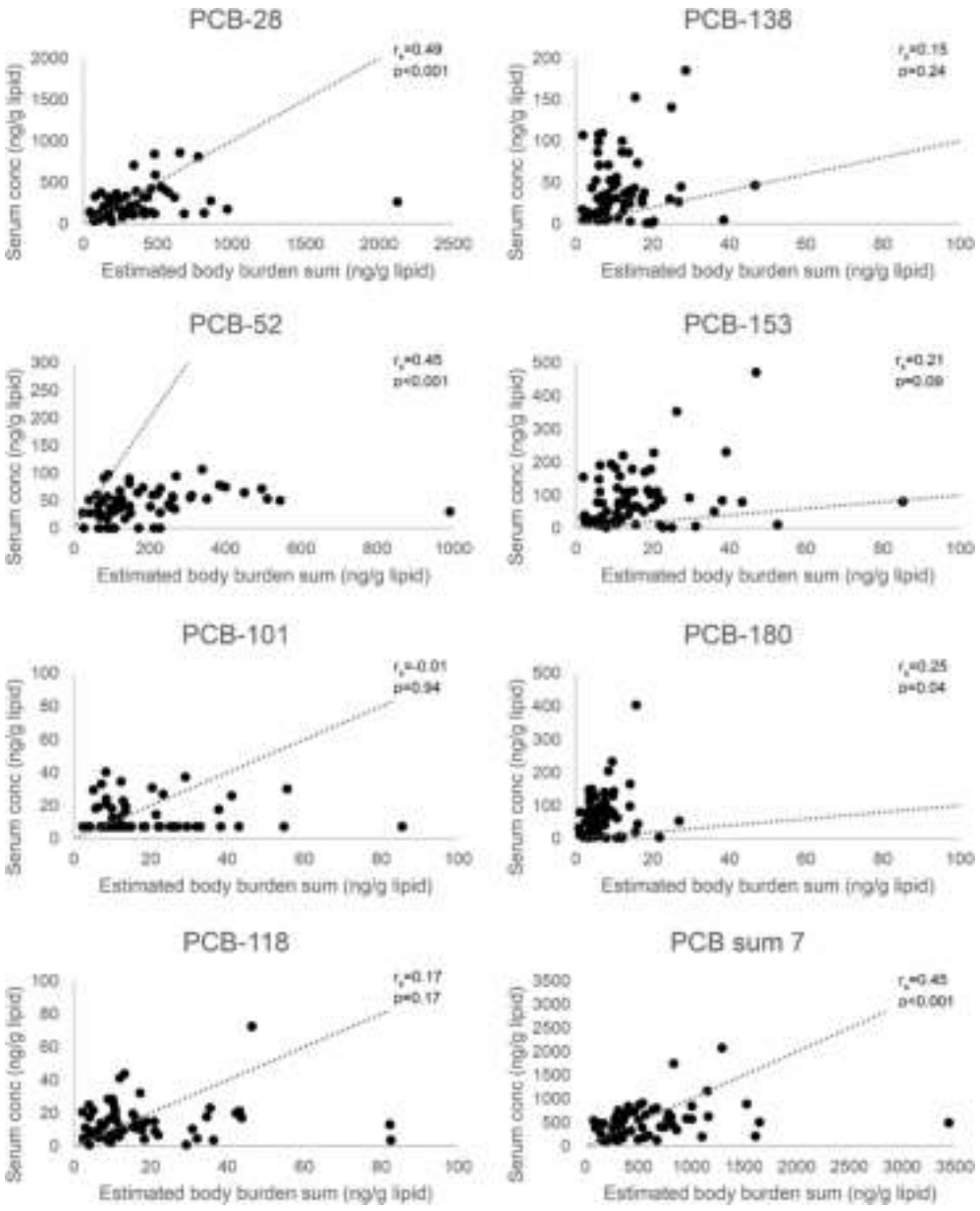

Fig. 3. Estimated body burden of individual exposed residents from all calculated pathways, normalized to individual body lipid mass, was compared to serum measurements for individual and summed PCBs. A 1:1 trendline is presented, demonstrating where body burden over- or under-predicted measured serum levels.

inherently the entire time in which participants have lived in their current residence. Samples within the homes were taken at one time point and then used to estimate exposure retrospectively. While the sample measurements themselves had a high level of validity, PCBs in indoor dust can be fairly consistent over time ($t_{1/2}$ = 5–18 years) whereas indoor air concentrations may fluctuate with temperature and season (Andersen et al., 2021; Whitehead et al., 2014). As such, these measurements may not be representative of many years of exposure, particularly if certain residents moved into the apartments when they were first built. Body burden calculations also assume similar body lipid mass, which is likely to fluctuate throughout a person's life by virtue of age and other factors. Other behaviors reported by participants at time of sampling such as time spent at home could also shift over time, and equating exposure period to number of days exposed does not take into account any time that participants spent away from their homes (e.g., holidays, visiting friends and family).

With regard to diet, calculations of dietary exposure were based on PCB content in food measured in Denmark in the late 1990s to early 2000s (Fromberg et al., 2011). PCB content has been slowly decreasing in food, including in fish and seafood, since PCBs have not been actively applied in decades (Saktrakulkla et al., 2020), and this has also been the case for dietary exposure in Denmark (Duedahl-Olesen et al., 2020). Thus, between the decreasing PCB content in food and less fish consumption, our estimations of dietary PCB exposure are potentially overestimations of the actual dietary contribution. This effect could be modulated by age and habits of residents, including dietary consumption prior to living in the contaminated apartments. We restricted the consideration of dietary intake only to the years in which residents lived in the apartments, for the purpose of comparing pathways of PCB exposure while recognizing that this assumption could underestimate lifetime dietary exposure, particularly for some of the higher chlorinated PCBs. Dietary information was only available for 8 of 15 PCB congeners; however, it is likely that the lower chlorinated PCBs were not as abundantly present in food as some of the higher chlorinated ones, such as PCB-138 and -153, and thus may not contribute significantly to overall exposure (Fromberg et al., 2011). The relative importance of exposure

pathways will be somewhat dependent on which congeners are included. In addition, exposure via diet did not consider potential deposition of PCBs onto food surfaces (i.e., partitioning of PCBs from air, dust, or airborne particles to food) while in contaminated apartments prior to consumption of the food.

Finally, physicochemical properties of the PCB congeners and any necessary adjustments with temperature (25 °C to skin surface temperature, 32 °C) were estimated primarily using SPARC software. In particular, we relied on Henry's Law constant, H, and log K_{oa} for calculating dermal absorption. Previous work has demonstrated that there are systematic inconsistencies across PCB congeners for certain experimental and modeled estimates of these parameters, which have been discussed in the context of air and dust partitioning (Andersen and Frederiksen, 2021). Here, we have utilized SPARC exclusively for calculating physicochemical properties, due to their consistency with other published values for PCBs (Li et al., 2003). So, any uncertainty within these properties, specifically with Henry's law constant as it relates to dermal uptake from indoor air, could have an effect on the magnitude of these results; however, the trends observed are not likely to deviate tremendously and dermal estimations from air tracked closely with hand wipe data, suggesting that these parameters would not impact the interpretation of our results here.

4. Conclusion

Here, we present estimations of daily intake and pathway-specific body burden of a suite of PCBs for residents of contaminated apartments and a related reference group. With high PCB concentrations in indoor air of contaminated residences, particularly of lower chlorinated PCBs, inhalation was the primary pathway of exposure for residents, and inhalation and dermal absorption combined accounted for roughly 95% of total estimated daily intake of the sum of 7 indicator PCBs. Among the reference group, diet was the primary pathway of exposure, confirming that general exposure to PCBs still comes predominantly from food sources. Assessments of body burdens across relevant pathways for PCB-28 and -52, as well as PCB_{sum7}, from these exposure estimations were significantly correlated to serum measurements, suggesting that these models could potentially predict internal dose over time. However, differences in the values between body burden and measured serum suggest that more detailed environmental and behavioral characterization, including lifelong dietary consumption, in exposure assessment as well as further investigations into PCB half-lives and partitioning characteristics should be considered. This evaluation also highlights the importance of considering dermal absorption for future risk assessment and remediation measures, which has been largely neglected in exposure assessment, but could contribute substantively to overall exposure.

Credit author statement

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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.2022.114056.

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Interpreting biomonitoring data: Introducing the international human biomonitoring (i-HBM) working group's health-based guidance value (HB2GV) dashboard

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ABSTRACT

Human biomonitoring (HBM) data measured in specific contexts or populations provide information for comparing population exposures. There are numerous health-based biomonitoring guidance values, but to locate these values, interested parties need to seek them out individually from publications, governmental reports, websites and other sources. Until now, there has been no central, international repository for this information. Thus, a tool is needed to help researchers, public health professionals, risk assessors, and regulatory decision makers to quickly locate relevant values on numerous environmental chemicals. A free, on-line repository for international health-based guidance values to facilitate the interpretation of HBM data is now available. The repository is referred to as the "Human Biomonitoring Health-Based Guidance Value (HB2GV) Dashboard". The Dashboard represents the efforts of the International Human Biomonitoring Working Group (i-HBM), affiliated with the International Society of Exposure Science. The i-HBM's mission is to promote the use of population-level HBM data to inform public health decision-making by developing harmonized resources to facilitate the interpretation of HBM data in a health-based context. This paper describes the methods used to compile the human biomonitoring health-based guidance values, how the values can be accessed and used, and caveats with using the Dashboard for interpreting HBM data. To our knowledge, the HB2GV Dashboard is the first open-access, curated database of HBM guidance values developed for use in interpreting HBM data. This new resource can assist global HBM data users such as risk assessors, risk managers and biomonitoring programs with a readily available compilation of guidance values.

Abbreviations

ADI	Acceptable Daily Intake
BE	Biomonitoring Equivalent
CHMS	Canadian Health Measures Survey
DEHP	di-(2-ethylhexyl) phthalate
GV	Guidance Value
HB2GV	Human Biomonitoring Health-Based Guidance Value
HBM	Human Biomonitoring
HBM-GV	Human Biomonitoring Guidance Value
HBM4EU	Human Biomonitoring for Europe
i-HBM	International Human Biomonitoring Working Group
IBN	International Biomonitoring Network
ISES	International Society of Exposure Science
LOD	Limit of Detection
LOQ	Limit of Quantitation
NHANES	National Health and Nutrition Examination Survey
PBPK	Physiologically-Based Pharmacokinetic
PFHxS	Perfluorohexane sulfonate
RfD	Reference Dose
RV	Reference Value
QA/QC	Quality Assurance and Quality Control
TDI	Tolerable Daily Intake

1. Introduction

Scientists use numerous approaches for evaluating human exposures to environmental chemicals. One approach – human biomonitoring (HBM), or the measurement of chemical biomarkers in human matrices such as urine or blood – has been referred to as the gold standard of exposure characterization (Sexton et al., 2004). Since the 1990s, the use of biomonitoring to characterize human exposures has experienced unprecedented growth (Angerer et al., 2007; Calafat 2016; Paustenbach and Galbraith 2006; Pirkle et al., 1995; Sobus et al., 2015; US EPA 2012), to some degree supplanting other approaches. Biomonitoring has been used in exposure and epidemiology research globally (NRC 2006). It has also been used to obtain nationally representative information on human exposures to a large number (e.g., hundreds) of chemicals (Apel et al., 2017; Bastiaensen et al., 2021; Centers for Disease Control and Prevention, 2022; Fillol et al., 2021; Gilles et al., 2021; Health Canada 2021; Hong et al., 2021; Jeon et al., 2021; Jung et al., 2022; Liao et al., 2021; Schoeters et al., 2017; Schulz et al., 2021; Seifert et al., 2000; Seo et al., 2021; VITO 2022).

HBM data can provide insight into overall population exposures (Angerer et al., 2007, 2011). HBM data or biomarker concentrations from a sample population may also be compared to reference concentrations from national surveys or large population studies. HBM data from such surveys or studies constitute a relevant source to determine reference values (RVs). RVs are particularly relevant in the absence of HBM health-based guidance values (HB2GV), either when such values are not available or cannot be derived (Vogel et al., 2019). The RVs are based on the upper end of the exposure distribution, such as 95th percentile concentrations and may be used for comparisons (Vogel et al., 2019).

Risk assessors can also compare biomarker concentrations measured in specific contexts or populations to health-based guidance values derived from human or animal data using uncertainty factors or

modelling approaches, such as physiologically-based pharmacokinetic (PBPK) modelling. Most guidance values (with a few exceptions described later in this paper) are derived from toxicological studies that use dose information (e.g., mg/kg bw/day of a chemical entering the body), but biomonitoring data describe the bioavailable concentration from all sources in a given human matrix (e.g., ng/mL blood). Connecting a dose such as a Reference Dose (RfD) to a biomonitoring concentration requires additional processes. Specifically, modelling efforts can convert dose-based guidance values to concentrations in blood or urine (Hays et al., 2008; Pletz et al., 2020). These efforts have resulted in a large number of human biomonitoring health-based guidance values (HB2GVs), but to locate these values, interested parties need to seek them out individually from publications, governmental reports, websites and other sources. Until now, there has been no central, international repository for this information. Thus, a tool is needed to help researchers, public health professionals, risk assessors, and regulatory decision makers quickly locate relevant data on numerous environmental chemicals.

An effort to develop a repository for health-based guidance values to facilitate the interpretation of HBM data started with the formation of the International Biomonitoring Network (IBN) in 2018. The IBN objectives were to enable knowledge exchange, collaboration, and harmonization across international biomonitoring programs (St-Amand, 2021; Nassif and St-Amand 2021). In 2020, the International Human Biomonitoring Working Group (i-HBM), proposed by the IBN, became formally affiliated with the International Society of Exposure Science (ISES).

The i-HBM's mission is to promote the use of population-level HBM data to inform public health decision-making by developing harmonized resources to facilitate the interpretation of HBM data in a health-based context (https://intlexposurescience.org/i-hbm/).

A first step was the development of a free, on-line repository (the "Human Biomonitoring Health-Based Guidance Value (HB2GV) Dashboard") for health-based biomonitoring guidance values. The objectives of the Dashboard are:

- To be an open-access, curated database of biomonitoring guidance values developed for use in interpreting and understanding human biomonitoring data for the general population;
- To provide a user-friendly search tool for human biomonitoring guidance values for specific chemicals and/or biomarkers of exposure; and
- To assist users (risk assessors, risk managers and biomonitoring programs) with the interpretation of HBM data by allowing users to compare population-level data to the guidance values and providing standardized outputs in the form of figures and descriptive text.

This paper provides a description of the HB2GV Dashboard including the methods used to compile the values contained in the database, and how the Dashboard can be accessed and used. Finally, we offer thoughts on caveats with using the Dashboard for interpreting human biomonitoring data.

2. Methods

To develop the database, we conducted literature searches during two periods: during March 2020 to October 2020, and February 2022 to March 2022 using PubMed and Google Scholar. Although these literature searches were non-systematic, we targeted several search terms that sought to capture as many health-based biomonitoring guidance values as possible, including "biomonitoring", "biological monitoring", "blood guidance values", "guidance values", "biomonitoring equivalent(s)", "human biomonitoring values", "HBM", "HBM-I", "HBM-II", and "HBM-GV". Of note, we did not target search terms for occupational exposures or for nutritional or medicinal chemicals. In addition to literature searches, we also consulted websites of certain organizations, including Health Canada and the HBM Commission of the German Federal Environment Agency (https://www.umweltbundesamt.de/en/topics/health/commissions-working-groups/human-biomonitoring-commission-hbm-commission), the Human Biomonitoring for Europe (HBM4EU) initiative (https://www.hbm4eu.eu/), and i-HBM Working Group member affiliations.

We extracted information from the identified literature to populate the Dashboard. While the preponderance of the Dashboard data (e.g., biomarker name, biological matrix, biomonitoring guidance value) were taken directly from the associated primary publications, some information required coding or re-coding for the purposes of consistency (e.g., risk level associated with guidance values developed from cancer endpoints).

The Dashboard includes numerous commonly used terms for HBM guidance values; the terminology and acronyms are described in (Table 1).

The HB2GV Dashboard was developed in R (version 4.1.2, R Foundation for Statistical Computing, Vienna, Austria) using the Shiny Applications package (version 1.7.1, RStudio Inc, Boston, MA, USA). The database will be updated on an annual basis as new guidance values are identified.

3. Results

Five hundred eighty eight (588) biomonitoring guidance values were identified from 58 sources.

The Dashboard was designed with a landing page (https://intlexposurescience.org/i-hbm/) that features the database of guidance values shown in a table, as well as a sidebar with multiple reactive search filter options that can be selected by the end user.

The Dashboard has two main functionalities. The first is a Guidance Value search function. The user can search for chemicals by chemical group, chemical name or CAS RN, biomarker name, matrix (e.g., urine, serum), or the units of measure. As the user selects search filters, the table automatically updates with only the relevant guidance values. The following information is shown in the table: Chemical, Chemical CAS RN, Biomarker, Matrix, Group, Exposure Guidance Value Info (Type, Value, Risk Type, Source) and Biomonitoring Guidance Value Info (Type, Value, Units, Reference). Either the complete or the filtered database can be downloaded in a spreadsheet (XLSX) format. When the user selects one chemical, biomarker, matrix, and units from the filters, a figure will be automatically generated below the table that depicts the applicable guidance values as horizontal lines at the appropriate concentrations.

The second main functionality is a comparison feature. The user can input one or more biomonitoring-based chemical concentration(s) of their choosing and compare it with the available guidance values. The user can provide custom labels that describe those concentrations, such as the collection period, name of the biomonitoring program, or citation for the biomonitoring study. These results and labels will be added as bars to the figure in the Dashboard, which allows the user to visually compare them against the applicable guidance values. In cases where chemical concentrations representative of the Canadian population are available, users can import results from the Canadian Health Measures Survey (CHMS) directly into the figure for comparison purposes. The figure, with or without accompany comparative biomonitoring concentrations, can be downloaded as an image (PNG) file. A user guide containing more detailed instructions and the abbreviations list are also made available on the Dashboard. The Dashboard is hosted online through RStudio's shinyapps.io platform.

4. Discussion

The HB2GV Dashboard aggregates existing guidance values and facilitates understanding of how they may be used as screening tools in the interpretation of HBM data. The Dashboard is for informational

Table 1
Human Biomonitoring Health-based Guidance Value (HB2GV) terminology.

Type of Guidance Value (GV)	Definition
Health GV	Derived from epidemiological studies establishing a quantitative relationship between biomonitoring levels in humans and an observed biological response. Exceedance at the individual or population level indicates a need for medical follow-up, and for jurisdictions to identify and mitigate sources of exposure.[a]
Biomonitoring Equivalent (BE)	Concentration or range of concentrations of a chemical or its metabolites that is consistent with an existing value such as a reference dose (RfD) or tolerable daily intake (TDI) for non-cancer endpoints and risk-specific doses for cancer endpoints (Hays et al., 2008). BEs are meant to be used as a screening value to inform chemical prioritization for risk assessment or risk management, and they cannot be used to evaluate the likelihood of adverse health effects or for diagnostic purposes (LaKind et al., 2008). When a human biomonitoring value is above the BE value, it may inform chemical prioritization for follow up, along with investigating underlying exposure sources (Aylward et al., 2013; St-Amand et al., 2014; Faure et al., 2020).
Human Biomonitoring (HBM-I and HBM-II) Values	Values are health-related guidance values derived by the German Human Biomonitoring Commission (Apel et al., 2017; Schulz et al., 2012). They may be derived from epidemiological studies, or similar to BEs, by toxicokinetic extrapolation of tolerable intakes, such as acceptable daily intakes (ADI), TDI, or by toxicokinetic extrapolation of TDI-like values derived from animal experiments. The HBM-I value represents the concentration below which there is no risk for adverse health effects and, consequently, no need for action. For a chemical with a concentration higher than the HBM-I and lower than the HBM-II value, the potential sources of exposure should be identified and either eliminated or reduced by appropriate means. The HBM-II value represents the concentration above which, according to the knowledge and judgement of the commission, there is an increased risk for adverse health effects and, consequently, an urgent need to reduce exposure and to provide individual biomedical care.
Human Biomonitoring Guidance Value (HBM-GV)	In reference to the general population, the HBM-GV refers to the concentration of a substance or its metabolites in human biological material at and below which there is no risk of health effects anticipated for a lifetime exposure (Apel et al., 2020). Any population exceeding an HBM-GV may be prioritized for risk management follow-up. These HBM-GVs are equivalent to the HBM-I values and BE values.

[a] The Centers for Disease Control and Prevention has set a blood lead "reference value" of 3.5 μg/dL, equivalent to the 97.5th percentile of the blood lead values among U.S. children aged 1–5 years from recent NHANES surveys (Centers for Disease Control and Prevention, 2021). This value describes a cut point for children who have high levels of blood lead compared to a nationally representative group of children but does not provide information on what this level might indicate for children's health.

purposes only and is not intended for drawing conclusions regarding health risk for individuals. For example, it would be appropriate to employ population data in the Dashboard to inform chemical prioritization for further follow up.

It should be noted that the guidance values have been developed by researchers from different sectors (private, government) and areas of expertise (toxicologists, PBPK modelers), and with different funding sources (private and public) and different levels of confidence underpinning the values. These specific details have not been incorporated into the Dashboard database. It is therefore incumbent upon the Dashboard user to examine the chemical-specific publications cited in the Dashboard to determine whether the guidance values selected are fit for the user's purpose. For this reason, a direct link to the source of each HB2GV is provided and can be accessed from the Dashboard (or in the downloaded XLSX file).

Of note, researchers and government agencies often interpret biomonitoring data using RVs. While understanding how chemical levels in a sample population or individual relate to population levels can be useful for a variety of purposes (e.g., better understanding of possible sources of exposure or populations that warrant additional study or outreach (Vogel et al., 2019)), comparisons to exposure RVs do not offer health-related information. It is critical that this distinction be understood and recognized when using guidance values to interpret HBM data.

The Dashboard will be updated as new guidance values become available. It is our hope that this repository will facilitate already existing and innovative screening approaches that orient prioritization of future efforts (Aylward et al., 2013; Faure et al., 2020; St-Amand et al., 2014). A link is provided in the User Guide for individuals to provide information and documentation on new or otherwise missing guidance values so that they can be added to the Dashboard.

4.1. Confidence in HB2GVs

The HB2GVs are each unique in terms of the toxicological and epidemiological data used for their derivation, as well as the models used to develop them; not all of these data and models are of equal quality. Thus, confidence in the derived HB2GVs will vary from one value to the next. Assessing confidence in a given value is a critical step in terms of its use. Yet the process of assigning confidence in these values is fraught with difficulty and is very much reliant on expert judgment. As done with previous efforts to assess confidence in various types of data (LaKind et al., 2014; International Programme on Chemical Safety, 2008) and RVs (US EPA, 1993, 2005), it would be recommended that confidence in HB2GVs be categorized as high, medium, or low (Apel et al., 2020; Hays et al., 2008). However, it is important to note that the processes for assessing high, medium, or low confidence are similar, but not identical for BEs, HBM-I, HBM-II, and HBM-GV values (Hays et al., 2008; Apel et al., 2017, 2020); guidance for the HBM-I and HBM-II values is much more explicit. Further, not all HB2GVs have been assigned a confidence level.

Therefore, the current version of the Dashboard does not include the existing available confidence assessments. Future iterations of the Dashboard may include confidence categories. Several factors that will need to be considered have been described (LaKind et al., 2014) and include study design aspects such as analytical considerations, and sample collection and handling issues. At this time, it is incumbent on the user to examine the underlying literature that describes the development of the HB2GVs in order to assess the confidence in the value, and to put risk estimates in the context of underlying uncertainty.

4.2. Interpretation of HB2GVs

Availability of health-based guidance values, as well as RVs, are useful for the interpretation, risk assessment, and comparison of any biomonitoring data. However, it is important to note that comparing HBM data to nationally representative RVs or health-based guidance values can present major interpretive challenges. Issues include, but are not limited to:

- Biomonitoring results provide information on integrated pathways and routes of exposures (Albertini et al., 2006). Thus, HBM data do not provide information on *individual* pathways or routes of exposure. This presents difficulties for those seeking to identify and limit important avenues for human exposures. In this case, the evaluation

of information obtained by means of questionnaires within the framework of a survey can be helpful.

- Chemical concentrations in human matrices are inextricably tied to the physiological half-life of the chemical, the time between the last exposure and sampling of matrix, and the nature of the exposure (e.g., constant versus episodic). For many chemicals, half-lives are short (on the order of hours) and data on time since last exposure are often lacking (Teeguarden et al., 2011). This can make use of the HBM data for interpreting human exposure difficult. For example when a chemical concentration is non-detectable, it could imply no exposure or alternatively it could mean that the sample was collected after the chemical was already excreted.
- Many studies include a single (spot) blood or urine sample, generally due to cost and participant burden considerations. A single sample often will not represent long-term exposures and can result in exposure misclassification, especially for chemicals with short physiological half-lives and infrequent exposure patterns (LaKind et al., 2019; Pleil et al., 2013; Verner et al., 2020).
- Placing HBM data into context by comparing results with datasets such as those from national surveys provides information on relative exposures and if the sample size of a study is large enough, the HBM data can represent the population's exposure status but does *not* provide information related to health.
- HBM data should be derived from laboratories with extensive QA/QC programs that successfully participate in established laboratory harmonization schema (e.g., "round robin" studies, data harmonization programs, external proficiency testing, quality assessment programs).
- Serum concentrations of many persistent halogenated organic pollutants are reported both lipid-corrected and on wet-weight basis (not lipid-corrected) (Bernert et al., 2007). Similarly, urinary concentrations are often corrected for urine dilution with creatinine or specific gravity. Dashboard users will need to ensure that the comparison between guidance values and their biomonitoring data are reported using a common metric (uncorrected or corrected) (Barr et al., 2005). It is for this reason that users *must* select only one unit of measure before generating a figure and making a comparison in the Dashboard.

The HBM values in the Dashboard – with the exception of the blood lead reference value (CDC 2021) – are not meant to be used for comparison with an individual's biomonitoring data. This is especially true when only a single sample for an individual has been obtained. Many factors influence a single measure and may make it unrepresentative for estimations of overall exposure. These factors include (ACGIH 2001; Aylward et al., 2014; LaKind et al., 2008): physiological makeup and health status, exposure elements such as co-exposures and routes of exposures, and methodological issues (e.g., specimen contamination or degradation during collection and storage). Further, the Reference Doses (RfD) that form the foundation for estimation of GVs are not designed to evaluate individual risk, but rather are meant to assess population risk. The HBM-I and HBM-II values are in Germany also used for individual health counseling, addressing uncertainties and limitations.

Interestingly, there are substances with multiple Dashboard Values. This is the case for a well-studied plasticizer, di-(2-ethylhexyl) phthalate (DEHP), which has 36 entries. To properly interpret the Dashboard in these cases one must have a clear understanding of differences in the various GVs available, how GVs were derived, and their intended use and application. On the other hand, the Dashboard can also reveal data gaps where chemicals of interest have no health-based guidance values available. This can help prioritize future efforts and target resources in deriving new values in the future.

The Dashboard user should also be aware of the possibility that guidance values may be updated from time to time. Specifically, existing guidance values should be checked periodically to ensure that they are up to date. For example, for so called legacy compounds (e.g., vinyl chloride, benzene, lead), the values have decreased over the time due to a better characterization of their toxicity. It is also the case that fewer guidance values have been developed for emerging chemicals of interest (e.g., poly- and perfluoroalkyl substances), where a paucity of available data might not allow a complete characterization of their toxicity and further correlation with certain health effects. Thus, the derived/or to-be-derived guidance values might need further revision over time availability of data increase. When the body of scientific evidence (also the availability of epidemiological, toxicodynamic, and toxicokinetic data) is sufficient to quantify an effect threshold with certainty for a chemical, a new or updated HB2GV can be derived.

5. Conclusions

To our knowledge, the HB2GV Dashboard is the first open-access, curated database of human biomonitoring guidance values developed for use in interpreting human biomonitoring data. It allows user-friendly searches for specific chemicals and/or biomarkers of exposure. It also allows users to compare biomonitoring data to the Dashboard values and provides standardized outputs in the form of figures and descriptive text. This new resource can assist global HBM data users such as risk assessors, risk managers and biomonitoring programs with a readily available compilation of guidance values. An additional value of the Dashboard is to facilitate future HBM efforts by helping to identify knowledge gaps and areas where more research could be conducted to improve reliance of GV estimates.

The creation of the Dashboard is a key tangible achievement of the i-HBM Working Group. It is our hope that the Dashboard will become a vital tool for the international harmonization of HBM data interpretation and will help public and governmental agencies better understanding the value of HBM. The i-HBM Working Group also envisages collaborations among international organizations such as the World Health Organization to further promote the use of population level human biomonitoring.

Conflict of interest

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC) or U.S. Environmental Protection Agency (USEPA). The findings and conclusions in this report are from a research perspective and do not necessarily represent the official position of Health Canada. The research presented was not performed or funded by US EPA and was not subject to US EPA's quality system requirements. Health Canada support is acknowledged for this publication.

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Long-term exposure to traffic-related air pollution and stroke: A systematic review and meta-analysis

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ABSTRACT

Background: Stroke remains the second cause of death worldwide. The mechanisms underlying the adverse association of exposure to traffic-related air pollution (TRAP) with overall cardiovascular disease may also apply to stroke. Our objective was to systematically evaluate the epidemiological evidence regarding the associations of long-term exposure to TRAP with stroke.

Methods: PubMed and LUDOK electronic databases were searched systematically for observational epidemiological studies from 1980 through 2019 on long-term exposure to TRAP and stroke with an update in January 2022. TRAP was defined according to a comprehensive protocol based on pollutant and exposure assessment methods or proximity metrics. Study selection, data extraction, risk of bias (RoB) and confidence assessments were conducted according to standardized protocols. We performed meta-analyses using random effects models; sensitivity analyses were assessed by geographic area, RoB, fatality, traffic specificity and new studies.

Results: Nineteen studies were included. The meta-analytic relative risks (and 95% confidence intervals) were: 1.03 (0.98–1.09) per 1 $\mu g/m^3$ EC, 1.09 (0.96–1.23) per 10 $\mu g/m^3$ PM_{10}, 1.08 (0.89–1.32) per 5 $\mu g/m^3$ $PM_{2.5}$, 0.98 (0.92; 1.05) per 10 $\mu g/m^3$ NO_2 and 0.99 (0.94; 1.04) per 20 $\mu g/m^3$ NO_x with little to moderate heterogeneity based on 6, 5, 4, 7 and 8 studies, respectively. The confidence assessments regarding the quality of the body of evidence and separately regarding the presence of an association of TRAP with stroke considering all available evidence were rated low and moderate, respectively.

Conclusion: The available literature provides low to moderate evidence for an association of TRAP with stroke.

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1. Introduction

According to the World Stroke Organization Fact Sheet 2022, stroke remains the second leading cause of death and the third leading cause of death and disability combined (Feigin et al., 2022). Stroke is defined by broad and inclusive clinical and tissue criteria and encompasses central nervous system infarction, ischemic stroke, and intracerebral, cerebral and subarachnoid haemorrhage (Sacco et al., 2013).

Important risk factors for stroke morbidity and mortality include health states (e.g., high blood pressure, diabetes), behaviours that contribute to those states (e,g., smoking, features of the diet), and socioeconomic conditions that shape the former, and other factors influencing risk. Among these other factors are environmental pollutants. Air pollution, in particular, is of interest because of its adverse association with several cardiovascular outcomes (Franklin et al., 2015; Kaufman et al., 2020; Newman et al., 2020). Also, it is estimated that 6% of global mortality attributable to air pollution is traffic-related (McDuffie et al., 2021).

Abbreviations

TRAP	Traffic Related Air Pollution
PM	Particulate Matter
EC	Elemental Carbon
NOx	Nitrogen oxides
NO_2	Nitrogen Dioxide
CO_2	Carbon Dioxide
UFP	Ultrafine Particles
GRADE	Grading of Recommendations Assessment, Development and Evaluation
OHAT	Office of Health Assessment and Translation

A major and growing source of air pollution is traffic. Traffic-related air pollution (TRAP) is a complex mixture and refers to ambient air pollution resulting from the use of motor vehicles including heavy-duty and light-duty vehicles, buses, passenger cars, and motorcycles. Motor vehicles are important contributors of pollutants from combustion including nitrogen dioxide (NO_2) and oxides (NO_x), elemental carbon (EC), particulate matter (i.e. PM_{10} and $PM_{2.5}$) and ultrafine particles (UFPs). These pollutants can be directly emitted through the vehicle exhaust (i.e. tailpipe emissions) or through resuspension of road dust, mechanical wear of brakes and tires, and abrasion of road surfaces (i.e. non-tailpipe emissions) (Health Effects Institute, 2018).

TRAP exposure is associated with mechanisms such as cerebrovascular dysfunction that appear to be manifested through several pathways that can increase stroke risk, including inflammation and oxidative stress, endothelial dysfunction, blood pressure, atherosclerosis, procoagulant changes, increased thrombogenicity, loss of vascular flexibility and alterations in autonomic nervous system balance (Landrigan et al., 2018; Miller, 2020).

TRAP continues to be of public health interest; notably, TRAP has been the target of successful interventions, thus also making it a concern to policy makers and motor vehicle manufacturers. Advances in systematic review methods for environmental health (Whaley et al., 2020; Woodruff and Sutton, 2014) provide more specific guidance for the conduct of literature reviews, thereby enhancing consistency and transparency. Using this refined guidance, we aimed to systematically evaluate the epidemiological evidence on long-term exposure to TRAP in relation to stroke in adults. Results were quantitatively combined to evaluate the magnitude of the association. We also assessed the quality of the evidence base and the level of confidence in the presence of an association between TRAP and stroke.

2. Methods

This study is part of an extensive systematic review (conducted by the Health Effects Institute (HEI)) on the effects of TRAP on key health outcomes, involving a Panel of 13 experts in epidemiology, exposure assessment, and statistics (Boogaard et al., 2022; Health Effects Institute, 2022). The methods were based on standards set by the Cochrane Collaboration (Higgins et al., 2019), the NIEHS Office of Health Assessment and Translation handbook (OHAT, 2019), the systematic reviews conducted as part of the World Health Organization Air Quality Guidelines (WHO AQG) (Chen and Hoek, 2020; Huangfu and Atkinson, 2020; WHO, 2021) and the newly published COSTER recommendations for the conduct of systematic reviews in toxicology and environmental health research (Whaley et al., 2020). This review complies with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) (PRISMA, 2021) as well as the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (Stroup et al., 2000). The review protocol was published in 2019 and registered in Prospero (Health Effects Institute, 2019). Outcomes, including ischemic and haemorrhagic stroke, were selected based on evidence on causality (causal or likely causal) according to the latest determination for general air pollution (Health Canada, 2016; The International Agency for Research on Cancer, 2016; U.S. Environmental Protection Agency, 2019; 2016). Where applicable, included studies were approved by the respective institutional review boards.

2.1. Search strategy

The PubMed and the Swiss Literature Database and Services on Health Effects of Ambient Air Pollution (LUDOK) electronic databases (https://www.swisstph.ch/de/projects/ludok/) were searched comprehensively for studies matching the PECOS question (Higgins et al., 2019) by independent reviewers (M.K.J., R.K., H.B. and A.P.) (Supplementary Table 1). The HEI review covered papers published from January 01, 1980, to July 31, 2019. We repeated the stroke component of the search by including papers published through January 06, 2022. Keywords included TRAP or proximity measures and stroke as described in the main HEI report (Health Effects Institute, 2022). We also considered references in other reviews of health effects of air pollution including the HEI 2010 report (Health Effects Institute, 2018) and in the individual bibliographic databases maintained by members of the Panel. Contact to authors or identification of unpublished studies or data was not attempted.

2.2. Eligibility criteria

Eligible studies met the following criteria: (1) original epidemiological study with individual-level data and adopting a cohort, case-cohort, case-control, cross-sectional, or intervention design; (2) reported on the general population, of all ages, with no geographical restrictions; (3) assessed long-term exposure (months to years) to a specific traffic pollutant or used proximity metrics of TRAP (distance to or density of traffic); (4) defined the outcome as total and/or type-specific stroke from ICD-9-CM 430–434 and 436 and ICD-10 I60–I69; (5) estimated the association between a continuously or categorically modelled/parameterized exposure and fatal and/or non-fatal stroke morbidity and mortality (odds ratio (OR), hazard ratio (HR), incident relative risk (IRR) and relative risk (RR)); and (6) published or accepted for publication in a peer-reviewed journal and written in English. Limitation to English publications was chosen as the state of the art of publication in the area of research.

The exclusion criteria eliminated studies reporting on: (1) exposure in occupational settings or exclusively indoor settings; (2) exposure for combined-source air pollution and not specific to traffic; (3) short-term (minutes to months) or self-reported exposures to TRAP; (4) only ecological or area-level analyses; (5) only unadjusted results and clear

evidence of an analytical error; and (6) methodological papers, or studies focusing on gene-environment-interaction.

2.3. Exposure framework

A novel framework to determine exposure to TRAP was developed to ensure that the included studies were informative about health effects specific to TRAP. The framework combined three aspects of TRAP measurement: (1) exposure metric (including pollutants, distance and density metrics) (Supplementary Table 2); (2) spatial scales of the pollution surface and participant addresses, to exploit/ensure TRAP contrasts (i.e., at local and neighbourhood scale); and (3) exposure assessment methods including appropriate models or monitoring (Supplementary Table 3).

The review included NO_2, EC, CO and other pollutants in which traffic is usually the main source; results pertaining to $PM_{2.5}$ and PM_{10} were also included except if exclusively based on surface monitoring. As none of these pollutants are universally TRAP, a traffic specificity indicator based on stricter criteria for the three elements of the general framework was developed.

2.4. Study selection and data extraction

DistillerSR, a web–based, systematic review software program (DistillerSR, 2021), was used for screening of studies to ensure standardization of process. Two reviewers independently screened titles and abstracts of the search results. The studies were classified by health outcomes and full-text articles and supplements were retrieved for those that provisionally met the inclusion criteria. Next, a full-text screening was conducted to confirm that effect estimates were reported for stroke and that the exposure framework criteria described above were met (Health Effects Institute, 2022). Disagreements were resolved through discussion or consultation with the Panel.

Data extraction was performed by MK, RK and PH as well as by a number of students to extract key information for meta-analysis such as study name, details on the study population, study design, method of exposure assessment, pollutants, method of outcome assessment, outcomes, statistical analysis, effect estimates with pollutant increments and 95% confidence intervals. After completion of data extraction, all data from *DistillerSR* were exported to Excel spreadsheets, quality controlled and processed into figures and summary tables.

2.5. Meta-analysis

To quantify the overall association with stroke, meta-analyses were performed in cases where three or more studies reported associations of a given exposure with stroke. The full list of inclusion and exclusion criteria for meta-analysis are found in the Supplement Table 4. Standardized results (Department for Environment, Food and Rural Affairs, 2014) were quantitatively combined using random effects models using restricted maximum likelihood to estimate the between studies' variance (Veroniki et al., 2016). Effect estimates from single pollutant models were selected for the meta-analysis, because we considered the associations of single pollutants to represent the associations of the TRAP mixture. Random effects models were chosen a priori because of the expected differences in populations and pollution mixtures. Statistical heterogeneity was assessed using Cochran's Q I^2, and τ^2 (tau-squared). Tau^2 is also presented in the form of a 95% prediction interval around the mean effect of the random effects meta-analysis (Borenstein et al., 2017). We reported RR in the review as a non-specific term to indicate any of the ratio measures. Thus HR, IRR and OR were included in the same meta-analyses on the assumption that when the RR is close to the null and the stroke prevalence in the population is less than 10%, all these measures approximate the risk ratio (Anderson et al., 2013; Davies et al., 1998; Khreis et al., 2017). Also, we expressed summary RR estimates over the increments of pollutant concentration used by the ESCAPE study, to reflect a realistic range of exposure contrasts in most studies (Beelen et al., 2014, 2015).

In primary meta-analyses, we used estimates for the combined endpoint of non-fatal and fatal stroke, if available; if separate estimates were generated for non-fatal and fatal stroke, we used the former, as non-fatal stroke cases numbers were/are typically higher. Sensitivity analyses were conducted for every pollutant and stratified by at least one of the following: region, risk of bias (RoB) assessment domain, smoking, study design and fatality. Additional estimates for $PM_{2.5}$ and NO_2 from the updated search in January 2022 were included as sensitivity-analyses. We conducted these analyses using R (version 3.6.0), and the libraries "metafor" (v.2.4–0),"meta", (v. 4.16–2), "forestplot" (v.1.10.1),"ggplot" (v. 3.3.3) for the analyses and plots.

2.6. Overall assessment of the evidence

We rated the overall evidence using complementary assessments of (1) its quality and (2) the degree to which it supported the presence of an adverse association between TRAP exposure and stroke.

For the rating of quality, we adapted the GRADE (Grading of Recommendations Assessment, Development and Evaluation) assessment of confidence in the quality of the body of meta-analysed evidence, using the Office of Health Assessment and Translation (OHAT) method as a guide (OHAT, 2019). We grouped studies by key design features, with each given an initial confidence rating. This initial confidence rating could then be downgraded corresponding to factors that decreased confidence in the quality of the body of evidence (high RoB, unexplained inconsistency, imprecision, and publication bias) or upgraded corresponding to factors that increased confidence in the body of evidence (monotonic exposure-response, consistency across populations, and consideration of residual confounding) (Supplementary Fig. 1). For RoB assessment, we used a modified tool developed for the RoB assessment in the WHO AQG review (WHO, 2020). The modified OHAT assessment in the quality of the body of evidence was rated high, moderate, low or very low.

Because the GRADE assessment focused on the quality of the body of evidence rather than on the presence of an association, and because it was heavily geared towards the studies entering a meta-analysis, the Panel conducted a narrative assessment to evaluate the level of confidence in the presence of an association of TRAP with stroke, considering both meta-analysed studies and all other studies not included in the meta-analysis. (Supplementary Table 5). For the comprehensive narrative assessment, we evaluated the number, size, and location of the evidence base; study design, study population and representativeness, the strength and nature of the association, quality of the studies. consistency of the findings. Monotonic exposure-response function, and other considerations. The comprehensive narrative assessment of the confidence in the presence of an association, based on the complete study base, was rated as high, moderate, low or very low.

Subsequently the findings from the modified OHAT assessment and the comprehensive narrative assessment were combined into an overall confidence assessment (Supplementary Table 6).

3. Results

3.1. Study selection

The initial search of the larger HEI review (Boogaard et al., 2022; Health Effects Institute, 2022), that included several key health outcomes, identified 13660 unique articles of which 206 were identified as cardiometabolic studies (i.e.: ischemic heart disease, stroke, diabetes mellitus and coronary events) after title and abstract screening. During full-text screening, 149 studies were excluded for the following reasons: study design (N = 18), exposure assessment (i.e. nationwide study with no or insufficient area-specific adjustments or spatial scale too crude for either the pollution surface or the health data) (N = 85), health outcome

(N = 34) and other (N = 12). Of the 57 remaining studies for the selected cardiometabolic outcomes – out of which 37 included estimates on stroke – 19 were included in the current review (Fig. 1, Table 1). A list of the 18 excluded articles and the reasons behind their exclusion can be found in Supplementary Table 7.

3.2. Study characteristics

Most of the 19 studies had starting dates in the 1990s (Table 1). The majority of the studies were located in Europe (N = 12). The 14 cohort studies (Alexeeff et al., 2018; Andersen et al., 2012; Atkinson et al., 2013; Carey et al., 2016; Dirgawati et al., 2019; Gan et al., 2012; Hoffmann et al., 2015; Katsoulis et al., 2014; Korek et al., 2015; Kulick et al., 2018; Sørensen et al., 2014; Stafoggia et al., 2014; Stockfelt et al., 2017) had sample sizes between 3287 and 819,370 participants and mean follow up times between 3 and 21 years. One study was a multi-cohort analysis of 11 European cohorts that were analysed within the harmonized framework of the ESCAPE study (Stafoggia et al., 2014). Data sources for stroke ascertainment varied, including self-reported events, medical care records, hospital admissions, disease and death registries, insurance claims or health administrative databases (Table 1).

The three case-control studies (Johnson et al., 2013; Oudin et al., 2009, 2011) had sample sizes between 6302 and 556,912 with recruitment times of two to four years. Oudin et al. (2009) included incident ischemic stroke cases (fatal and non-fatal). For the second analysis, Oudin et al. (2011) obtained personal covariates data from questionnaires sent to surviving cases of ischemic stroke (fatal and non-fatal), thus included prevalent cases only. Oudin et al. (2011, 2009) used national and local stroke registries to identify cases; controls who shared the same date of birth as the cases and were residing in Scania, were sampled from the national statistics databases. The third case-control study included incident all-stroke cases (fatal and non-fatal) (Johnson et al., 2013). Johnson et al. (2013) identified first-time stroke cases from hospital emergency administrative data and sampled controls from persons visiting the same emergency administrative data for minor trauma.

The three cross-sectional studies (Lazarevic et al., 2015; Pindus et al., 2016; Qin et al., 2015) included 905 to 26,991 participants. The study populations included survivors of non-fatal all-stroke events only. Stroke ascertainment relied primarily on self-reports.

The majority of studies assigned TRAP exposures based on land-use regression or dispersion models. Most studies estimated exposures at

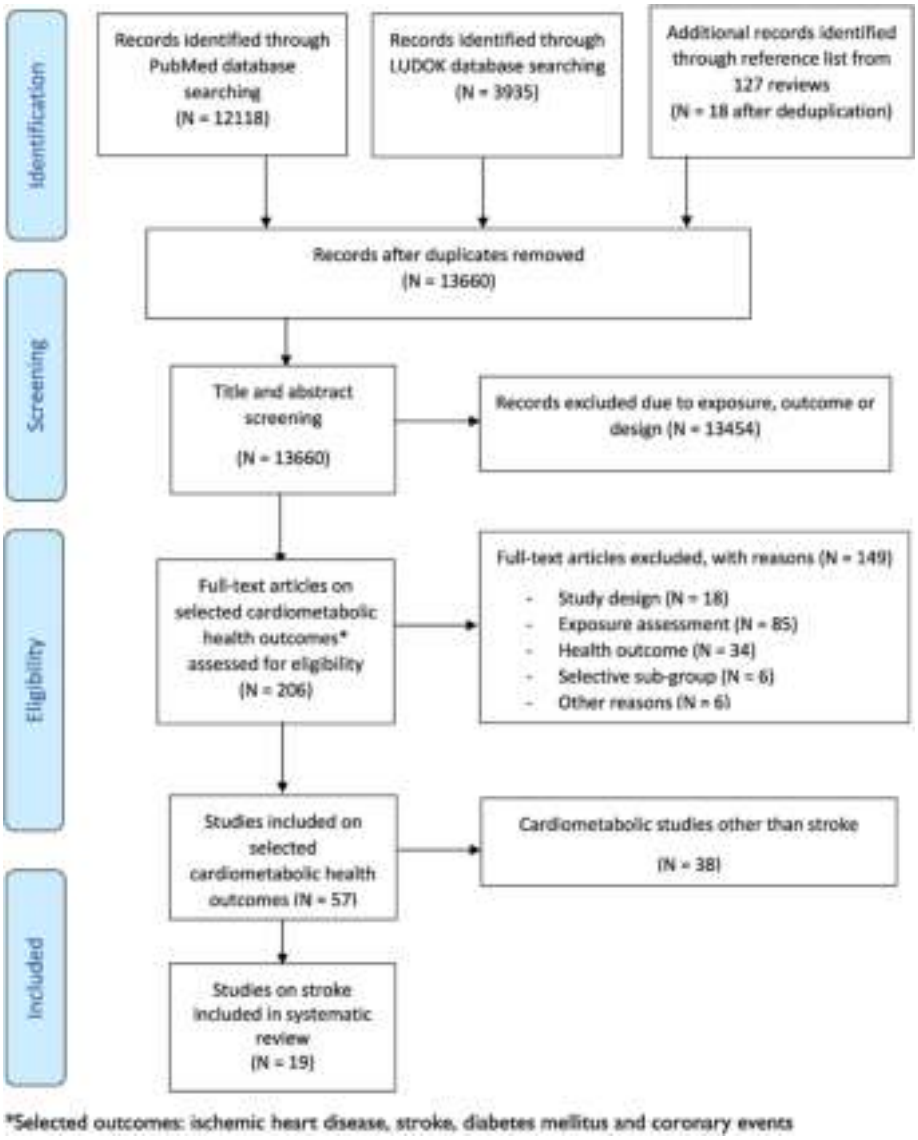

Fig. 1. Study Selection Flow Chart
*Selected outcomes: ischemic heart disease, stroke, diabetes mellitus and coronary events.

Table 1
Key study characteristics of articles included in the systematic review for stroke-pollutants.

Reference	Study Name	Location	Study period	Study design and Sample size	Exposure Assessment	Age at baseline	Sex	Stroke outcome ascertain-ment	Confounder adjustment	Results (estimate[a], 95% CI, increment)
Alexeeff et al., 2018	KPNC Oakland	Oakland, California, United States	2010–2015	Cohort	Surface monitoring	Age Range: 18–65+	Males and Females	Medical record and death certificates	Age, sex, race, BMI, smoking, co-morbidities[b], use of medication, neighbourhood socioeconomic status (nSES)	**Fatal and non-fatal all stroke (HR)** BC[c] 0.96 (0.85, 1.08) per 0.17 μg/m^3 NO 0.98 (0.87, 1.11) per 3.8 ppb NO_2[c] 0.97 (0.85, 1.11) per 3.8 ppb
				41869						**Fatal all stroke (HR)** BC 0.92 (0.58, 1.45) per 0.17 μg/m^3 NO 1.13 (0.86, 1.49) per 3.8 ppb NO_2 1.38 (0.93, 2.06) per 3.8 ppb
Andersen et al., 2012	DDCH	Copenhagen and Aarhus, Denmark	1993–2006	Cohort	Dispersion/ Chemical Transport Model (CTM)	Age Range: 50-65	Males and Females	Hospital admission and death certificates	Age, sex, smoking, environmental tobacco smoke (ETS), BMI, education, sports, alcohol, fruit/veg intake, fat intake, co-morbidities[b]	**Non-fatal all stroke (HR)** NO_2 1.05 (0.99, 1.11) per 6.2 μg/m^3 Density 1.02 (0.99, 1.04) per 1700 vehicle-km/ day Distance 1.09 (0.94, 1.26) <50 vs. >50 m
				52215	And density measures					**Fatal all stroke (HR)** NO_2 1.22 (0.99, 1.49) per 7.5 μg/m^3 Density 0.99 (0.91, 1.09) per 1700 vehicle-km/ day Distance 1.17 (0.70, 1.98) <50 vs. >50 m
Atkinson et al., 2013	CPRD London	England	2003–2007	Cohort	Dispersion	Age Range: 40-89	Males and Females	Primary care records, hospital admissions and death certificates	Age, sex, smoking, BMI, co-morbidities[b], index of multiple deprivation (IMD)	**Fatal and non-fatal all stroke (HR)** PM_{10}[c] 1.00 (0.93, 1.06) per 3.0 μg/m^3
				819370	and CTM					
Carey et al., 2016	CPRD London	London, United Kingdom	2005–2011	Cohort	Dispersion /CTM And density and distance measures	Age Range: 40-79	Males and Females	Primary care records, hospital admissions and death certificates	Age, sex, smoking, BMI, IMD, night-time noise[d]	**Fatal and non-fatal all stroke (HR)** NO_2[c] 0.88 (0.82, 0.95) per 10 μg/m^3 NO_x[c] 0.90 (0.85, 0.96) per 20 μg/m^3 $PM_{2.5\ traffic}$ 0.88 (0.81,

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Table 1 (*continued*)

Reference	Study Name	Location	Study period	Study design and Sample size	Exposure Assessment	Age at baseline	Sex	Stroke outcome ascertain-ment	Confounder adjustment	Results (estimate[a], 95% CI, increment)
				207047						0.97) per 1 μg/m^3 Density 1.00 (0.88, 1.15) >100000 heavy vehicle-km/year vs. none Density 1.02 (0.96, 1.11) <100000 heavy vehicle-km/year vs. none Distance 0.98 (0.86, 1.12) <100 vs. >250 m Distance 1.02 (0.95, 1.10) 100–250 vs. >250 m
Dirgawati et al., 2019	HIMS	Perth, Australia	1996–2012	Cohort	Land-Use Regression Model (LUR)	Age: ≥65	Males only	Hospital records and death register	Age, smoking, education, BMI, co-morbidities[b], physical inactivity, high-fat diet, alcohol	**Fatal and non-fatal all stroke (HR)** $PM_{2.5\ abs}$[c] 0.86 (0.71, 1.03) per 1 1e-5/m NO_2[c] 0.96 (0.85, 1.08) per 10 μg/m^3 NO_x[c] 1.00 (0.95, 1.04) per 10 μg/m^3 $PM_{2.5\ mass}$[c] 1.01 (0.84, 1.21) per 5 μg/m^3
				10126						**Fatal all stroke (HR)** $PM_{2.5\ abs}$ 0.70 (0.47, 1.03) per 1 1e-5/m NO_2 0.93 (0.72, 1.19) per 10 μg/m^3 NO_x 0.97 (0.88, 1.07) per 10 μg/m^3 $PM_{2.5\ mass}$ 0.71 (0.49, 1.02) per 5 μg/m^3
Gan et al., 2012	Vancouver Administrative	Vancouver, British Columbia, Canada	1999–2002	Cohort	LUR	Age Range: 45-85	Males and Females	Death registration database	Age, sex, co-morbidities[b], nSES, noise[d]	**Fatal all stroke (RR)** $PM_{2.5\ abs}$[c] 1.04 (1.00, 1.09) per 0.97 1e-5/m
				445868						
Hoffmann et al., 2015	HNR	Ruhr Areas, Germany	2000–2012	Cohort	LUR and density measures	Age Range: 45-74	Males and Females	Patient records and death certificates	Marital status, education, employment, smoking, co-morbidities[b], BMI, physical activity, alcohol, noise[d]	**Fatal and non-fatal all stroke (HR)** $PM_{2.5\ abs}$ 1.57 (0.86, 2.86) per 0.98 1e-5/m $PM_{10\ mass}$ 2.38 (1.06, 5.35) per 6.32 μg/m^3
				4222						$PM_{2.5\ mass}$ 2.90 (1.18,

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Table 1 (*continued*)

Reference	Study Name	Location	Study period	Study design and Sample size	Exposure Assessment	Age at baseline	Sex	Stroke outcome ascertain-ment	Confounder adjustment	Results (estimate[a], 95% CI, increment)
										7.12) per 3.51 μg/m^3 $PM_{coarse\ mass}$ 1.79 (0.72, 4.46) per 5.26 μg/m^3 Density 1.06 (0.69, 1.64) 4302 vehicle-km/day
Johnson et al., 2013	Edmonton Stroke	Edmonton, Alberta, Canada	2007–2009	Case-control 42419	LUR	Mean Age cases: 69.7 controls: 39.8	Males and Females	Cases: Emergency administrative data Controls: hospitalization data	Age, sex, contextual SES, smoking, BMI	**Fatal and non-fatal all stroke (OR)** NO_2[c] 1.01 (0.94, 1.08) per 5 ppb
Katsoulis et al., 2014	EPIC Athens	Athens, Greece	1994–2011	Cohort 2752	LUR	Age Range: 21-82	Males and Females	Self-reported data and death certificates	Sex, age, smoking, BMI, education, physical activity, total energy intake, co-morbidities[b], alcohol	**Fatal and non-fatal all stroke (HR)** NO_2[c] 0.98 (0.71, 1.34) per 10 μg/m^3 $PM_{10\ mass}$[c] 1.17 (0.60, 2.26) per 10 μg/m^3
Korek et al., 2015	SDPP, SIXTY, SALT, SNAC-K	Stockholm, Sweden	1992–2011	Cohort 20070	Dispersion and CTM	Age Range: 35-56	Males and Females	Hospital registry and death registry	Gender, education, smoking, socio-economic index	**Fatal and non-fatal all stroke (HR)** NO_x[c] 1.20 (0.64, 2.29) per 20 μg/m^3 $PM_{10\ traffic}$ 1.20 (0.89, 1.63) per 10 μg/m^3
Kulick et al., 2018	NOMAS	Manhattan, United States	1993–2016	Cohort 3287	Distance measures	Median Age: 69	Males and Females	Self-reported, medical records, death certificates	Age, sex, race, education, insurance status, year of enrolment, nSES, smoking, alcohol, physical activity, BMI, co-morbidities[b]	**Fatal and non-fatal ischemic stroke (HR)** Distance 1.42 (1.01, 2.02) <100 vs. >400 m Distance 1.14 (0.81, 1.60) 100–200 vs. >400 m Distance 1.08 (0.80, 1.45) 200–400 vs. >400 m
Lazarevic et al., 2015	ALSWH	Australia	2006–2011	Cross sectional 26991	LUR and distance measures	3 age cohort (younger, middle aged, older)	Females only	Self-reported	Age, BMI, smoking, alcohol, physical activity, fruit/veg, degree of residential urbanisation, mean temperature, marital status, education, self-assessed financial resources	**Non-fatal (prevalence) all stroke (RR)** NO_2 0.83 (0.58, 1.19) per 3.3 ppb Distance 1.01 (0.90, 1.14) 1 km
Oudin et al., 2009	Scania Stroke	Scania, Sweden	2001–2005	Case-control	Dispersion and CTM	Birth year: 1923–1965	Males and Females	Cases: hospital admissions from national stroke register	Sex, marital status, country of birth, smoking, co-morbidities[b]	**Fatal and non-fatal (prevalence) ischemic stroke (OR)**

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Table 1 (*continued*)

Reference	Study Name	Location	Study period	Study design and Sample size	Exposure Assessment	Age at baseline	Sex	Stroke outcome ascertain-ment	Confounder adjustment	Results (estimate[a], 95% CI, increment)
				556912						NO_x 0.87 (0.73, 1.03) 30–60 vs. <10 μg/m^3 NO_x 0.97 (0.90, 1.05) 10–20 vs. <10 μg/m^3 NO_x 0.95 (0.86, 1.06) 20–30 vs. <10 μg/m^3
Oudin et al., 2011	Scania Stroke	Scania, Sweden	2001–2006	Case-control 6302	Dispersion and CTM	Birth year: 1923–1965	Males and Females	Cases: hospital admissions from national stroke register	Sex, marital status, country of birth, smoking, co-morbidities[b], physical inactivity	**Non-fatal (prevalence) ischemic stroke (OR)** NO_x[c] 0.93 (0.82, 1.95) 10 μg/m^3
Pindus et al., 2016	RHINE Tartu	Tartu, Estonia	2011–2012	Cross sectional 905	Dispersion and CTM	Mean age: 50	Males and Females	Self-reported	Gender, age, BMI, education, smoking, ETS	**Non-fatal (prevalence) all stroke (OR)** $PM_{10\ traffic}$ 1.21 (0.53, 2.77) per 2.2 μg/m^3
Qin et al., 2015	33 CCHS	Shenyang and Anshan and Jinzhou, China	2009–2009	Cross sectional 14646: normal weight, 1435: obese, 8764: overweight	Surface monitoring	Age Range: 18-74	Males and Females	Self-reported	Age, sex, race, education, income, smoking, drinking, exercise, diet, sugar, family co-morbidities[b], study district	**Non-fatal (per weight category; prevalence) all stroke (OR)** NO_2 1.01 (0.84, 1.22) per 9 μg/m^3 NO_2 1.15 (0.64, 2.07) per 9 μg/m^3 NO_2 1.22 (0.98, 1.51) per 9 μg/m^3
Sørensen et al., 2014	DDCH	Copenhagen and Aarhus, Denmark	1993–2009	Cohort 51569	Dispersion and CTM	Age Range: 50-64	Males and Females	National registries, medical records	Sex, length of school attendance, nSES, smoking, fruit/veg, alcohol, coffee, physical activity, BMI, calendar year, noise[d]	**Fatal and non-fatal all stroke (IRR)** NO_2[c] 1.08 (1.01, 1.16) per 10 μg/m^3 NO_x[c] 1.02 (0.98, 1.07) per 20 μg/m^3 **Fatal all stroke (IRR)** NO_2 1.47 (1.21, 1.80) per 10 μg/m^3 NO_x 1.17 (1.05, 1.31) per 20 μg/m^3
Stafoggia et al., 2014	ESCAPE	Multiple cities, Multiple countries	1992–2010	Cohort 99446	LUR and density measures	Mean Age range: 44–74	Males and Females	Self-reported, medical record, death certificates	Sex, calendar year, marital status, education, occupation, smoking, area level SES, noise[d]	**Fatal and non-fatal all stroke (HR)** $PM_{2.5\ abs}$[c] 1.08 (0.83, 1.41) per 1 1e-5/m NO_2[c] 0.99 (0.89, 1.11) per 10 μg/m^3 NO_x[c] 0.98 (0.89, 1.07) per 20 μg/m^3 $PM_{10\ mass}$[c] 1.11 (0.90,

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Table 1 (*continued*)

Reference	Study Name	Location	Study period	Study design and Sample size	Exposure Assessment	Age at baseline	Sex	Stroke outcome ascertain-ment	Confounder adjustment	Results (estimate[a], 95% CI, increment)
										1.36) per 10 μg/m^3 $PM_{2.5\ mass}$[c] 1.19 (0.88, 1.62) per 5 μg/m^3 PM_{coarse} 1.02 (0.90, 1.16) per 5 μg/m^3 Density 1.02 (0.95, 1.10) 4000 vehicle-km/day
Stockfelt et al., 2017	GOT-MONICA	Gothenburg, Sweden	1990–2011	Cohort 4500	Dispersion and CTM	Age Range: 25-64	Males and Females	Death register, self-reported, hospital discharge register	Age, smoking, marital status, physical activity, calendar year, mean income of area, sex, enrolment year	**Fatal and non-fatal all stroke (HR)** BC[c] 1.25 (0.89, 1.76) per 1 μg/m^3 NO_x[c] 1.04 (0.90, 1.20) per 20 μg/m^3 $PM_{10\ nontailpipe}$ 1.10 (0.97, 1.24) per 1.48 μg/m^3 $PM_{10\ exhaust}$ 1.07 (0.92, 1.23) per 0.29 μg/m^3 $PM_{10\ traffic}$ 1.09 (0.97, 1.23) per 1.77 μg/m^3 $PM_{10\ mass}$[c] 1.48 (0.88, 2.49) per 10 μg/m^3 $PM_{2.5\ mass}$[c] 1.50 (0.90, 2.51) per 5 μg/m^3
Stockfelt et al., 2017	PPS	Gothenburg, Sweden	1990–2011	Cohort 5850	Dispersion and CTM	Age Range: 64-75	Males only	Death register, self-reported, hospital discharge register discharge register	Age, smoking, marital status, physical activity, calendar year, mean income of area, occupational class	**Fatal and non-fatal all stroke (HR)** BC[c] 1.09 (0.90, 1.31) per 1 μg/m^3 NO_x[c] 1.04 (0.97, 1.12) per 20 μg/m^3 $PM_{10\ nontailpipe}$ 1.03 (0.96, 1.10) per 1.41 μg/m^3 $PM_{10\ exhaust}$ 1.04 (0.97, 1.28) per 0.29 μg/m^3 $PM_{10\ traffic}$ 1.03 (0.97, 1.10) per 1.77 μg/m^3 $PM_{10\ mass}$[c] 1.08 (0.80, 1.45) per 10 μg/m^3 $PM_{2.5\ mass}$[c] 1.06 (0.78, 1.44) per 5 μg/m^3

[a] Effect estimates can be ORs, RRs, HRs or IRRs, depending on the analysis; Estimates of incidence of stroke are reported unless otherwise mentioned.
[b] Co-morbidities include at least one of the following: diabetes, hypertension, COPD, hyperlipidemia, medications for the latter.

[c] Included in the meta-analysis; see Supplementary Table 4 for inclusion and exclusion criteria.
[d] Also adjusted for noise in sensitivity analyses but estimates are not shown in Table 1.

participants' residential locations, while others (Andersen et al., 2012; Atkinson et al., 2013; Carey et al., 2016) estimated exposures at participants' high-resolution postal codes. NO_x was the most frequently investigated pollutant (N = 9), followed by NO_2, EC, PM_{10} and $PM_{2.5}$. Annual mean exposures varied considerably across the studies: from 8 to 39 μg/m^3 for NO_2 and 5–31 μg/m^3 for $PM_{2.5}$. Six studies analysed proximity metrics such as distance to or density of traffic. Four studies evaluated the influence of concurrent noise exposure as a source of confounding or effect modification on the association between TRAP and stroke (Gan et al., 2012; Hoffmann et al., 2015; Sørensen et al., 2014; Stafoggia et al., 2014).

3.3. Meta-analyses and sensitivity analyses

A sufficient number of studies (≥3) were available to perform meta-analyses on NO_2, NO_x, EC, PM_{10}, and $PM_{2.5}$ in association with stroke (Fig. 2). The summary effect estimates indicated positive associations for EC, PM_{10}, and $PM_{2.5}$ with confidence intervals overlapping unity, and null associations for NO_2 or NO_x.

3.4. NO_2

For NO_2 the summary effect estimate was 0.98 (95% CI: 0.92; 1.05) per 10-μg/m^3 increment (N = 7) (Fig. 3A). The individual associations were moderately heterogeneous (I^2 = 64%) and varied in direction. Three studies estimated associations of NO_2 with fatal stroke separately, and with fatal and non-fatal stroke combined. In two of those studies, the Danish DDCH (Hoffmann et al., 2015) and the KPNC Oakland (Alexeeff et al., 2018), the estimated effects on fatal stroke were large and positive (RR = 1.47 and 1.57 respectively), in contrast to the smaller estimated effects on the combined stroke (RR = 1.08 and 0.96 respectively) (Supplementary Fig. 2). The two studies investigating a positive and negative exposure-response function were the DDCH (Andersen et al., 2012; Sørensen et al., 2014) and HIMS (Dirgawati et al., 2019), a highly selected population of older men, respectively.

3.5. NO_x

The meta-analysis of NO_x and stroke (Fig. 3B) yielded a summary estimate of 0.99 (95% CI: 0.94; 1.04) per 20-μg/m^3 increment (N = 8). The heterogeneity of the associations was moderate (I^2 = 50%): the most heavily weighted association was inverse, from the CPRD London study (Carey et al., 2016), while the others were closer to null and/or estimated with less precision. One study was a case-control study analysing prevalent cases (Oudin et al., 2011). Regarding sensitivity analyses, no clear picture emerged from a comparison of associations with fatal stroke and associations with fatal and non-fatal stroke combined. However, similar to the findings for NO_2, the positive association of NO_x with fatal events in the DDCH study (Sørensen et al., 2014) was stronger than any of the individual associations with combined stroke (Supplementary Fig. 2). There was mixed evidence regarding the exposure-response function (e.g., negative slope in Dirgawati et al., 2019, and positive for categories of NO_x in Oudin et al., 2011).

3.6. EC

For EC the summary RR was 1.03 (95% CI: 0.98; 1.09) per 1-μg/m^3 increment. (N = 6) (Fig. 4A). Heterogeneity was low; four studies

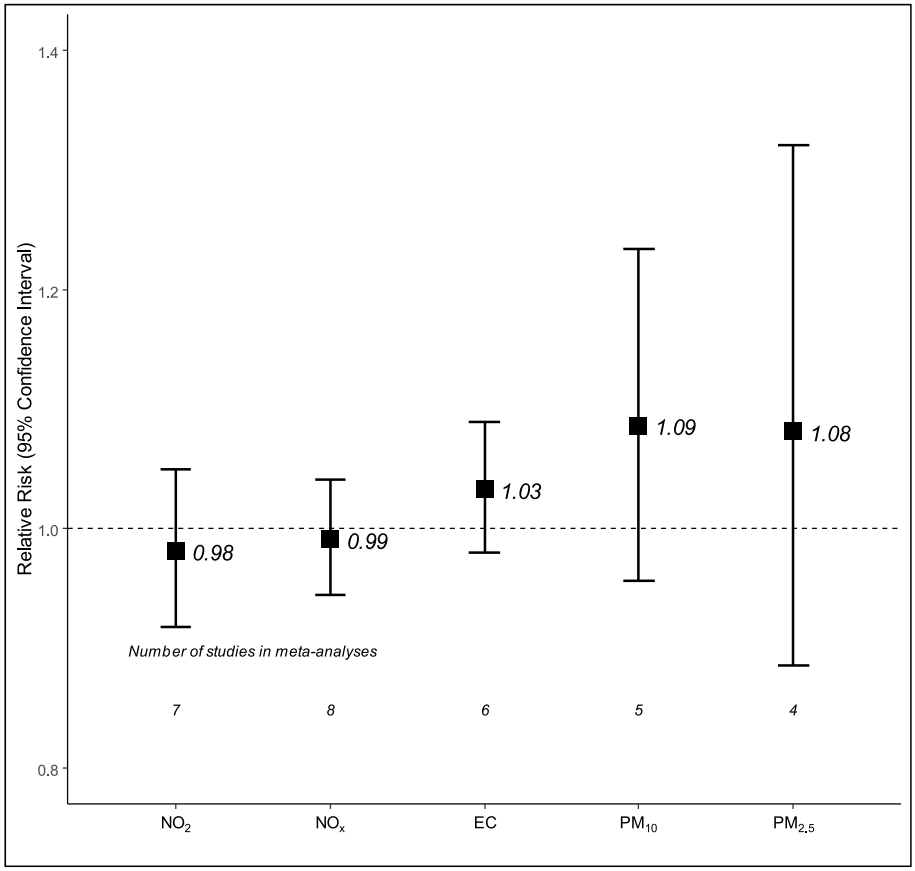

Fig. 2. Meta-analysis of associations between TRAP and incidence of stroke.

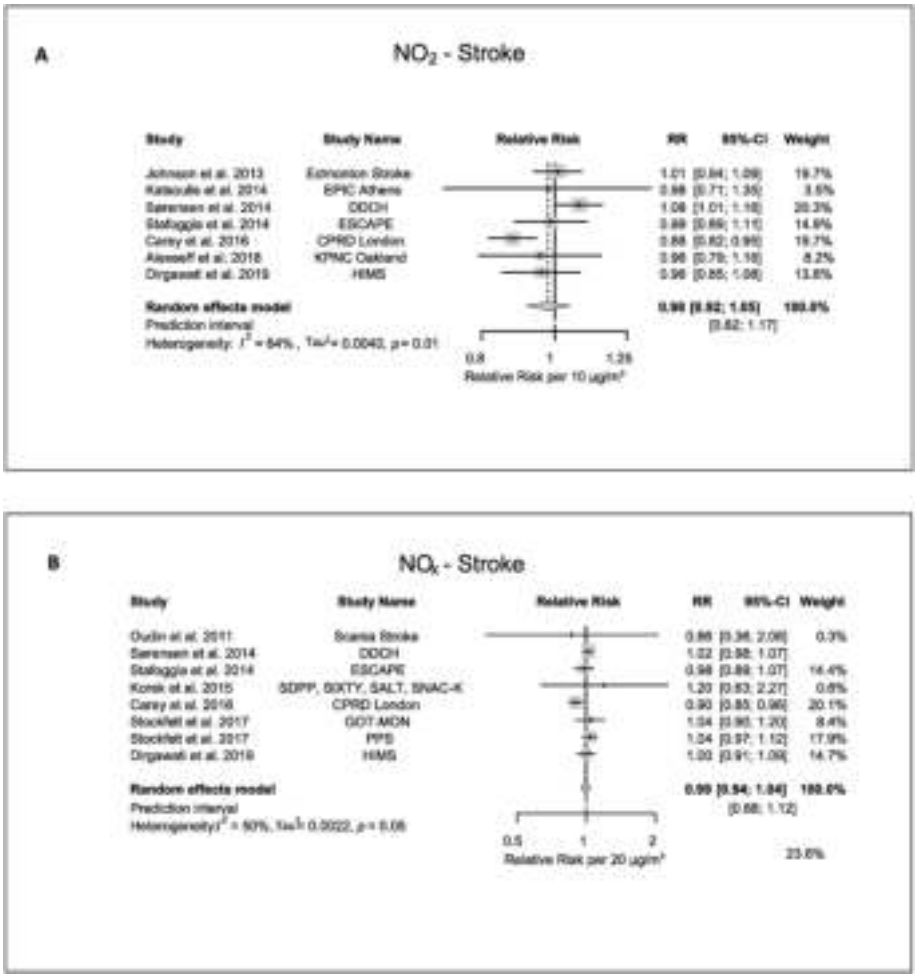

Fig. 3. Associations between gaseous traffic-related pollutants (NO_2 (A) and NO_x (B)) and incidence of stroke: Meta-analysis.

reported positive, though mostly imprecise, associations. The meta-analysis was dominated by the positive estimate from the Vancouver Administrative cohort study (Gan et al., 2012) with 84% of the overall weight. The Vancouver study had limited individual-level information on potential important sources of confounding, such as smoking, and adjusted for health conditions (i.e., diabetes mellitus, chronic obstructive pulmonary disease, and hypertensive heart disease) as proxies of behavior-related stroke risk factors. When we excluded the estimate from this study from meta-analysis, the summary estimated effect was virtually the same (RR = 1.02) although substantially less precise (95% CI: 0.86; 1.20) (Supplementary Fig. 2). Similar to NO_2 and NO_x, Dirgawati et al. (2019) reported a negative slope for incidence of non-fatal strokes over the study's relatively low concentration range of 0.1–1.5 10^5 m^{-1} for PM absorbance. On the other hand, Stafoggia et al. (2014) reported that a linear exposure-response function was a good approximation of the EC-stroke association in most of the 11 European cohorts in the ESCAPE study.

3.7. PM_{10}

The meta-analysis of PM_{10} exposure (Fig. 4B) and combined fatal and non-fatal stroke incidence (N = 5) yielded a summary RR of 1.09 (95% CI: 0.96–1.23) with no heterogeneity; the RRs from all but one study exceeded unity (Atkinson et al., 2013). A linear and monotonically increasing exposure-response function over the 5–26 µg/m^3 range was reported in the GOT-MONICA cohort (Stockfelt et al., 2017), and Stafoggia et al. (2014) reported a roughly linear shape of the exposure-response function for most of the 11 cohorts in ESCAPE.

3.8. $PM_{2.5}$

The effect estimates included in the meta-analysis of $PM_{2.5}$ (Fig. 4C) and stroke all exceeded unity, with no heterogeneity, and the summary RR was 1.08 (95% CI: 0.89–1.32) per 5-µg/m^3 increment (N = 4). Upon exclusion of the Australian study (Dirgawati et al., 2019) in analyses by geographic region, the estimate for the remaining Western European studies was substantially higher (1.17, 95% CI: 0.82; 1.67) (Supplementary Fig. 2). Both the ESCAPE study (Stafoggia et al., 2014) and the GOT-MONICA cohort (Stockfelt et al., 2017) reported a linear and monotonically increasing exposure-response function.

3.9. Results of studies not entering meta-analyses

There were too few cross-sectional studies on stroke prevalence to conduct meta-analysis. Briefly, a positive association was observed in the very small Estonian study of traffic specific PM_{10} and stroke (Pindus et al., 2016). The large 33CCHS study in China observed positive associations between NO_2 and stroke, specifically in overweight and obese subjects (Qin et al., 2015). The cross-sectional medium-sized study on Australian women showed an inverse, though imprecise association between NO_2 and stroke (Pindus et al., 2016).

A small number of studies examined other pollutants (PM_{coarse}, $PM_{traffic\text{-}specific}$), with the findings generally supportive of an association of TRAP with stroke (Table 1). Specifically, the ESCAPE study reported risks for PM_{coarse} of 1.02 (95% CI: 0.90, 1.16) per 5 µg/m^3 increment (Stafoggia et al., 2014) and the Heinz Nixdorf Recall (HNR) also reported an elevated estimate for PM_{coarse} (Hoffmann et al., 2015).

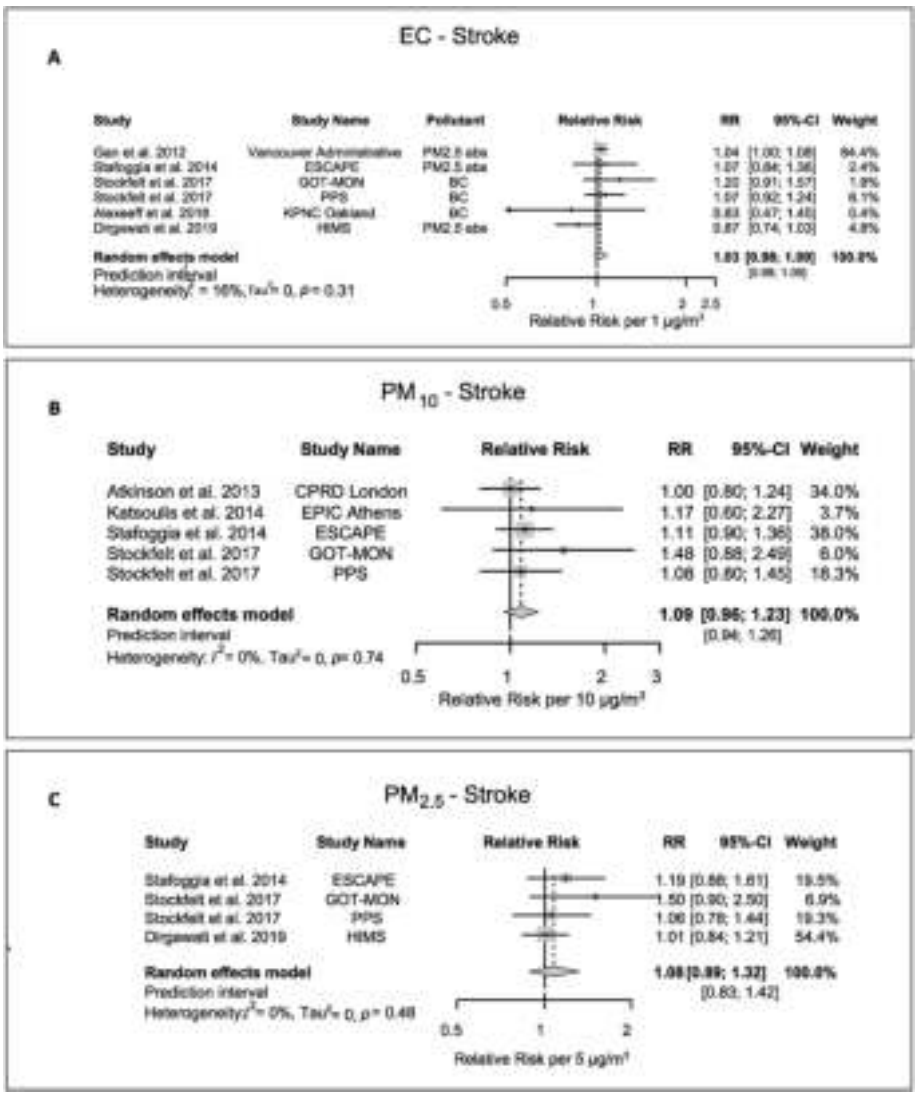

Fig. 4. Associations between particulate pollutants (EC (A), PM_{10} (B) and $PM_{2.5}$ (C)) and incidence of stroke: Meta-analysis.

Overall, four studies investigated indicators of $PM_{traffic\text{-}specific}$, all of which found positive associations with stroke (Korek et al., 2015; Pindus et al., 2016; Stockfelt et al., 2017) except one in the inverse direction (Atkinson et al., 2013).

Altogether six studies investigated associations with proximity to roads and/or traffic density, one of which was the ESCAPE multi-cohort (Stafoggia et al., 2014). Two studies reported positive associations (Andersen et al., 2012; Kulick et al., 2018), one of them with a monotonic exposure-response relationship. (Table 1). The four studies examining the effect of noise adjustment for one or more traffic-related pollutants showed stable or even larger effect estimates (Andersen et al., 2012; Gan et al., 2012; Hoffmann et al., 2015; Sørensen et al., 2014).

3.10. Overall assessment

The modified OHAT formal confidence assessment was conducted for the studies and exposure-stroke pairs for which a meta-analysis was conducted (N = 12). As the studies included in the meta-analysis were cohort or case-control, the initial rating for confidence was moderate for all exposure-stroke pairs (Table 2).

Among the factors that may reduce confidence, RoB was ranked low or moderate in most exposure-stroke pairs and domains (Supplementary Table 8). Two studies ranked as high RoB, due to lack of confounder control for smoking and BMI and/or selection bias (Gan et al., 2012; Johnson et al., 2013). No downgrade was applied because results were robust in sensitivity analyses excluding high RoB studies. We downgraded the level of confidence for all pollutants except NO_x for imprecision because although all meta-analyses met the sample size criterion and had sufficient power, the confidence intervals were wide, clearly including unity. Given the small number of studies in each exposure-stroke pair, an analysis of publication bias was infeasible; this did not lead to a downgrade.

We upgraded the evidence for associations of PM_{10} and $PM_{2.5}$ with stroke following the demonstration of a monotonic exposure-response function in the GOT-MONICA cohort (Stockfelt et al., 2017) and the results of a subset analyses in the 11 studies of the ESCAPE analysis. In this study, evaluations of individual cohort exposure-response functions with spline models (Stafoggia et al., 2014) showed that the estimates did not clearly deviate from linearity. Several mechanisms of potential bias towards the null were identified in the analysis including potential for over-adjustment or inclusion of potential intermediates (Alexeeff et al., 2018; Andersen et al., 2012; Atkinson et al., 2013; Carey et al., 2016). However, an upgrade was not considered appropriate, based on the small number of studies with potential underestimation of the association. Similarly, too few studies were available to evaluate consistency across geographic regions, populations or study period and therefore no upgrade was performed.

The final rating of the quality of the evidence base of the individual

Table 2
Confidence rating for TRAP and stroke incidence.

Pollutant	High ++++ Moderate +++ Low ++ Very low +		Factors decreasing confidence "0" if no concern; if serious concern to downgrade confidence				Factors increasing confidence "0" if not present; "+" if sufficient to upgrade confidence			**Final confidence rating**
	Study design	**Initial confidence rating** (# studies)	Risk of Bias	Unexplained inconsistency	Imprecision	Publication bias	Monotonic exposure-response	Consideration of residual confounding	Consistency across populations	
NO_2	Cohort, CC	+++ (N = 7)	0	0	–	0	0	0	0	++ (Low)
	Rationale	Cohort and case-control initially rated as moderate.	Not sensitive to exclusion of two studies with high RoB.	Moderate heterogeneity (I^2 = 64%).	Sample size met but confidence interval wide and includes unity.	No formal evaluation possible.	No evidence of plausible shape of ERF.	Confounding in both directions possible.	Too few studies across different populations.	
NO_X	Cohort, CC	+++ (N = 8)	0	0	0	0	0	0	0	+++ (Moderate)
	Rationale	Cohort and case-control initially rated as moderate.	No studies rated high RoB.	Moderate heterogeneity (I^2 = 50%), at least partly explained by one influential study with concerns.	Sample size met and confidence interval includes unity, but precise.	No formal evaluation possible.	No evidence of plausible shape of ERF.	Confounding in both directions possible.	Too few studies across different populations.	
EC	Cohort	+++ (N = 6)	0	0	–	0	0	0	0	++ (Low)
	Rationale	Cohort design initially rated as moderate.	Not sensitive to exclusion of one study with high RoB.	Low heterogeneity (I^2 = 16%).	Sample size met but confidence interval wide and includes unity.	No formal evaluation possible.	One multi-cohort study with monotonic ERF (Stafoggia et al., 2014).	Confounding in both directions possible.	Too few studies across different populations.	
PM_{10}	Cohort	+++ (N = 5)	0	0	–	0	+	0	0	+++ (Moderate)
	Rationale	Cohort design initially rated as moderate.	No studies rated high RoB.	Low heterogeneity (I^2 = 0).	Sample size met but confidence interval wide and includes unity.	No formal evaluation possible.	Two studies with either monotonic ERF or stable estimates in subset analysis (Stafoggia et al., 2014; Stockfelt et al., 2017).	Confounding in both directions possible.	Too few studies across different populations.	
$PM_{2.5}$	Cohort	+++ (N = 4)	0	0	–	0	+	0	0	+++ (Moderate)
	Rationale	Cohort design initially rated as moderate.	No studies rated high RoB.	Low heterogeneity (I^2 = 0).	Sample size met but confidence interval wide and includes unity.	No formal evaluation possible	Two studies with either monotonic ERF or stable estimates in subset analysis (Stafoggia et al., 2014; Stockfelt et al., 2017).	Confounding in both directions possible.	Too few studies across different populations.	

pollutant-stroke pairs was low for NO_2 and EC, and moderate for NO_x, $PM_{2.5}$ and PM_{10}, with EC, $PM_{2.5}$ and PM_{10} showing a positive meta-analytic estimate and NO_2 and NO_x indicating no effect in the meta-analysis (Table 2, Fig. 2). Combined confidence rating for the quality of the evidence base for measures of TRAP across all meta-analysed pollutants started with moderate confidence. We downgraded to low, because all $PM_{2.5}$ and PM_{10} studies were rated only as moderately traffic-specific studies whereas the highly traffic-specific NO_2 and NO_x meta-analytic estimates were null.

In our comprehensive narrative assessment, we concluded a moderate level of evidence in an association of exposure to TRAP with stroke. Overall, the study base and the meta-analyses provided evidence of an association of PM_{10} and suggestive evidence of an association of EC and $PM_{2.5}$ with stroke from a moderately large number of studies. Several high-quality studies from different regions across Europe and in North America yielded positive estimates for EC, PM_{10} and $PM_{2.5}$ in different populations, albeit the precision of the estimates was low, and the CIs of the meta-analytic estimates included unity. The determination was supported by some evidence from individual pollutant or proximity metric studies not included in meta-analyses, and relative stability in

noise-adjusted models. What made the evidence less compelling was the absence of evidence for NO_2 and NO_x, the pollutants considered highly traffic specific, yielding null findings in the meta-analyses.

Based on both assessments, the overall evaluation of an association between TRAP exposure and stroke was rated low to moderate.

3.11. Study characteristics and sensitivity-analyses following the new search

On January 06, 2022, we identified 64 newly published studies on stroke, 6 of which met the original inclusion criteria (Amini et al., 2020; Andersson et al., 2020; Magnoni et al., 2021; Rodins et al., 2020; Vivanco-Hidalgo et al., 2019) (Table 3). Estimates reported a positive association between different pollutants and stroke except the very large study in Milan (Magnoni et al., 2021) showing no association. The DNC, ELAPSE and HNR studies reported an RR for $PM_{2.5}$, NO_2 and PM_{10} of 1.12 (95%CI: 1.05; 1.25), 1.08 (95%CI: 1.04, 1.12) and 1.08 (95%CI: 1.01, 1.16) respectively. All 6 studies adjusted for traffic noise, reporting stable estimates. After including the new studies in sensitivity meta-analyses for $PM_{2.5}$ and NO_2 (Supplementary Fig. 2), we found slightly more robust adverse estimates for $PM_{2.5}$ (1.22; 95% CI: 1.03–1.21) and a null association for NO2 (1.01; 95% CI: 0.96–1.06).

4. Discussion

Based on 19 publications, we found low to moderate evidence for an association of long-term exposure to TRAP with stroke. This was based on a formal confidence rating according to the modified OHAT framework and on a comprehensive narrative assessment of the body of evidence. The meta-analytic estimates of EC, PM_{10} and $PM_{2.5}$ indicated positive associations for stroke, but for all pollutants the confidence intervals included unity. The evidence was strengthened by several high-quality studies with a positive exposure-response function or subset analysis indicating stable effects across levels of exposure. In addition, several individual studies investigating pollutants highly likely indicative of traffic, such traffic-specific PM fractions provided support for an association. Several studies also observed associations of proximity metrics such as residential distance to high traffic roadways or traffic density with stroke. Because cardiometabolic disease is likely influenced by traffic noise, some studies investigated possible confounding or effect modification by noise with mostly very stable results. However, the evidence for TRAP and stroke was generally weakened by null associations for the gaseous pollutants NO_2 and NO_x in the meta-analyses.

Following the systematic search in July 2019, six new studies have been published on stroke in association with TRAP. Overall, the recently published studies support the overall results from this review, showing no association for NO_2 and a significant adverse association for $PM_{2.5}$ in sensitivity analyses.

In a review and meta-analysis of general air pollution and stroke, Scheers et al. (2015) found statistically significant, but slightly lower associations with $PM_{2.5}$ and PM_{10} in a set of 20 studies. In contrast to our study, they targeted all studies exposed to $PM_{2.5}$ and PM_{10} from all source and not only TRAP related exposure studies, thus the higher number of studies included in their meta-analyses. They also reported unexplained geographical variability in these associations due to null results for PM_{10} exposures in Asia, while studies of PM_{10} exposures in North America and Europe were positive.

Contrary to our findings, in a recent review by Rugel and Brauer (2020), who analysed the effects of TRAP, noise, natural spaces and neighbourhood walkability in urban populations, the authors concluded that “when TRAP and noise were considered jointly, evidence was sufficient for increased cardiovascular morbidity with higher noise exposures; sufficient for no effect of TRAP on cardiovascular disease morbidity”. This review was limited to studies of at least two environmental exposures and outcomes were grouped more broadly, preventing a direct comparison of results with our study. Nevertheless, the conclusion of a vanishing TRAP effect upon adjustment for noise is contrary to ours, where studies generally showed little influence on the TRAP effect upon adjustment for noise in the few studies that did so.

Major strengths of this review include the systematic approach to study selection and evaluation using an a priori specified framework for exposure assessment and for a systematic evaluation of the epidemiological evidence. The use of several indicators of TRAP allowed the evaluation of consistency across pollutants and enabled us to base conclusions on a larger number of studies with diverse exposure metrics, rather than focusing only on a few meta-analysed pollutants. The outcomes of the overall review were grouped into relatively specific subgroups of cardiovascular disease to allow a more detailed evaluation. The identified studies were located in diverse areas of the world with different populations and different study designs. Several studies with in-depth characterization of the study population were available. The more recent studies also were more likely to include an evaluation of traffic noise.

One of the limitations of this review was the low number of studies per exposure-stroke pair for most pollutants. This prevented us from conducting more in-depth, stratified analyses by region, traffic-specificity or study design, the evaluation of publication bias, and inconclusive stratified and sensitivity analyses in many cases. A second specific limitation of this body of evidence was the potential underassessment and misclassification of stroke, depending on study design, age of the study population and data source. Third, the studies provided only limited opportunity to study the influence of potentially important co-exposures such as traffic noise, area-level SES or green space in a detailed manner, although each have been shown to be related to cardiovascular disease (World Health Organization, 2018; Yuan et al., 2021).

We followed the earlier 2010 HEI Report in recognizing that a major challenge for epidemiological research on TRAP and for the objective of selecting and evaluating studies remains – i.e., that no commonly measured or modelled pollutant is fully specific to traffic sources. Other sources, such as heating and energy production also contribute to commonly used indicators of TRAP (for example NO_2 and UFPs). Therefore, the use of accepted indicators of TRAP would ideally be evaluated in the context of the major drivers of exposure contrast in the geographic region and the specific design of each epidemiological study. However, given that detailed evaluation of the sources and data underlying exposure assessment in individual studies is not feasible, we consider it a strength that a novel exposure framework was developed to guide transparent selection and evaluation of the included studies.

One further challenge is identifying the most important time period for the elicitation of adverse effects on stroke. This question of relevant time of exposure also includes the role of short-term traffic exposures, which was not covered in this review. While in the triggering of acute events due to short term exposure has been demonstrated in many studies (Mills et al., 2015), it remains unclear how repeated high short-term exposures contribute to disease development. Also a better understanding of the molecular and cellular actions of nitrogen oxides on the cardiometabolic system is necessary to provide mechanistic evidence for a plausible adverse health effect. So far, only limited evidence is available from toxicological studies at relevant ambient concentrations (Burbure et al., 2007; Channell et al., 2012; Huang et al., 2012; Li et al., 2011; Riedl et al., 2012).

5. Conclusions

The available literature provides low-to moderate evidence for an association of TRAP with stroke. As traffic in cities remains the most important source of contrasts in air pollution, future studies should specifically focus on small-scale exposure assessment, ideally also including other factors associated with traffic, such as traffic noise, area-level SES and green space, to improve the evidence base. The role these

Table 3
Key study characteristics of the newly identified studies (up to January 2022).

Reference	Study Name Location	Study period	Study design and sample size	Exposure Assessment	Age at baseline, sex	Stroke outcome ascertainment	Monotonic ER-function	Confounder adjustment	Results (estimate, 95% CI, increment)	Results (estimate, 95% CI, increment) Adjusted for road traffic noise
Magnoni et al. (2021)	Data collected by the Agency for Health Protection (ATS) Milan, Italy	2011–2018	Cohort 1,087,110	LUR model	Mean Age: 54, both	Medical record	No	Age, sex, citizenship, Italian Deprivation Index	**Fatal and non-fatal ischemic (HR)** NO_2 0.99 (0.96, 1.03) per 10 μg/m^3 **Fatal and non-fatal heamorrahgic (HR)** NO_2 0.99 (0.92, 1.06) per 10 μg/m^3	**Fatal and non-fatal ischemic (HR)** NO_2 0.98 (0.94, 1.02) per 10 μg/m^3 **Fatal and non-fatal heamorrahgic (HR)** NO_2 0.96 (0.90, 1.04) per 10 μg/m^3
Amini et al. (2020)	Danish Nurse Study Denmark, nationwide	1993–2014	Cohort 23, 423	Danish air pollution modeling system, called DEHM/UBM/AirGIS	Mean Age: 52.6, female	National patient registry	Yes	Age, year of entry, calendar year, income, degree of urnabanicity, physical activity, alcohol, smoking, marital status, fruit consumption	**Fatal and non-fatal (all stroke) (HR)** $PM_{2.5}$ 1.12 (1.05, 1.25) per 3.9 μg/m^3 PM_{10} 1.05 (0.97, 1.13) per 3.3 μg/m^3 NO_2 1.05 (0.97, 1.13) per 8.0 μg/m^3 NO_x 1.02 (0.99, 1.06) per 11.0 μg/m^3	**Fatal and non-fatal (all stroke) HR** $PM_{2.5}$ 1.13 (1.01, 1.25) per 3.9 μg/m^3 PM_{10} 1.05 (0.97, 1.13) per 3.3 μg/m^3 NO_2 1.05 (0.97, 1.15) per 8.0 μg/m^3 NO_x 1.03 (0.99, 1.06) per 11.0 μg/m^3
Andersson et al. (2020)	PPS Gothenburg, Sweden	1970–2011	Cohort 6304	High resolution dispersion model	Age range: 47–55; men	Hospital discharge register, Swedish national death register	No	Calendar year, marriage/cohabitation, SES, smoking, BMI, cholesterol, stress, heredity, diabetes, physical activity, age	**Fatal and non-fatal (all stroke) (HR)** NO_x 1.02 (0.97, 1.07) per 10 μg/m^3 **Fatal and non-fatal (all stroke) (HR) categorized** NO_x 1.14 (0.93, 1.40) 36.1–44.1 versus <36.7 μg/m^3 NO_x 1.05 (0.85, 1.3) 44.1–53.3 versus <36.7 μg/m^3 NO_x 1.05 (0.85, 1.30) 53.3–64.8 versus <36.7 μg/m^3 NO_x 1.25 (1.02, 1.54) >64.8 versus <36.7 μg/m^3	**Results only for categories of exposure** NO_x 1.14 (0.93, 1.41) 36.1–44.1 versus <36.7 μg/m^3 NO_x 1.06 (0.85, 1.31) 44.1–53.3 versus <36.7 μg/m^3 NO_x 1.04 (0.83, 1.32) 53.3–64.8 versus <36.7 μg/m^3 NO_x 1.20 (0.93, 1.56) >64.8 versus <36.7 μg/m^3
Vivanco-Hidalgo et al. (2019)	Barcelona, Spain	2005–2014	Cross-sectional 2786	LUR model	Mean age: 75; both	BASICMAR database	no	Age, sex, smoking status, nSES, comorbidities[a]	**Severe Ishemic stroke (OR)** $PM_{2.5}$ Q2 1.01 (0.80, 1.26) $PM_{2.5}$ Q3 0. 93 (0.74, 1.17) $PM_{2.5}$ Q4 1.04 (0.83, 1.31)	**Severe Ishemic stroke (OR)** $PM_{2.5}$ Q2 0.97 (0.77, 1.21) $PM_{2.5}$ Q3 0.88 (0.70, 1.11) $PM_{2.5}$ Q4 0.95 (0.75, 1.20) **Adjusted for noise and green space**

(*continued on next page*)

Table 3 (*continued*)

Reference	Study Name Location	Study period	Study design and sample size	Exposure Assessment	Age at baseline, sex	Stroke outcome ascertainment	Monotonic ER-function	Confounder adjustment	Results (estimate, 95% CI, increment)	Results (estimate, 95% CI, increment) Adjusted for road traffic noise
(Wolf et al., 2021)	ELAPSE Multiple cities	1992–2015	Cohort 137,148	LUR model	Mean Age: 54, both	Hospital discharge and death registries	yes	Subcohort strata, age, sex, year of baseline visit, marital status, BMI, smoking, employment status, education, 2001 income mean on a nSES	**Fatal and non-fatal stroke (HR)** $PM_{2.5}$ 1.10 (1.01, 1.21) per 5 μg/m^3 NO_2 1.08 (1.04, 1.12) per 10 μg/m^3 BC 1.06 (1.02, 1.10) per 0.5[a]10^{-5}/m	**Fatal and non-fatal stroke (cohort with available data on noise) (HR)** $PM_{2.5}$ 1.09 (0.99, 1.21) per 5 μg/m^3 NO_2 1.08 (1.03, 1.12) per 10 μg/m^3 BC 1.05 (1.01, 1.10) per 0.5[a]10^{-5}/m
Rodins et al. (2020)	HNR Western Germany	2000-14 years follow-up	Cohort 4105	EURAD-CTM	Mean Age: 59.1, both	Self-report, physician interviews and medical records	not specified	Age, sex, iSES, nSES, BMI, smoking, alcohol, physical activity, nutrition, night-time traffic noise	**Fatal and non-fatal stroke (HR)** PM_{10} 1.08 (1.01, 1.16) per 1 μg/m^3 $PM_{10\ traffic}$ 2.55 (1.11, 5.86) per 1 μg/m^3 $PM_{2.5}$ 1.16 (1.02, 1.34) per 1 μg/m^3 $PM_{2.5\ traffic}$ 2.53 (1.07, 5.97) per 1 μg/m^3 PN_{AM} 1.06 (1.01, 1.10) per 100n/cm^3 $PN_{AM\ traffic}$ 1.27 (1.05, 1.55) per 100n/cm^3 AOC 1.07 (1.01, 1.13) per 0.1 μg/m^3 $AOC_{traffic}$ 1.33 (1.00, 1.76) per 0.1 μg/m^3 EC 1.07 (1.01, 1.14) per 0.1 μg/m^3 $EC_{traffic}$ 1.78 (1.02, 3.12) per 0.1 μg/m^3 **All results adjusted for traffic noise**	

[a] Comorbidities: hypertensions, diabetes mellitus, dyslipidemia, prior history of coronary heart disease/stroke/transient ischemic attack.

urban co-exposures needs more attention, given that there is clear evidence that noise and area-level SES, and to a lesser degree lack of green space, have adverse health effects on cardiometabolic health and quality of life (Diez Roux et al., 2016; Schultz et al., 2018; World Health Organization, 2018; Yuan et al., 2021). The interplay of these exposures in terms of confounding and potential synergism needs to be better understood for effective prevention and urban planning. With cities starting to rethink urban planning and the interactions of personal motor vehicles, active transport and increased green space (for example Paris, Barcelona, Copenhagen, etc.), the effects of these changes on cardiometabolic health should be evaluated.

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Declaration of competing interest

No author declares a conflict of interest.

The following increments were used: 10 μg/m^3 for NO_2, 20 μg/m^3 for NO_x, 1 μg/m^3 for EC, 10 μg/m^3 for PM_{10}, and 5 μg/m^3 for $PM_{2.5}$. Effect estimates cannot be directly compared across the different traffic-related pollutants because the selected increments do not necessarily represent the same contrast in exposure.

A. Forest plot of the association between NO_2 and stroke, **B**. Forest plot of the association between NO_x and stroke.

Note: Oudin et al., (2011) are estimates for non-fatal stroke; others combined fatal and non-fatal stroke.

A. Forest plot of the association between EC and stroke; **B.** Forest plot of the association between PM_{10} and stroke; **C.** Forest plot of the association between $PM_{2.5}$ and stroke.

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Appendix A. Supplementary data

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PFAS levels and determinants of variability in exposure in European teenagers – Results from the HBM4EU aligned studies (2014–2021)

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ABSTRACT

Background: Perfluoroalkyl substances (PFAS) are man-made fluorinated chemicals, widely used in various types of consumer products, resulting in their omnipresence in human populations. The aim of this study was to describe current PFAS levels in European teenagers and to investigate the determinants of serum/plasma concentrations in this specific age group.

Methods: PFAS concentrations were determined in serum or plasma samples from 1957 teenagers (12–18 years) from 9 European countries as part of the HBM4EU aligned studies (2014–2021). Questionnaire data were post-harmonized by each study and quality checked centrally. Only PFAS with an overall quantification frequency of at least 60% (PFOS, PFOA, PFHxS and PFNA) were included in the analyses. Sociodemographic and lifestyle factors were analysed together with food consumption frequencies to identify determinants of PFAS exposure. The variables study, sex and the highest educational level of household were included as fixed factors in the multivariable linear regression models for all PFAS and each dietary variable was added to the fixed model one by one and for each PFAS separately.

Results: The European exposure values for PFAS were reported as geometric means with 95% confidence intervals (CI): PFOS [2.13 μg/L (1.63–2.78)], PFOA ([0.97 μg/L (0.75–1.26)]), PFNA [0.30 μg/L (0.19–0.45)] and PFHxS [0.41 μg/L (0.33–0.52)]. The estimated geometric mean exposure levels were significantly higher in the North and West versus the South and East of Europe. Boys had significantly higher concentrations of the four PFAS compared to girls and significantly higher PFASs concentrations were found in teenagers from households with a higher education level. Consumption of seafood and fish at least 2 times per week was significantly associated

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with 21% (95% CI: 12–31%) increase in PFOS concentrations and 20% (95% CI: 10–31%) increase in PFNA concentrations as compared to less frequent consumption of seafood and fish. The same trend was observed for PFOA and PFHxS but not statistically significant. Consumption of eggs at least 2 times per week was associated with 11% (95% CI: 2–22%) and 14% (95% CI: 2–27%) increase in PFOS and PFNA concentrations, respectively, as compared to less frequent consumption of eggs. Significantly higher PFOS concentrations were observed for participants consuming offal (14% (95% CI: 3–26%)), the same trend was observed for the other PFAS but not statistically significant. Local food consumption at least 2 times per week was associated with 40% (95% CI: 19–64%) increase in PFOS levels as compared to those consuming local food less frequently.

Conclusion: This work provides information about current levels of PFAS in European teenagers and potential dietary sources of exposure to PFAS in European teenagers. These results can be of use for targeted monitoring of PFAS in food.

1. Introduction

Perfluoroalkyl substances (PFAS) are human-made fluorinated chemical compounds, listed by Stockholm Convention (2019) as persistent organic pollutants. They are persistent in the environment, widespread and bioaccumulating in both humans and wildlife. The use of PFAS include surface coating and protectant formulations, fire-fighting foams, paper and cardboard packaging products, carpets, leather products, and water- and stain-proof textiles (ATSDR, 2018). The wide use of these chemicals resulted in their presence in the body of almost every human (Berg et al., 2014; Kato et al., 2014; Liu et al., 2011; Mørck et al., 2015; Schoeters et al., 2017). Due to its adverse health effects, perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) were phased out by major manufacturers since 2000 and 2006, respectively (Dassuncao et al., 2018; Xu et al., 2021) and have been restricted under the EU's Persistent Organic Pollutants Regulation in 2009 and 2019 (PFOS and PFOA, respectively). Another substance in PFAS group, perfluorohexane sulfonic acid (PFHxS), is under consideration for inclusion in the Stockholm Convention as well (Stockholm Convention, 2019). Despite the phase out, PFOS and PFOA were still predominant PFAS substances in European newborns years after the regulation (Cariou et al., 2015; Colles et al., 2020; Richterová et al., 2018). Besides, new substitutes of restricted PFAS are emerging, such as shorter-chain PFAS analogues (Xu et al., 2021). In the general population, exposure to PFAS can occur through contaminated air, drinking water, food, soil and dermal uptake (ATSDR, 2018; Ragnarsdóttir et al., 2022). Most of the previous studies on PFAS exposure have been done in pregnant women, newborns, or adults, however, less attention is paid to teenagers. However, some recent studies showed associations between high PFAS exposure in teenagers and higher risk of health outcomes, such as dyslipidemia, hypertension, obesity (Averina et al., 2021), asthma (Averina et al., 2019) or association with levels of reproductive hormones (Tsai et al., 2015).

Determinants of exposure to chemicals in teenagers might differ from those of children, since the typical hand-to-mouth behaviour of young children can lead to different ways of exposure. Exposure determinants of PFAS of teenagers may also differ from those of adults. Unlike teenagers, adults can be occupationally exposed to PFAS, and the use of PFAS containing products probably vary between these two age groups. Previous studies reported associations with parity, breastfeeding or educational level and PFAS levels in serum or plasma (Berg et al., 2014; Bjerregaard-Olesen et al., 2016; Kato et al., 2014; Richterová et al., 2018; Sagiv et al., 2015). However, in teenagers, these determinants most likely do not play a major role in their PFAS exposure, except of educational level of household, which could be linked to the lifestyle of members of household. Also, the shift from more parent-dependent behaviour of a child to more (but not-fully) independent behaviour of teenagers must be taken into account. Altogether, it gives a unique opportunity to explore determinants of PFAS exposure in this very specific age group in both sexes, before occupational exposure or giving birth can step in as the main determinants of PFAS concentrations.

EFSA reported detectable PFAS levels in various types of food samples obtained from 16 European countries and tolerable weekly intake (TWI) of 4.4 ng/kg body weight was derived for the sum of PFOA, PFOS, PFNA and PFHxS (EFSA, 2020). This suggests that diet can be an important source of PFAS exposure also for teenagers. In this study, we describe exposure levels of PFAS in European teenagers of the HBM4EU aligned studies (2014–2021), explore differences in exposure levels between the geographical regions (North, East, South, West) and examine the associations between sociodemographic characteristics, dietary patterns and serum/plasma PFAS levels in pooled data from 9 European countries.

2. Methods

2.1. Study population

This study was conducted as a part of the European Human Biomonitoring Initiative (HBM4EU) aligned studies. The aim of this initiative was to harmonise human biomonitoring in Europe to support policy making (Ganzleben et al., 2017). Design and general characteristics of the HBM4EU aligned studies was described elsewhere (Gilles et al., 2021, 2022). Briefly, the HBM4EU aligned studies collected comparable exposure data, questionnaire data and data on health outcomes across the European studies. The population was divided into three age groups: children (6–11 years), teenagers (12–19 years) and adults (20–39 years), and in each age group prioritized chemical substances were analysed. PFAS exposure was selected to be measured in the teenagers age group only. Blood samples were taken by clinical staff. Questionnaires were filled out by interviewer or the study participants themselves and/or their parents. 9 out of 11 European studies of teenagers included in the aligned studies determined PFAS concentrations in blood samples: ESTEBAN (Étude de santé sur l'environnement, la biosurveillance, l'activité physique et la nutrition; France; Fillol et al., 2021), GerES V-sub (German Environmental Survey, 2014–2017, unweighted subsample; Germany; Schultz et al., 2021), Riksmaten Adolescents 2016–17 (Sweden; Moraeus et al., 2018), NEBII (Norwegian Environmental Biobank II; Norway; Magnus et al., 2016), FLEHS IV (Flemish Environment and Health Study IV; Belgium; Schoeters et al., 2022), BEA (Biomonitorización en Adolescentes; Spain; Pérez-Gómez et al., 2013), SLO CRP (Exposure of children and adolescents to selected chemicals through their habitat environment; Slovenia; Stajnko et al., 2020), PCB cohort follow-up (Endocrine disruptors and health in children and teenagers in Slovakia; Slovakia; Hertz-Picciotto et al., 2003) and CROME (Cross-Mediterranean Environment and Health Network; Greece) (Table 1). All studies had obtained ethical approval and all participants or their legal guardians signed an informed consent prior to participation.

2.2. Exposure assessment

Blood samples of teenagers were collected and analysed in laboratories that participated and obtained successful results in the HBM4EU Quality Assurance/Quality Control (QA/QC) programme (Esteban López et al., 2021). The proficiency of laboratories for the analysis of PFAS in serum comprised one round of interlaboratory comparison

Table 1
Description of participating studies with PFAS concentrations data available.

Study	Country[a]	Region	N	Sampling year	Age (years)
Riksmaten Adolescents	Sweden	North	300	2016–2017	12–17
NEB II	Norway	North	177	2016–2017	12–14
PCB cohort follow-up	Slovakia	East	292	2019–2020	15–17
BEA	Spain	South	299	2017–2018	13–17
SLO CRP	Slovenia	South	94	2018	12–15
CROME	Greece	South	52	2020–2021	12–18
ESTEBAN	France	West	143	2014–2016	12–17
GerES V-sub	Germany	West	300	2014–2017	12–17
FLEHS IV	Belgium	West	300	2017–2018	13–16

[a] The HBM4EU aligned studies are not all country representative studies.

investigations (ICI) and three rounds of external quality assurance schemes (EQUAS) (Nübler et al., 2022). 21 laboratories from 12 countries achieved satisfactory results for at least six of PFAS biomarkers. ESTEBAN and GerES V-sub PFAS exposure data were generated before the HBM4EU QA/QC programme, but the laboratories successfully participated in the HBM4EU QA/QC programme, thus exposure data were deemed comparable and approved a posteriori by the HBM4EU Quality Assurance Unit (QAU) (Table S1). Data for Riksmaten Adolescents 2016-17 were generated before the HBM4EU QA/QC scheme and were evaluated as comparability not guaranteed by the HBM4EU QAU. Sensitivity analysis was performed to see if the data from Sweden affected the calculated European exposure values and geographical comparisons. Concentrations of twelve PFAS were measured in blood serum or plasma: perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), perfluorohexanoic acid (PFHxA), perfluoropentanoic acid (PFPeA), perfluoroheptanoic acid (PFHpA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluoroheptanane sulfonic acid (PFHpS), perfluorododecanoic acid (PFDoDA) and perfluorobutane sulfonic acid (PFBS). Information with regard to biological matrix, analytical method, type of forms measured, and HBM4EU QA/QC label for each of the studies can be found in Supplementary material (Table S1). The limit of quantification (LOQ) differed across the studies, more details can be found in Supplementary material (Table S2).

2.3. Questionnaire data

Only few studies used the modified questionnaires developed within the HBM4EU project, other studies were already ongoing or finished, therefore different questionnaires were applied across the studies and post-harmonization was necessary. The post-harmonization process for questionnaire data was done by each study according to the HBM4EU codebook (https://doi.org/10.5281/zenodo.6598532). Data on socio-demographic and lifestyle factors were gathered for this study. The variables included sex and age of participant, degree of urbanization, the highest educational level of household, occupation of the participant (part time job relevant only for older participants), alcohol consumption and smoking of participant. A subject's living environment is classified according to the degree of urbanisation (DEGURBA) classification of Eurostat distinguishing three levels of urbanisation. i.e. densely populated area (cities), intermediate density area (towns and suburbs) and thinly populated area (rural area) (Lewis Dijkstra, 2014). Data on educational level were categorized based on The International Standard Classification of Education (ISCED, 2012): low education (ISCED 0–2), medium education (ISCED 3–4), high education (ISCED ≥5). Smoking, alcohol consumption and occupation of participant were categorized as yes or no. We also received information about recent renovation works in the residency, however variable renovation was not provided by Riksmaten Adolescents and GerES V-sub study. Data on many other variables that may be important for PFAS-exposure such as use of impregnated clothes or sport equipment, data on food packaging, popcorn consumption or use of Teflon cookware were missing in most of the studies.

Dietary factors were analysed as potential determinants of PFAS exposure. We received data on consumption of seafood, fish (including freshwater and sea fish when available), meat, offal, milk and dairy products, eggs, local food and fast food (consumption over the last year or 24 h). Frequency of food consumption for each variable was divided into 6 categories: 0 = Never, 1 = Rarely (<1x/month), 2 = Sometimes (≤1x/week but ≥1x/month), 3 = Often (2-3x/week), 4 = Very Often (4-6x/week), 5 = Everyday (≥7x/week). Since we observed low percentage of subjects in some categories of food consumption (Table 3), we decided to group some categories together based on the proportion of subjects in each category of frequency. Seafood and fish, eggs consumption and consumption of local food was dichotomised: <2x/week or ≥2x/week, meat consumption was grouped into 3 categories: ≤1x/week, 2-3x/week, ≥4x/week, offal was categorized as never or sometimes, milk and dairy consumption was categorized as <4x/week or ≥4x/week, and fast food consumption was grouped into 3 categories: <1x/month, ≥1x/month but ≤1x/week, ≥2x/week. No data on seafood consumption were available in PCB cohort follow-up. In Riksmaten Adolescents cohort, seafood and fish consumption were the only dietary variables provided. Fast food consumption was missing in FLEHS IV and data on local food consumption were provided by 4 out of 9 studies: CROME, SLO CRP, PCB cohort follow-up and FLEHS IV. Variable local food included consumption of plant-based and animal-based locally produced food in PCB cohort follow-up and plant-based local food consumption in CROME, SLO CRP and FLEHS IV. We also obtained data on type (bottled, tap, ground water or other) and source of drinking water (public, private well or both). Data on the type of drinking water consumed were missing in Riksmaten Adolescents and GerES V-sub study and source of drinking water was missing in ESTEBAN study. Overall, only 5 people responded that the source of water at home is both public and private well. The answer itself does not provide any information on the potential source of exposure, thus we decided to treat this category as missing.

2.4. Data management and statistical analysis

Harmonized data were uploaded to a data platform, quality checked by the central data management team (checking for outliers, coding, inconsistencies, etc.) and after that, the final dataset was provided for statistical analyses. Statistical analyses were performed using statistical programs, SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and SPSS (version 22). Concentrations below LOQ were imputed, using a truncated lognormal distribution (Lubin et al., 2004). The imputation was done per biomarker and per data collection. Random values were imputed (between 0 and the LOQ) from the lognormal distribution with the estimated mean and standard deviation. Distribution of PFAS concentrations were skewed and concentrations were ln-transformed using natural logarithm. Only PFAS with an overall quantification frequency of at least 60% were included for further analysis, and this includes PFOS, PFOA, PFHxS and PFNA.

European exposure values were derived as geometric means with their 95% confidence intervals (CIs) using the survey procedure in SAS with country as cluster to account for the complex survey design when calculating variance estimates. To look into the effect of geographical region on the estimated exposure values, region was tested in a survey regression model adjusted for sex of the participant and educational level of the household. A p-value < 0.05 was taken as significance level. A sensitivity analysis was performed to check the influence of the PFAS data from Sweden, for which the comparability was not guaranteed by the HBM4EU QAU, on the estimated European exposure values and geographical comparisons.

To identify possible exposure determinants, association between

each PFAS and each variable (sex, age, education, sampling year, degree of urbanisation, smoking, renovation) was examined in the individual studies and in the pooled sample of teenagers by univariate linear regression. Directed acyclic graphs were plotted to prioritize potential predictors of exposure (data not shown). The variables study, sex and the highest educational level of household were included as fixed factors in the multiple linear regression (MLR) models for all PFAS, because of their significance in the univariate analysis. Age of participants in years was not significant in the multiple regression analysis and was not included as a fixed factor in further analyses. Next, we built separate model for each dietary variable and each PFAS, adjusted for the fixed factors. Using this approach, we were able to identify dietary determinants of PFAS exposure, while taking into account important general characteristics, such as sex and educational level of household, and differences between countries (studies). Beta coefficients were exponentiated and presented are the effect estimates that represent proportional changes in PFAS concentrations in each category compared to the reference category.

As a sensitivity analysis, we included sampling year in MLR model as another potential determinant of PFAS exposure. We also compared results of MLR with/without educational level. Final MLR models used in the pooled data analysis were applied to study-by-study analysis as well.

3. Results

General characteristics of the teenagers (households), and their food consumption behaviour are described in Tables 2 and 3 respectively.

Table 2
General characteristics of the study population (n = 1957).

Characteristics	n (%) or AM ± SD	Missing[a] n (%)
Teenager		
Sex		0 (0)
Boys	937 (48)	
Girls	1020 (52)	
Age (in years)	14.5 ± 1.4	4 (0)
Active smoking		
Yes	98 (7)	572 (29)
No	1287 (93)	
Passive smoking		
Yes	331 (25)	647 (33)
No	979 (75)	
Alcohol		
Yes	680 (56)	753 (38)
No	524 (44)	
Occupation		
Yes	124 (7)	99 (5)
No	1734 (93)	
Household		
Region		0 (0)
North	477 (24)	
South	445 (23)	
West	743 (38)	
East	292 (15)	
Educational level of household		43 (2)
Low (ISCED 0–2)	162 (8)	
Medium (ISCED 3–4)	737 (39)	
High (ISCED ≥ 5)	1015 (53)	
Degree of urbanization		2 (0.1)
Cities	620 (32)	
Towns and suburbs	750 (38)	
Rural areas	585 (30)	
Recent renovation		
Yes	492 (39)	681 (35)
No	784 (61)	

AM = arithmetic mean; SD = standard deviation.

[a] In some studies variable was not included in the questionnaire (see Supplementary Material Table S3).

Table 3
Food and drinking water consumption in population of European teenagers (n = 1957).

	n (%)	Missing n (%)*
Drinking water		912 (47)
Bottled	141 (13)	
Tap	810 (78)	
Ground	24 (2)	
Other	70 (7)	
Drinking water - source		391 (20)
Public	1488 (95)	
Private well	73 (4)	
Both	5 (1)	
Food consumption		
Fish		131 (7)
never	187 (10)	
<1x/month	279 (16)	
≤1x/week but ≥ 1x/month	826 (45)	
2-3x/week	332 (18)	
4-6x/week	150 (8)	
≥7x/week	52 (3)	
Seafood		362 (19)
never	145 (9)	
<1x/month	69 (4)	
≤1x/week but ≥ 1x/month	741 (47)	
2-3x/week	416 (26)	
4-6x/week	164 (10)	
≥7x/week	60 (4)	
Meat		346 (18)
never	19 (1)	
<1x/month	91 (6)	
≤1x/week but ≥ 1x/month	332 (21)	
2-3x/week	241 (15)	
4-6x/week	394 (24)	
≥7x/week	534 (33)	
Offal		638 (33)
never	870 (66)	
<1x/month	365 (28)	
≤1x/week but ≥ 1x/month	76 (5)	
2-3x/week	8 (1)	
4-6x/week	-	
≥7x/week	-	
Milk and dairy products		344 (18)
never	50 (3)	
<1x/month	34 (2)	
≤1x/week but ≥ 1x/month	403 (25)	
2-3x/week	160 (10)	
4-6x/week	191 (12)	
≥7x/week	775 (48)	
Eggs		640 (33)
never	39 (3)	
<1x/month	123 (9)	
≤1x/week but ≥ 1x/month	770 (58)	
2-3x/week	311 (24)	
4-6x/week	60 (5)	
≥7x/week	14 (1)	
Fast food		638 (33)
never	130 (10)	
<1x/month	408 (31)	
≤1x/week but ≥ 1x/month	528 (40)	
2-3x/week	185 (14)	
4-6x/week	58 (4)	
≥7x/week	10 (1)	
Local food		1221 (62)
never	193 (26)	
<1x/month	226 (30)	
≤1x/week but ≥ 1x/month	-	
2-3x/week	4 (1)	
4-6x/week	6 (1)	
≥7x/week	307 (42)	

* In some studies variable was not included in the questionnaire (see Supplementary Material Table S4).

Mean age of teenagers at sampling was 14.5 (±1.4) years and 52% were girls. 7% of teenagers already had a part-time job. More than a half (56%) of the study population have ever drunk an alcoholic beverage, 7% were smokers and 25% of teenagers reported exposure to passive smoking. The largest number of participants were from Western Europe (38%), while Eastern Europe was represented only by one study (15%). More than half of the households had high educational level, only 8% of households had low educational level. 30% of teenagers was living in cities, 38% in towns or suburbs and 30% in rural areas, however, categories were not equally represented in some studies. For example, in PCB cohort follow-up, none of the participants was living in cities and in SLO CRP study, all the participants were living in rural area. On the other hand, in BEA and CROME studies, majority of participants was living in cities (86 and 73%, respectively). Recent renovation was reported by 39% of the households. The main source of drinking water was public source (95%) and the most common type of drinking water was tap water (78%). For food consumption, 10% of teenagers never ate fish and 9% never ate seafood. Fish consumption several times per week was reported by 29% of study population and even more (40%) ate seafood more than once a week. Meat was consumed everyday by 33% of teenagers, only 1% never ate meat. On the other hand, 66% of teenagers responded that they never ate offal. Almost half of the study population (48%) consumed milk and/or dairy products daily. Majority of participants (58%) consumed eggs several times per month but less than once a week. Fast food consumption several times per week was reported by 19% of teenagers, 10% never consumed fast food. 44% of teenagers consumed local food several times per week, however, data on consumption of local food was available only in 4 studies. Detailed characteristics of study participants in each study are presented in the Supplementary Tables S3 and 4.

Supplementary Table S2 gives an overview of the LOQ, percentage of samples above LOQ, and observed percentiles per compound per data collection. Only PFOS, PFOA, PFNA and PFHxS were quantified for at least 60% (PFOS 100%, PFOA 98%, PFHxS 81% and PFNA 80%) in the overall population, and were explored in further analyses.

For PFNA, GerES V-sub was excluded from the statistical analyses as only 12% of the values were quantified due to a high LOQ of 0.5 μg/L in comparison with the other LOQs (0.012–0.288 μg/L) (Table S2). For PFHxS, BEA was excluded from the statistical analyses as only 20% of the values were quantified due to a high LOQ of 0.34 μg/L in comparison with the other LOQs (0.014–0.25 μg/L) (Table S2).

PFOS is the most abundant PFAS compound, with an overall GM and 95% CI of 2.13 μg/L (95% CI: 1.63–2.78), followed by PFOA [0.97 μg/L (95% CI: 0.75–1.26)], PFHxS [0.41 μg/L (0.33–0.52)] and PFNA [0.30 μg/L (0.19–0.45)]. In the basic model adjusted for sex and educational level of the household, geographical region was a strongly influencing factor on the estimated exposure levels. Significantly higher levels were observed in the North (represented by 2 studies from NO and SE) and West (represented by 3 studies from FR, DE and BE) of Europe versus the South (represented by 3 studies from ES, EL, SI) and East (only represented by one study from SK) of Europe (Fig. 1) for all four PFASs, except for PFNA for which the observed difference between West and South was not statistically significant, while the East was significantly lower in comparison with all other regions. Excluding the data from Sweden, for which the comparability was not guaranteed by the HBM4EU QAU, did not affect the interpretation of the results (data not shown).

Detailed results of multiple linear regressions are presented in Supplementary Tables S5–8. We present results from pooled sample analysis only. Boys had significantly higher concentrations of the four PFAS compared to girls. In addition, we observed that medium and high educational level of household to be associated with increased levels of all four PFAS analysed compared to low educational level. Regarding dietary variables, we observed significant associations between PFOS and PFNA levels and consumption of seafood and fish (both $p < 0.001$), the same trend was observed for PFOA and PFHxS although not statistically significant (Fig. 2). Consumption of seafood and fish at least 2 times per week was associated with 21% (95% CI: 12–31%) increase in PFOS levels and 20% (95% CI: 10–31%) increase in PFNA levels. Consumption of eggs at least 2 times per week was associated with 11% (95% CI: 2–22%) ($p = 0.020$) and 14% (95% CI: 2–27%) ($p = 0.019$) increase in PFOS and PFNA levels, respectively. Consumption of offal was associated with 14% (95% CI: 3–26%) increase in PFOS levels ($p = 0.012$). Local food consumption at least 2 times per week was associated with 40% (95% CI: 19–64%)) increase in PFOS levels ($p < 0.001$), the same trend was observed between the other PFAS and local food consumption, although not statistically significant. We did not find any significant association between consumption of milk and dairy products or fast food consumption and PFAS levels. The observed associations between dietary determinants and PFAS concentrations were not affected by educational level in the model. As a sensitivity analysis, we included sampling year in MLR model. However, it was not significant in the MLR models. MLR models used in the pooled data analysis were applied to study-by-study analysis and we observed the same direction of the associations with dietary variables across the studies, although mostly not significant.

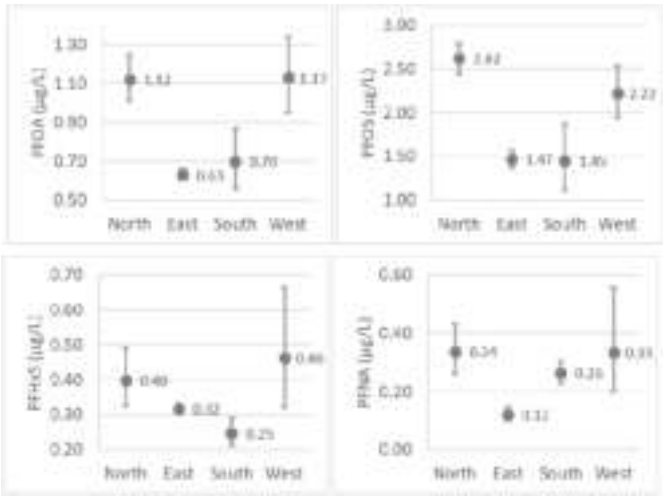

Fig. 1. Estimated geometric mean (GM) and 95% confidence interval (CI) for plasma/serum concentrations of PFOS, PFOA, PFHxS and PFNA by European region adjusted for sex of the participant and educational level of the household Contributing studies for Northern Europe: NEBII (Norway), Riksmaten Adolescents 2016–17 (Sweden); for Eastern Europe: PCB cohort follow-up (Slovakia); for Southern Europe: BEA (Spain), CROME (Greece), SLO CRP (Slovenia); and for Western Europe: ESTEBAN (France), GerES V-sub (Germany), FLEHS IV (Belgium).

4. Discussion

The HBM4EU aligned studies (2014–2021) generated PFAS exposure data for 1957 teenagers (12–19 years) from 9 European countries geographically spread over Europe. European exposure values, calculated as GM with 95% CI, were obtained for PFOS [2.13 μg/L (1.63–2.78)], PFOA ([0.97 μg/L (0.75–1.26)]), PFNA [0.30 μg/L (0.19–0.45)] and PFHxS [0.41 μg/L (0.33–0.52)]. Significantly higher concentrations were observed in the North and West of Europe versus the South and East.

These GM levels of PFOS and PFOA are comparable to data reported for Canadian teenagers for sampling in the same period (2016–2017 and 2018–2019) in a comparable age group (12–19 years) (Health Canada, 2019, 2021), and slightly lower than the levels observed in the United States (2015–2016 and 2017–2018; 12–19 years) (US-CDC, 2022). The GM levels for PFNA and PFHxS are lower than those reported from Health Canada and US-CDC. Respectively 9 and 8% of the HBM4EU participants exceeds the HBM-I values for PFOS (5 μg/L) and PFOA (2

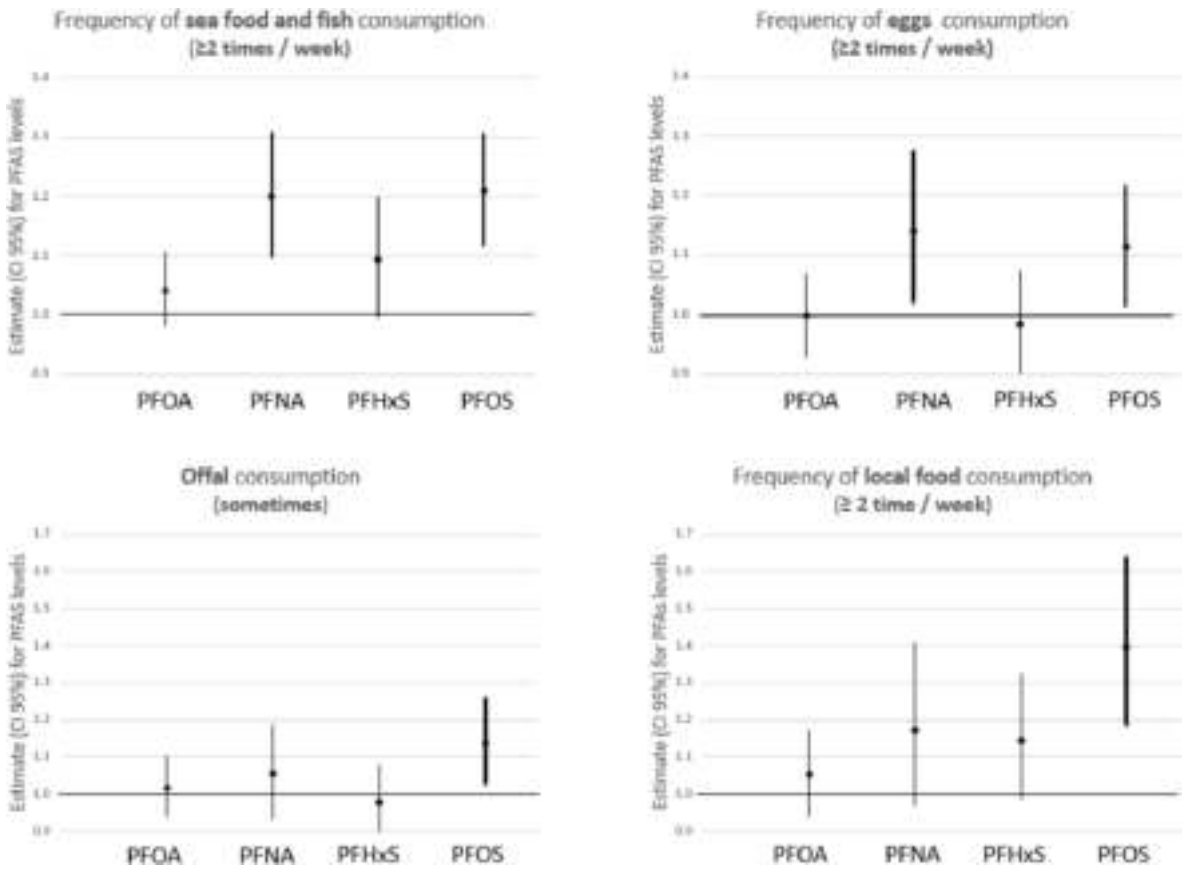

Fig. 2. Associations between PFAS concentrations in teenagers and food consumption
Reference category: seafood & fish consumption = <2x/week; eggs consumption = <2x/week; offal consumption = never; local food consumption = <2x/week
All models adjusted for study, sex and educational level of household.

μg/L) derived by the German HBM Commission (Apel et al., 2017). For the sum of PFOS, PFOA, PFNA and PFHxS the proportion of participants exceeding the blood serum concentration of 6.9 μg/L derived from the external Tolerable Weekly Intake established by EFSA (2020) was 14%, indicating that concerns for adverse health effects cannot be excluded (Govarts and Gilles, 2022; submitted). In the subpopulation of teenagers with high seafood & fish, offal and egg consumption, the proportion of exceedance was even elevated to 17%, 16% and 16%, respectively.

For regulated PFAS, PFOS and PFOA, time trends were previously observed indicating that sampling year might be one of the determinants of PFAS exposure (Land et al., 2018; Schoeters et al., 2017). In our study, pooled data analysis did not show significant association between sampling year and PFAS concentrations in teenagers. This could probably be explained by the short sampling time frame for the aligned studies that include samples only from 2014 until 2021, therefore the difference in sampling year between study participants is not substantial and it appears that it does not affect the PFAS levels as much as other assessed factors. Effect of sampling year can also partially be addressed by the study variable in the model and addition of sampling year together with the study variable in the model could lead to over-adjustment.

In this study, significantly higher concentrations of all four PFAS were observed in boys compared to girls. Similar results were previously observed in children and adolescents (Canova et al., 2021) as well as in adults (Calafat et al., 2007). For adolescent girls, menstruation could be an elimination pathway for PFAS, resulting in lower levels of PFAS in blood (Colles et al., 2020), however, we did not have data on menarche available in all studies. We observed association between higher PFAS concentrations and higher educational level, which was also reported in other studies in different populations (adults - Calafat et al., 2007; pregnant women - Bjerregaard-Olesen et al., 2016; newborns – Richterová et al., 2018).

We observed higher frequency of seafood and fish and eggs consumption to be significantly associated with higher levels of PFOS and PFNA in teenagers. Higher PFOS levels were significantly associated with offal consumption and higher consumption of local food. We did not find any significant association between diet and PFOA or PFHxS levels, although the same increased trend was observed with seafood and fish consumption and local food. Even though food packaging could be an important source of PFAS, fast food consumption was not associated with PFAS levels in our study population.

Associations between seafood and fish consumption and PFAS levels were reported by several studies (Haug et al., 2010; Jain, 2014; Manzano-Salgado et al., 2016; Shu et al., 2018). In the current study, not only countries with high consumption of fish and seafood (e. g. Spain or Norway) but also data from countries with low consumption of fish (e. g. Slovakia or Germany) were included in pooled analysis with similar results as previous studies. Fish and seafood consumption was identified as one of the main contributors to dietary exposure to PFAS by EFSA (2020).

We observed an association between eggs consumption at least 2 times per week and higher PFOS and PFNA levels. Similarly, PFNA levels were significantly associated with consumption of eggs more than once per week in Belgian adults (Colles et al., 2020). On the other hand, Liu et al. (2017) did not observe association between eggs consumption and PFAS blood concentrations. EFSA (2020) reported high concentrations of PFOS and PFOA in eggs and egg products, which suggests it can be an important source of exposure to PFAS for those, whose eggs consumption is high.

In our study, consumption of offal was associated with significantly

higher PFOS levels, and the same trend was observed for PFOA and PFNA, although not statistically significant. This association was observed in the study by Tian et al. (2018) as well. They observed consumption of offal at least once a week to be associated with higher PFOS and PFNA levels in pregnant women. In addition, a Belgian study observed increased PFOS, PFOA and PFNA levels in cord blood when mothers reported consumption of offal (Colles et al., 2020). PFAS are absorbed in the gastrointestinal tract of mammals, then distributed via plasma to the other parts of the body and tend to accumulate in the liver (EFSA, 2020).

We found that consumption of local food at least 2 times per week was associated with higher levels of PFOS. However, data on local food consumption were available only in 4 studies and the definitions of local food varied across the studies with different types of food included in the variable. 3 out of 4 studies (CROME, SLO CRP and FLEHS IV) included only plant-based local food, one study (PCB cohort follow-up) included both plant-based and animal-based local food. Despite these limitations, association between PFOS and local food consumption was observed in both, the pooled dataset, and each study separate (results not shown). This suggests that even in countries with no PFAS production (e. g. Slovakia), local food might be contaminated by these chemicals.

One of the limitations of this study is that we were not able to perform statistical analyses for the other eight PFAS compounds measured, due to a high LOQ in some studies and subsequent low quantification frequency. Although PFAS were measured in blood serum or plasma (Table S1), this probably does not affect the results, since 1:1 serum to plasma ratio was observed for PFHxS, PFOS, and PFOA, independently of concentrations measured (Ehresman et al., 2007). Another limitation is underrepresentation of households with lower educational level in all studies, except of BEA and SLO CRP studies. Due to missing data on use of consumer products containing PFAS, we could not investigate the association between the use of these products in the households and PFAS concentrations in teenagers. Another limitation is that not all studies measured the total PFOS levels, but only the linear isomer was measured (ESTEBAN and FLEHS IV) (Table S1). Branched PFOS may account for more than 30% of the total sum of PFAS (Schultz et al., 2020), therefore, these levels could be an underestimation of the actual exposure levels for these studies. As both studies are situated in the West of Europe, this further strengthens the observation on geographical differences, with higher values observed in the North and West of Europe.

Despite the limitations, our study has several strengths as well. One of our main advantages is the size of our study population. We analysed PFAS and questionnaire data on 1957 teenagers from 9 European countries. We had geographic coverage of all four European regions and we covered countries with different lifestyle and dietary patterns. Our study provides the most recent data on exposure as the sampling period was from 2014 to 2021. The comparability and accuracy of measured PFAS concentrations between laboratories was assured by QA/QC programme performed within HBM4EU project. Two studies in which PFAS exposure data were generated before the HBM4EU QA/QC program were deemed comparable by the HBM4EU QAU, since the laboratories successfully passed the QA/QC programme. Only for one study (Riksmaten Adolescents, 2016–17) comparability was not guaranteed by the HBM4EU QAU. Moreover, questionnaire data used in the study were harmonized, which improved results of the statistical analysis for PFAS exposure determinants.

5. Conclusion

This is the first study using harmonized and quality controlled comparable PFAS exposure data across Europe. Significantly higher PFAS concentrations were observed in teenagers in the North and West of Europe compared to the South and East. Some dietary factors were identified as determinants of PFAS exposure. Higher frequency of seafood and fish consumption was associated with higher levels of PFAS. Eggs consumption was associated with higher levels of PFOS and PFNA. Additionally, PFOS levels were associated with offal consumption and higher consumption of local food. These results provide information about potential sources of exposure to PFAS for targeted monitoring of PFAS in food.

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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.2022.114057.

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Short-term effects of ambient particulate matter (PM_1, $PM_{2.5}$ and PM_{10}) on influenza-like illness in Guangzhou, China

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ABSTRACT

Background: Particulate matter (PM) has been linked to respiratory infections in a growing body of evidence. Studies on the relationship between ILI (influenza-like illness) and PM_1 (particulate matter with aerodynamic diameter $\leq$1 μm) are, however, scarce. The purpose of this study was to investigate the effects of PM on ILI in Guangzhou, China.

Methods: Daily ILI cases, air pollution records (PM_1, $PM_{2.5}$, PM_{10} and gaseous pollutants), and metrological data between 2014 and 2019 were gathered from Guangzhou, China. To estimate the risk of ILI linked with exposure to PM pollutants, a quasi-Poisson regression was used. Additionally, subgroup analyses stratified by gender, age and season were carried out.

Results: For each 10 μg/m^3 increase of PM_1 and $PM_{2.5}$ over the past two days (lag01), and PM_{10} over the past three days (lag02), the relative risks (RR) of ILI were 1.079 (95% confidence interval [CI]: 1.050, 1.109), 1.044 (95% CI: 1.027, 1.062) and 1.046 (95% CI: 1.032, 1.059), respectively. The estimated risks for men and women were substantially similar. The effects of PM pollutants between male and female were basically equivalent. People aged 15–24 years old were more susceptive to PM pollutants.

Conclusions: It implies that PM_1, $PM_{2.5}$ and PM_{10} are all risk factors for ILI, the health impacts of PM pollutants vary by particle size. Reducing the concentration of PM_1 needs to be considered when generating a strategy to prevent ILI.

1. Introduction

Ambient air pollution, particularly particulate matter (PM), has grown to be one of the biggest threats for worldwide public health. Globally, 2.94 million deaths from all causes and 83 million disability-adjusted life-years (DALYs) were attributable to ambient particle mass with an aerodynamic diameter less than 2.5 μm ($PM_{2.5}$) in 2017 (Lancet, 2018). Numerous epidemiological studies have demonstrated a link between PM exposure and an increased risk of mortality and morbidity, such as cardiovascular diseases and respiratory diseases (Chen et al., 2017; Hu et al., 2018; Lin et al., 2018; Lin et al., 2016a,b; Shah et al., 2015). With 1.24 million deaths and 1513.1 per 100,000 age-standardised DALY rate estimated to be attributed to air pollution in 2017 (Yin et al., 2020), China is suffering greatly from disease and economic burdens brought on by ambient air pollution. Around 310 billion yuan in losses were caused by air pollution in China (Niu et al., 2017).

A variety of particle size fractions, including PM_{10} (inhalable particles, $<$ 10 μm), $PM_{2.5}$ (fine particles, $<$ 2.5 μm) and PM_1 (very fine particles, $<$ 1 μm) were associated with respiratory mortality and respiratory diseases (Liu et al., 2019; Wang et al., 2021; Zhang et al., 2020; Zhao et al., 2017). PM can induce airway epithelial cell damage and

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barrier dysfunction, and inhibit the defense systems of the respiratory system (Ciencewicki and Jaspers, 2007). Additionally, there is a positive association between $PM_{2.5}$/PM_{10} exposure and influenza-like illness (ILI) (Chen et al., 2017; Huang et al., 2016; Liu et al., 2019; Su et al., 2019; Toczylowski et al., 2021). ILI is a common respiratory syndrome that has a big impact on public health and causes a significant amount of morbidity and mortality annually (Paget et al., 2019).

According to recent studies, smaller PM particles may be more toxic to humans (Wang et al., 2021; Yang et al., 2019). In China, PM_1 makes up around 80% of the $PM_{2.5}$ mass (Chen et al., 2018). However, due to the absence of ground-based PM_1 measurement, only a small number of research have concentrated on the relationship between PM_1 and health. Besides that, there is presently no research on how PM_1 may affect ILI. In this study, we aimed to investigate the short-term effects of ambient PM_1, $PM_{2.5}$ and PM_{10} on the incidence of ILI in a megacity in southern China.

2. Materials and methods

2.1. Study settings

The capital of Guangdong Province, which is Guangzhou City is important as the economic hub of South China's Peral River Delta Region. Along with the rapid economic development, the concentration of air pollutants in Guangzhou exceeds the standards set by the World Health Organization (WHO). Furthermore, Guangzhou has a subtropical humid-monsoon climate, the seasonal pattern of ILI in Guangzhou was typical of Southern China: with a bimodal increase in both the summer and winter (Shu et al., 2010).

2.2. Data collection

2.2.1. Data of ILI cases

Data of daily ILI cases from January 1, 2014 to December 31, 2019 were collected from Guangzhou Center for Disease Control and Prevention. Herein, ILI cases were defined as an acute respiratory infection with a body temperature higher than 38 °C, and cough or sore throat without other diagnoses. Influenza is a Class-C notifiable infectious disease in China, therefore, sentinel hospitals are obligated to collect a nasopharyngeal swab from each case, transmit it to certified laboratories for viral isolation and subsequent identification, and submit the results online within 24 h. The ILI cases were classified into five age groups: 0–4, 5–14, 15–24, 25–59 and ≥60 years old.

2.2.2. Air pollution data

With a spatial resolution of 10 km × 10 km, the ChinaHighAirPollutants (CHAP, available at https://weijing-rs.github.io/product.html) grid dataset provided us with the daily average concentrations of PM (PM_1, $PM_{2.5}$, PM_{10}) (Wei et al., 2019, 2021a,b) and gaseous pollutants (NO_2: nitrogen dioxide, SO_2: sulfur dioxide, O_3: ozone) (Wei et al., 2022a,b,c) in Guangzhou. Herein, the CHAP dataset presented here is a comprehensive, high-resolution, long-term, and high-quality dataset of ground-level air pollutants for China. Moreover, it is generated using artificial intelligence by considering the spatiotemporal heterogeneity of air pollution based on big data from sources such ground observations, satellite remote sensing products, atmospheric reanalysis and model simulations. With the determination coefficient R^2 between 0.74 and 0.77, the cross-validation results showed that the model maintained a high level of prediction accuracy.

2.2.3. Meteorological data

The China Meteorological Data Sharing Service system of the China Meteorological Administration was used to acquire daily meteorological data, including mean temperature and relative humidity, over the study period (http://data.cma.cn).

2.3. Statistical analysis

Herein, descriptive analysis was performed to uncover the distributions of ILI cases, air pollutants and meteorological data. Meanwhile, the association between each air pollutant and meteorological covariates was estimated using the Spearman correlation.

A generalized additive model (GAM) with a quasi-Poisson regression was used to assess the association between daily PM pollutants and ILI cases. In order to account for seasonality, we also utilized a natural cubic spline with 7 degrees of freedom. To control for the short-term trend, the day of the week (DOW), a categorical variable, was employed. Moreover, we used a natural cubic spline with 3 degrees of freedom to control for the confounding effects of meteorological variables (mean temperature and relative humidity).

The model used is shown as follows:

$$\log[E(Y_t)] = \beta X_i + \text{ns (time, df = 7/year)} + \text{ns (temperature, df = 3)} + \text{ns (relative humidity, df = 3)} + \text{DOW} + \text{intercept} \quad (1)$$

where $E(Y_t)$ represents the expected value of the daily count of ILI cases; ns () is the smoothing function and df is the degree of freedom; β is the regression coefficient for each air pollutant; X_i is the air pollutants such as PM_1, $PM_{2.5}$, PM_{10}; and DOW indicates the day of the week.

According to previous studies (Huang et al., 2016; Chen et al., 2017), PM-ILI associations were significant during lags of 0–3 days. And a systematic review (Lessler et al., 2009) revealed that the incubation period for most ILI-associated acute respiratory infections is within 4 days. Therefore, the models with single-day lag (lag0–lag4) and moving average lag (lag01–lag04) were fitted to comprehend the characteristics of the possible lag effect between PM concentration and the onset of ILI cases.

Furthermore, we conducted several subgroup analyses stratified by gender (male and female) and age group (0–4, 5–14, 15–24, 25–59 and ≥ 60 years old) on the lag day with the strongest effects. In addition, the study period was further separated into warm (from April to September) and cold (October to March of the following year) seasons to determine the seasonal pattern of PM-ILI associations (Liu et al., 2019; Zhang et al., 2020). Then, between-group significance tests were carried out using the formula $(Q_1 - Q_2) \pm 1.96\sqrt{SE_1^2 + SE_2^2}$, where Q_1, Q_2 stood for the effect estimates for each stratum, and SE_1, SE_2 represented the related standard errors (Lin et al., 2016a; Zhang and Zhou, 2020).

Meanwhile, in sensitivity analysis, to check whether the PM-ILI associations would be modified by other air pollutants, we constructed two-pollutant models by adjusting for gaseous air pollutants (NO_2, SO_2 and O_3). By altering the df of time (from 6 to 10) and meteorological variables (from 3 to 6), we examined the potential influence of df. Considering the comparability of PMs concentration changes, we also estimated the effects of PMs on ILI with an interquartile range (IQR) increase.

The attributable number (AN) of ILI cases caused by PM and corresponding attributable fractions (AF) of ILI cases were calculated using previously published methodologies to determine the burden of ILI cases attributable to PM (Qiu et al., 2019; Wu et al., 2020). Based on the WHO's air quality recommendations, we established the reference PM values as 50 μg/m^3 for PM_{10}, and 25 μg/m^3 for $PM_{2.5}$. Considering that the current WHO pollution criteria lack pertinent information on PM_1, we used the 50th quantiles of observed PM_1 concentrations as the reference concentration for PM_1. The formula are as follows:

$$AF_i = 1 - \exp(-\beta * \Delta P_i) \quad (2)$$

$$AN_i = AF_i * n_i \quad (3)$$

where AF_i and AN_i represent the fractions and number of ILI cases, that might be attributed to excessive PM exposures on day i, respectively; β indicates the coefficient of the PM-ILI associations; ΔP_i is the difference in PM concentration between the measured value and the reference

Table 1
Descriptive statistics for meteorological data, air pollutants and influenza-like illness cases in Guangzhou, 2014–2019.

Variable	Mean ± SD	Percentile				
		Min	P_{25}	P_{50}	P_{75}	Max
Temperature (°C)	22.29 ± 5.99	3.46	17.83	23.55	27.41	31.44
Relative humidity (%)	79.16 ± 10.66	26.88	73.63	80.38	86.81	99.75
Air pollution(μg/m^3)						
PM_1	20.92 ± 10.91	2.97	12.93	18.17	26.99	83.69
$PM_{2.5}$	34.79 ± 17.05	6.10	22.19	30.99	44.23	132.60
PM_{10}	54.45 ± 22.53	12.09	37.88	49.5	67.65	160.45
NO_2	33.90 ± 10.73	9.27	26.47	31.23	39.38	81.55
SO_2	13.42 ± 5.49	5.13	9.60	12.16	15.87	40.19
O_3	94.85 ± 41.73	11.78	62.35	90.75	123.06	228.04
Influenza cases and subgroups						
All cases	101.47 ± 246.31	0	7	24	77	2985
Male	55.34 ± 135.11	0	4	13	42	1627
Female	46.13 ± 111.46	0	3	11	34	1358
0–4 years old	35.57 ± 70.62	0	3	10	30	642
5–14 years old	40.62 ± 133.56	0	1	6	26	1735
15–24 years old	8.28 ± 25.86	0	0	1	5	329
25–59 years old	13.94 ± 30.37	0	1	3	11	249
60 years old and above	3.06 ± 5.74	0	0	1	3	50
Warm	74.99 ± 137.93	0	9	27	54	909
Cold	128.06 ± 318.03	0	5	19	108	2985

value on day i; and n_i is the count of ILI cases on day i. Meanwhile, the sum of AN was divided by the total number of ILI cases to determine the overall AF.

All analyses were completed in R software version 4.1.1. Two-sided statistical tests were conducted, and statistical significance was defined as $p < 0.05$.

3. Results

The mean daily concentrations of PM_1, $PM_{2.5}$ and PM_{10} were 20.92, 34.79 and 54.45 μg/m^3, respectively (Table 1). Herein, the daily average concentrations of both $PM_{2.5}$ and PM_{10} exceeded the WHO's air quality guidelines ($PM_{2.5}$: 25 μg/m^3; PM_{10}: 50 μg/m^3). During the study period, the average values were 22.29 °C and 79.16% for mean temperature and relative humidity, respectively. A total of 222,316 ILI cases were recorded, with a daily mean of 101.47. Among all age groups, cases aged 5–14 years old accounted for 40.03% of the total ILI cases.

Table 2 demonstrates the correlation between air pollutants and meteorological variables. Herein, strong correlations were observed between PM_1 and $PM_{2.5}$ (r = 0.99, $p < 0.001$), $PM_{2.5}$ and PM_{10} (r = 0.98, $p < 0.001$), and PM_1 and PM_{10} (r = 0.96, $p < 0.001$). PM_1, $PM_{2.5}$ and PM_{10} were positively correlated with NO_2, SO_2 and O_3, whilst were negatively correlated with mean temperature and relative humidity.

Fig. 1 shows the relative risk (RR) for daily ILI cases at different lag days associated with per 10 μg/m^3 increase exposure to PM pollutants. For a single-day lag, PM_1 and $PM_{2.5}$ had a significant effect on ILI cases on lag0–lag2, whilst PM_{10} had a significant effect on lag0–lag3. Herein, the risks related to PM exhibited a declining tendency as the lag day grew. Meanwhile, for multi-day lag, the effects of all PM pollutants on ILI cases were significant on lag01–04. Herein, the effects of PM_1 were stronger than that of $PM_{2.5}$ and PM_{10}. The strongest effects of PM_1 and $PM_{2.5}$ were observed at the two-day moving average (lag01), with corresponding RR 1.079 (95% confidence interval [CI]: 1.050, 1.109) for PM_1 and 1.044 (95% CI: 1.027, 1.062) for $PM_{2.5}$, respectively (Table S1). Meanwhile, the strongest effect of PM_{10} was observed at a three-day moving average (lag02), the RR was 1.046 (95% CI: 1.032, 1.059).

In addition, subgroup-specific RR estimates for the associations between PM pollutants and ILI cases with the strongest effect were summarised in Fig. 2. Subgroup results stratified by gender and age group were similar between the 3 p.m. pollutants. Herein, the risks estimated between males and females were substantially similar. For instance, the RR was 1.075 (95% CI: 1.044, 1.105) among males and 1.085 (95% CI: 1.054, 1.117) among females, with a 10 μg/m^3 increase in exposure to PM_1 (Table S2). Although the differences are not significant except for PM_{10}, the age-stratified analysis revealed that the 15–24-year group may be more sensitive to PM pollutants.

Moreover, PM_1-and $PM_{2.5}$-ILI associations were slightly stronger in the warm season than in the cold seasons for total ILI cases, whereas the PM_{10}-ILI association was stronger in cold seasons (Fig. 3, Table S3). Meanwhile, the stratified analysis showed that the PM-ILI associations among males (except for PM_{10}) and the 5–14 age group were stronger in warm seasons, whilst other results were stronger in cold seasons.

Table 3 shows the attributable number and attributable fractions of ILI cases related to PM pollutants. Herein, the attributable fractions of total ILI cases were estimated to be 1.43% (95% CI: 0.88, 1.93) due to PM_1, 2.48% (95% CI: 1.44, 3.50) due to $PM_{2.5}$, and 2.33% (95% CI: 1.97, 2.68) due to PM_{10}. During the study period, 3190 (95% CI: 1934, 4319) ILI cases were estimated to be attributable to PM_1, whereas 5510 (95% CI: 3319, 7692) ILI cases were attributable to $PM_{2.5}$ and 5176 (95% CI: 4346, 6001) ILI cases were attributable to PM_{10}.

The results from the two-pollutant models illustrated that the estimated effects decreased after adding NO_2 and SO_2 to the models (Table S4). With the exception of PM_{10}, the impacts of PM_1 and $PM_{2.5}$ were no longer significant when the aforementioned pollutants were included. On the contrary, the PM-ILI associations increased after adjusting for O_3, and the results remained significant. Meanwhile, sensitivity analyses showed that our main findings were robust when

Table 2
Spearman correlation coefficients between air pollutants and meteorological variables in Guangzhou, 2014–2019.

	PM_1	$PM_{2.5}$	PM_{10}	NO_2	SO_2	O_3	Temperature	Relative humidity
PM_1	1.00							
$PM_{2.5}$	0.99**	1.00						
PM_{10}	0.96**	0.98**	1.00					
NO_2	0.70**	0.69**	0.71**	1.00				
SO_2	0.66**	0.68**	0.68**	0.41**	1.00			
O_3	0.29**	0.34**	0.42**	0.08**	0.25**	1.00		
Temperature	−0.42**	−0.34**	−0.27**	−0.38**	−0.11**	0.42**	1.00	
Relative humidity	−0.40**	−0.39**	−0.44**	0.01	−0.40**	−0.52**	0.15**	1.00

Note: **p < 0.001.

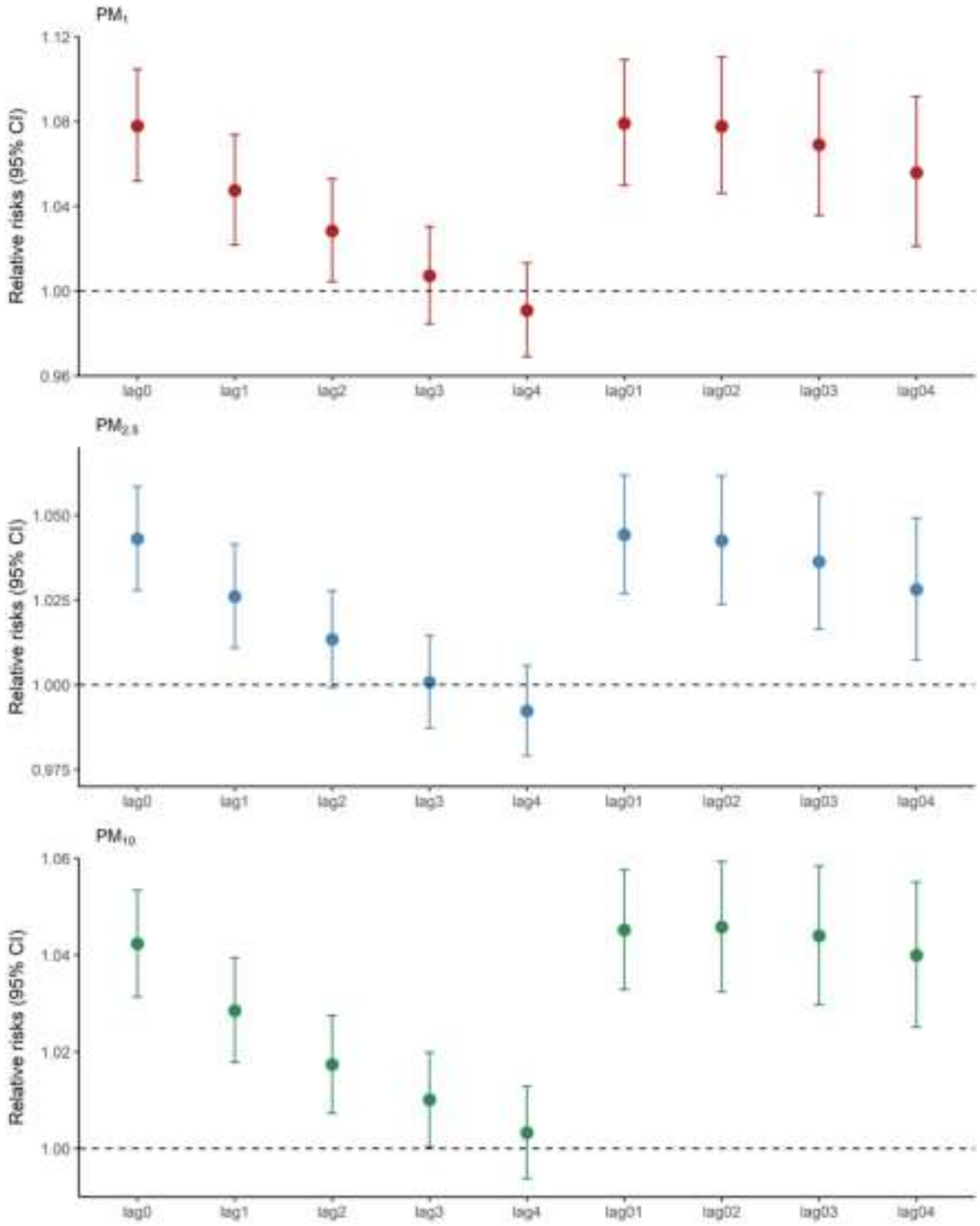

Fig. 1. Relative risk (with 95% CIs) of ILI cases at different exposure days for every 10 μg/m^3 increase in exposure to PM_1, $PM_{2.5}$ and PM_{10}. The x-axis represents PM measurements at several single-lag (lag0 to lag4) and moving-average (lag01 to lag04) days before illness onset. For instance, lag0 corresponds to the current day concentration, lag1 corresponds to the concentration of the day before illness onset; lag01 corresponds to the two-day moving averages of current and previous day concentrations of air pollutants.

modifying the degrees of freedom for time and meteorological variables. The effects of PMs on ILI with an IQR increase showed that, PM_{10} had the strongest effect, followed by PM_1 and $PM_{2.5}$ (Table S5).

4. Discussion

Influenza-like illness is a serious public health issue that annually accounts for a significant amount of morbidity and mortality. In addition, air pollution may affect respiratory infection and raise ILI incidence. In this study, we examined the associations between PM_1, $PM_{2.5}$, PM_{10} and ILI, and our findings suggested that the 3 p.m. pollutants mentioned above were associated with an increased risk of ILI. The adverse effects of air pollution on ILI varied by the size of particulate matter. Meanwhile, age and gender-specific subgroup analyses revealed

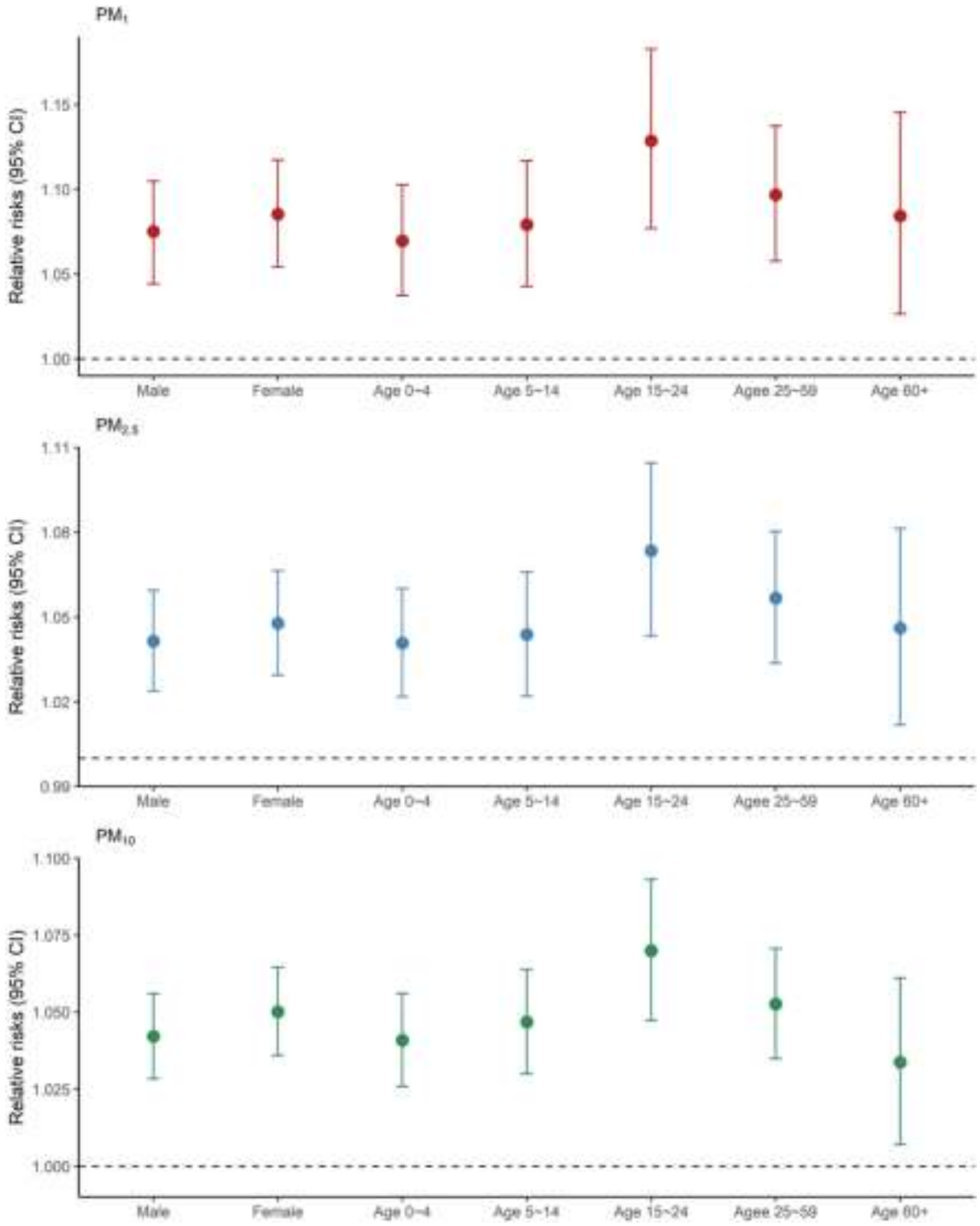

Fig. 2. Relative risk (with 95% CIs) of ILI cases among subgroups stratified by gender and age, associated with a per 10 μg/m^3 increase in exposure to PM_1 (lag01), $PM_{2.5}$ (lag01) and PM_{10} (lag02).

comparable PM-ILI associations, with people between the ages of 15–24 being the most susceptible. These results would enrich the evidence about the link between PM pollutants and ILI.

The adverse effects of $PM_{2.5}$ and PM_{10} on ILI have been consistently demonstrated by epidemiological studies (Chen et al., 2017; Huang et al., 2016; Liu et al., 2019; Su et al., 2019; Toczylowski et al., 2021); however, there is a lack of evidence regarding the impact of PM_1. We discovered significant associations between exposure to ambient PM_1 and increased ILI risks in Guangzhou. Potential mechanisms for the toxic effects on the human respiratory system may be that PMs could enhance airway responsiveness by inducing oxidative stress and inflammation (Ghio et al., 2012), which may weaken host immunological defenses and diminish susceptibility to bacterial and viral infections (Ciencewicki and Jaspers, 2007). Furthermore, the smaller size of PM_1 enables the pollutant to reach deeper into the respiratory system, thus harming the respiratory system more severely.

The health effects of PM pollutants vary depending on different particle size fractions, as well as source and chemical composition

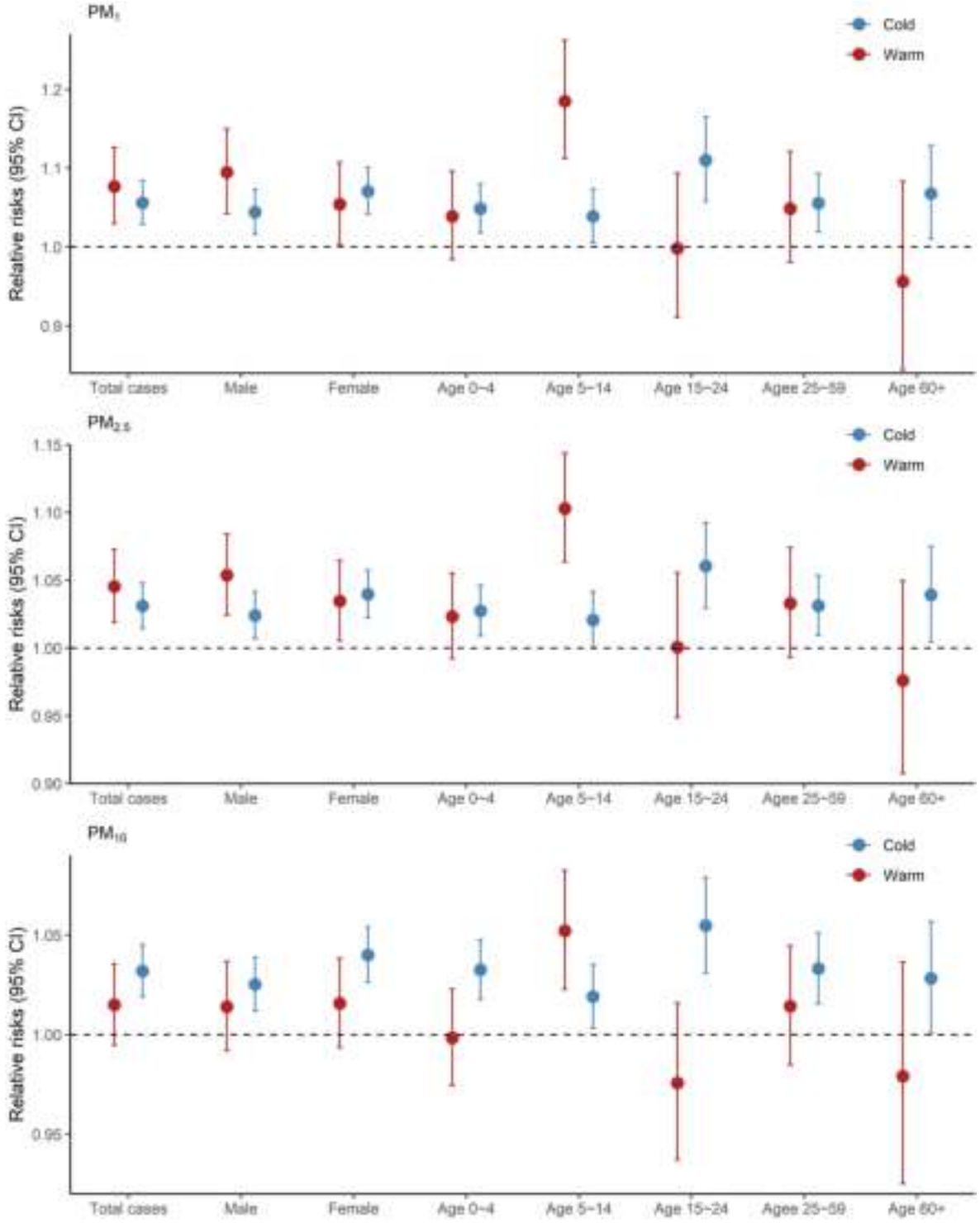

Fig. 3. Season-specific relative risk (with 95% CIs) of ILI cases by 10 μg/m^3 increase in exposure to PM_1 (lag01), $PM_{2.5}$ (lag01) and PM_{10} (lag02).

(Frank and Julia C, 2012). Our findings were in line with earlier research, showing that PM_1 had stronger adverse health effects than $PM_{2.5}$ and PM_{10} with an increase of 10 μg/m^3. For instance, Wang et al. reported that among PM_1, $PM_{2.5}$ and PM_{10}, PM_1 had the greatest impact on pneumonia hospitalisations (Wang et al., 2021). Meanwhile, Hu et al. (2018) and Lin et al., 2016b) found that PM_1, rather than $PM_{2.5}$ and PM_{10}, had a stronger associations with respiratory and cardiovascular mortality. Herein, Hu et al. indicated that by further comparing the ratio of $PM_1/PM_{2.5}$ concentration and attributable deaths, PM_1 accounts for 95% of the mortality caused by $PM_{2.5}$. PM_1 may consist of primary organic aerosols, ammonium, nitrate, sulphate, and chloride, which could be from coal combustion and traffic, cooking emissions (Niu et al., 2020). In addition, compared to $PM_{2.5}$, the proportion of PM_1 derived from combustion, such as burning biomass fuel, was substantially higher (Perrone et al., 2013). Despite the need for more research, these may be responsible for PM_1's more powerful hazardous effects. However, when using an IQR increase as the magnitude of change, the PM_1 effect was no longer the strongest. More studies need to be conducted to verify whether PM_1 had stronger harmful impacts.

The largest impact of $PM_{2.5}$ on ILI was observed in this study at a

Table 3
Fractions and counts of ILI cases (stratified by gender and age groups), attributed to ambient PM_1, $PM_{2.5}$, and PM_{10} in Guangzhou.

Cases and subgroups	Attributable number of influenza cases (95% CI)			Attributable fraction (95% CI)		
	PM_1	$PM_{2.5}$	PM_{10}	PM_1	$PM_{2.5}$	PM_{10}
All cases	3190(1934, 4319)	5510(3319, 7692)	5176(4346, 6001)	1.43(0.88, 1.93)	2.48(1.44, 3.50)	2.33(1.97, 2.68)
Male	1687(982, 2420)	2889(1577, 4086)	2824(2268, 3329)	1.39(0.78, 1.95)	2.38(1.35,3.39)	2.33(1.88, 2.73)
Female	1540(908, 2163)	2682(1559, 3768)	2354(1932, 2780)	1.52(0.90, 2.08)	2.65(1.62, 3.70)	2.33(1.93, 2.73)
0–4 years old	870(416, 1263)	1737(838, 2546)	1069(823, 1290)	1.12(0.58, 1.65)	2.23(1.17, 3.33)	1.37(1.04, 1.67)
5–14 years old	1921(968, 2886)	2666(1199, 4146)	4170(3278, 5021)	2.16(1.09, 3.33)	3.00(1.29, 4.61)	4.69(3.72, 5.55)
15–24 years old	158(13, 275)	377(68, 663)	323(217, 403)	0.87(0.11, 1.53)	2.08(0.33, 3.71)	1.78(1.18, 2.24)
25–59 years old	209(113, 273)	715(395, 1031)	83(59, 90)	0.68(0.41, 0.90)	2.34(1.31, 3.35)	0.27(0.19,0.29)
60 years old and above	68(23, 98)	184(46, 301)	36(9, 45)	1.02(0.37,1.45)	2.74(0.77, 4.53)	0.54(0.14,0.67)

two-day moving average (lag01), with a 4.4% increase in daily cases. Similar to research from Hefei (Liu et al., 2019), which found that exposure to $PM_{2.5}$ increased ILI cases by 4.0%. However, the $PM_{2.5}$-related effect of ILI in our study was higher than those studies in Jinan (Su et al., 2019) and nationwide (Chen et al., 2017). The RR value of ILI was 1.046 with a 10 $\mu g/m^3$ increase of PM_{10} on lag02 days in our study, which was similar to a study in Nanjing (Huang et al., 2016). The effects of PMs on ILI in our study were significant during single lags of 0–3 days and multi-lags of 01–04 days. And the strongest effects were observed with a 2-day moving average of PM_1 and $PM_{2.5}$, and a 3-day moving average for PM_{10}. It was consistent with previous studies and can be explained by the incubation period of ILI-associated viruses. According to Lessler and his colleagues (Lessler et al., 2009), the median incubation period was 1.4 and 0.6 days for influenza A and influenza B, respectively. For other ILI-associated viruses such as rhinovirus and parainfluenza, the median incubation period was 1.9 and 2.6 days. The information about lag effects of PMs could be useful when proposing a strategy for the control and prevention of ILI.

The epidemiology of air pollution has given considerable attention to gender differences in the relationship between air pollution and respiratory health, although the results were mixed. Similar to that of Zhang et al., the effects of PM pollutants between gender were basically equivalent (Zhang et al., 2020), whilst other studies indicated that females may be more sensitive to PM (Bell et al., 2015; Di Q et al., 2017). In addition, children and the elderly are especially vulnerable to air pollution because of their relatively weakened immune systems (Wang and Chau, 2013). However, the results of this study showed that people aged 15–24 was shown to have a higher risk of ILI with exposure to PM pollutants. Similar results were also found in studies by Huang et al. (2016) and Samoli et al. (2011). Possible explanations include the fact that young children would spend less time outdoors than older ones, shielding them from exposure to air pollution. In contrast, adults and the elderly frequently prefer to self-administer medication unless their disease becomes severe or unmanageable. As a result, ILI monitoring statistics may not adequately reflect the incidence in these age groups. On the other hand, children (aged 6 months to 5 years) and elderly (≥60 years) were recommended as a priority group for influenza vaccination in mainland China, and a study showed that the influenza vaccination coverage was higher among these age groups (Wang et al., 2018). This could protect them from being infected with influenza.

Herein, seasonal patterns of PM_1-and $PM_{2.5}$-ILI associations were stronger in the warm season than in the cold season, which were consistent with a previous study in Zhejiang Province (Hu et al., 2018). The higher effects in the warm season may be related to people being outdoors for a longer time (Calkins et al., 2007). Furthermore, studies have shown that the permeability of outdoor PM to the inside environment is increased by natural ventilation, and that the correlation between indoor and outside PM is larger in the summer than the winter (Peng et al., 2005). Nevertheless, other studies showed contradictory findings. A case-crossover study in Shenzhen identified larger effects for PM_1-and $PM_{2.5}$-associated risks of hospital admission for respiratory diseases in the cold season (Zhang et al., 2020). Another study also found that there was only a relationship between PM_{10} and emergency department visits during the cold season (Chen et al., 2019). Interestingly, the PM_{10}-ILI association was stronger in the cold season in our study. This might be the result of elements like the chemical composition and concentration of environmental PM, the exposure pattern of the population, and climatic conditions. Future studies, however, need delve deeper into the specific causes.

NO_2 and SO_2, which attenuated and lost significance in the two-pollutant models, proved to be confounding factors in the associations between PM_1 and $PM_{2.5}$ exposures and ILI. Other studies have also reported this phenomenon (Samoli et al., 2013; Zhao et al., 2017). It is challenging to assess the separate effects of PM_1, $PM_{2.5}$, and NO_2 because they are primarily traffic-derived contaminants (Tian et al., 2011) with substantial correlations.

Our study estimated the burden of ILI cases attributable to PM pollutants exposure, which yielded more appropriate information to estimate the potential health benefits of actions to enhance air quality. Such approaches have been applied to estimate the burden of mental disorders caused by PM exposure. This is the first study to evaluate the burden of ILI caused by PM pollutants, which gives policymakers specific information about the possible health benefits of lowering PM concentrations.

However, several limitations should also be acknowledged in this study. Firstly, exposure misclassification is inevitable because data were obtained from monitoring stations rather than personal exposure. Secondly, we could not identify their contributions to the risk of ILI because of data unavailability on specific chemical components of PMs. Thirdly, the ILI surveillance data may not be fully recorded, and the findings may not accurately reflect the population as a whole. Fourthly, because this study was limited to a particular city, it may be difficult to extrapolate from the findings. Hence, multi-regional studies will become increasingly important.

5. Conclusions

In summary, our study provided suggestive proof of the adverse effect of PM_1 on ILI cases. The health impacts of PMs on ILI were different between sizes of particulate matter. These findings could better understand the health effects of PM_1 and encourage the creation of public health policies to combat PM pollution. However, more research is required to establish the causality because the results from the current study are insufficient to accomplish so.

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Consent for publication

Not applicable.

Ethics approval and consent to participate

The ethics approval was waived by the ethics committee of Guangzhou CDC after consultation according to the law on the prevention and control of infectious diseases. Because cases should truthfully provide relevant information in the prevention of infectious disease, and the analytical data sets were constructed anonymously.

Declaration of competing interest

The authors declare that they have no competing interests.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.114074.

Abbreviations

PM	particulate matter
ILI	influenza-like illness
DALYs	disability-adjusted life-years
NO_2	nitrogen dioxide,
SO_2	sulfur dioxide,
O_3	ozone
CI	confidence interval
RR	relative risk
AN	attributable number
AF	attributable fractions

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Temporal trends in legionellosis national notification data and the effect of COVID-19, Switzerland, 2000–2020

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ABSTRACT

The notification rate of legionellosis in Switzerland and other European countries has markedly increased over the last 20 years. Here, we investigated the Swiss notification data on legionellosis from 2000 to 2020 in regards of overall time trend, content and data quality. We further explored the impact of the COVID-19 pandemic on the reported case numbers using an interrupted time series approach. Between 2000 and 2020, 5980 cases were included in our analysis. The annual crude notification rate for legionellosis cases increased from 1.1/100,000 population (CI: 0.9–1.4) in 2000 to 5.6/100,000 population (CI: 5.1–6.1) in 2020. In recent years, the summer peaks have been more pronounced and some shifted earlier in the year. The highest notification rate was recorded in 2018 with 6.7/100,000 population (CI: 6.2–7.3). The hospitalisation rate for notified cases remained high across all study years (89.9%), while the case fatality rate slightly decreased (from 7.7% to 3.6%). COVID-19 containment measures, such as travel restrictions and/or related behavioural changes, are associated with a temporary decline in cases of 35%. Overall, the quality of the notification data was good. Clinical data were more susceptible to interferences than data from laboratory reporting, which could be observed most clearly in the decline of clinical reports by 4.3 percentage points in 2020. As the case classification for Legionnaires' disease includes pneumonia symptoms, this decline could lead to an underestimation of Legionnaires' disease cases, yet the continuous reporting though the diagnostic laboratories suggested a robust surveillance system for legionellosis in Switzerland.

1. Introduction

The term legionellosis comprises all diseases caused by *Legionella* spp. The majority of the known burden of disease stems from Legionnaires' disease (LD), which presents as pneumonia often requiring hospitalisation. Legionellosis is caused by inhalation or aspiration of aerosols from contaminated water sources, and has the potential to occur as larger outbreaks, even though most cases are sporadic. To detect such outbreaks, monitor disease trends, and take appropriate public health measures, legionellosis is included in the passive disease surveillance system of many, mostly high-income, countries (Thacker et al., 1983).

In the last two decades, the notification rate of legionellosis steadily increased in Switzerland, other European countries and the US (Centers for Disease Control and Prevention (CDC), 2020; European Centre for Disease Prevention and Control (ECDC), 2021). Several hypotheses for the increase in disease incidence were formulated such as changes in weather and climate, changes in energy policy and buildings/water systems infrastructure, both thought to promote *Legionella* spp. growth, and, demographic changes with an increasing susceptible population for LD (European Centre for Disease Prevention and Control (ECDC), 2021; Fukushima et al., 2021). Yet, the observed disease trend is not only shaped by changes in incidence, but also prone to react to any changes in the processes leading up to the case being reported, e.g. health-seeking behaviour, diagnosis and reporting procedures (Schmutz, 2018).

In Switzerland, cases of legionellosis are notifiable to the National Notification System for Infectious Diseases (NNSID) since December 1987. The NNSID is managed by the Federal Office of Public Health (FOPH). Trigger for a mandatory notification is a positive confirmation for a *Legionella* spp. infection. The diagnostic laboratory has to notify simultaneously to the cantonal health authorities and the FOPH with the "reporting form on laboratory findings". The treating physician must

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also submit a "reporting form on clinical findings" to the cantonal health authorities. The cantonal health authorities check for completeness of the clinical information provided and if immediate measures are necessary. They then forward the information to the FOPH. At the FOPH, the paper-based clinical and laboratory notification forms are recorded electronically and are matched by patient. The timeframe for reporting of both laboratory and clinical findings for legionellosis is one week (Federal Office of Public Health, 2020).

Before 2000, there were substantial changes to the notification process, hampering the evaluation of prior disease trends. Since then, there were only few adjustments made to the notification form and to the case classification for LD, which was last updated in 2012 (see Table 1) (Gysin, 2018). Cases classified as "possible" were either without pneumonia or without clinical information on pneumonia. They count towards legionellosis cases, but not as LD. Since 2006, the FOPH also requested diagnostic laboratories to report the annual number of tests performed for *Legionella* spp. to obtain complementary denominator data to improve contextualisation of the surveillance data (Gysin, 2018). The quality of this reporting, however, was insufficient; therefore, a research study investigated the positivity for the years 2007–2016 (Fischer et al., 2020). The authors found a strong and parallel increase of the test volume and the number of positive cases. However, without an assessment of the reasons for the increase in test volume, a conclusion on the observed notification trend could not be made.

The COVID-19 pandemic in 2020 has affected the notification rates of almost all mandatory notifiable diseases in Switzerland, including LD (Bundesamt für Gesundheit (BAG), 2021). LD cases in 2020 reduced by one third compared to the expected number of LD cases based on the five years prior to the pandemic. Multiple mechanism could explain the impact of the pandemic on LD: First, changes in people's behaviour could affect incidences and health-seeking behaviour; second, the clinical presentation of LD being similar to COVID-19 (Cassell et al., 2021) could lead to higher testing rates and third, the heavy burden on the health care system could affect testing and reporting behaviours. In particular, the ubiquitous travel and entry restrictions were hypothesised to have reduced cases of travel-associated Legionnaires' disease (TALD) (Steffen et al., 2020). Additionally, the closure of public buildings for leisure activities e.g. sport centres, shopping malls, office buildings, and schools might have reduced exposure during the closure but could have led to increased proliferation of *Legionella* spp. in the then stagnant water in unused buildings. Upon re-opening and without thorough flushing of the pipes, the risk for an infection is thought to be increased (Dey and Ashbolt, 2020; ESCMID ESGLI, 2020; Palazzolo et al., 2020; Proctor et al., 2020). However, as of now, there has been no quantification of this effect.

Table 1
Case definition for Legionnaires' disease in Switzerland since 2012 (Gysin, 2018).

Case classification	
Confirmed case	Any person meeting the clinical criterion AND at least one laboratory criteria for a confirmed case
Probable case	Any person meeting the clinical criterion AND at least one laboratory criteria for a probable case
Possible case	Any person meeting at least one of the laboratory criteria for either a confirmed or probable case AND missing information on the clinical criterion OR clinical criterion not met
Criteria	
Clinical criterion	Any person with pneumonia
Laboratory criteria for a confirmed case	Either isolation of *Legionella* spp. from respiratory secretion or any normally sterile site OR detection of *Legionella pneumophila* antigen in urine
Laboratory criteria for a probable case	Detection of *Legionella* spp. nucleic acid in clinical samples (using for example PCR) OR detection of *Legionella pneumophila* antigen for example by DFA staining using monoclonal-antibody-derived reagents OR significant rise in specific antibody level to *Legionella pneumophila* or other *Legionella* spp. in paired serum samples OR single high level of specific antibody to *Legionella pneumophila* serogroup 1 in serum.

Additionally, in 2017, thresholds of *Legionella* spp. contamination in potable, publicly accessible water were regulated in the Food Safety Law (Das Eidgenössische Departement des Innern (EDI), 2016). Consequently, *Legionella* spp. became a new concern for the Federal Food Safety and Veterinary Office (FSVO). Due to these developments in the past years, the increasing attention towards legionellosis and efforts to understand and prevent illness cases, an analysis of the past 20 years of LD notification in Switzerland is timely. The first aim of this study is to describe the Swiss notification data for LD for the two decades between 2000 and 2020, specifically the content of the notification data (i.e., cases per week and their characteristics), and the quality of the data (i.e., completeness, validity and timeliness). The second aim is to explore the impact of the COVID-19 pandemic on the content and the quality of these data.

2. Material and methods

2.1. Study design and setting

This is a retrospective longitudinal study utilising routinely collected health data for legionellosis from the NNSID in Switzerland between January 01, 2000 and December 31, 2020. The year 2000 was chosen as the starting time point, as there have been significant changes to the notification system earlier on, rendering older data incomparable.

2.2. Legionellosis notification data sources, access and processing

The raw data presented by the NNSID (Bundesamt für Gesundheit (BAG), 2022a) reports all legionellosis notifications before case classification, including cases later classified as possible and "no case", irrespective of their residency. After classification based on the case definition shown in Table 1, the FOPH retains only confirmed and probable cases, i.e., LD cases, with residency in Switzerland or the Principality of Liechtenstein, in their reports. For the purpose of this study, we used the same inclusion criteria for residency, but kept confirmed, probable and possible cases in the dataset and only excluded "no cases".

The legionellosis notification data underwent the routine cleaning processes at the FOPH. For data confidentiality reasons, variables like date of birth and place of residence are stored in separate files and deleted after three years. For the years 2000–2016, we therefore, obtained only the age in years and the canton of residence. We did not exclude case records that violated the internal validity (illustrative example: an observation with the hospitalisation date after the death date), in order to present the full dataset and explore its quality.

The legionellosis notification dataset contained cases notified on any given day. Due to low case numbers and to eliminate the effect of the day of the week on health-seeking behaviour and case confirmation, we aggregated data on a weekly level. The case notification further contains information on the patient's demographics (date of birth, sex, residential address, nationality), clinical information (date of disease onset and diagnosis, hospitalisation status, death), diagnostic information (sample material and diagnostic method), information about exposures prior to disease onset (locations, activities, installations), risk factors for development of LD, and information about the notification process (date of data entry, case classification, number of received notification forms).

Age categories were pre-set by the FOPH according to the standard of the European Centre for Disease Prevention and Control (ECDC). The FOPH further categorises cases based on the most probable exposure in the 2–10 days prior to onset of illness: travel-associated, retirement-home-associated, nosocomial, professional-associated, and community-acquired (Bundesamt für Gesundheit (BAG), Bundesamt für

Lebensmittelsicherheit und Veterinärwesen (BLV), 2018). Community-acquired cases include both, cases with a probable or confirmed infection in the community and cases, without another exposure category indicated.

2.3. Quantification of the impact of the COVID-19 pandemic on legionellosis cases

To address the second aim, the exploration of the impact of the pandemic, we collected information on the development of the COVID-19 pandemic, either quantitative (case numbers, hospitalisations, deaths and tests) or qualitative (non-pharmaceutical interventions implemented).

Information on the evolution of the COVID-19 pandemic in Switzerland were taken from the Oxford COVID-19 Government Response Tracker (OxCGRT) (Hale et al., 2021), which has been adapted for the Swiss context (Dünner and Penny, 2020). This information was complemented with our own compilation of events. Data on the number of COVID-19 cases, hospitalisation, deaths and testing are publicly available and were extracted on February 4, 2021 (Federal Office of Public Health, 2021). Data on the COVID-19 pandemic contain daily information from the start of the pandemic in Switzerland (early February 2020) until end of December 2020 and was also aggregated by week.

2.4. Linkage of legionellosis case data and COVID-19 data

For the legionellosis data, to identify events and cases on the timeline, we used the variable "case date", which is generated within the NNSID. The case date denotes the earliest date available from a series of date-related variables per case. Ideally, and in most cases, this is the date of symptom onset. The OxCGRT and COVID-19 case database had unique time identifiers, which allowed linkage with the LD database on the timeline.

We used population statistics from the Swiss Federal Statistical Office (FSO) to calculate crude and adjusted notification rates. At the time of the analysis, these statistics were not yet available for 2020; therefore, we used the statistic from 2019 instead.

2.5. Statistical methods

2.5.1. Descriptive analyses

Data were descriptively analysed in terms of data content and data quality using the statistical software R (Version 4.0.3 (R Core Team, 2020)). Notification rates, defined as the number of notified cases per 100,000 resident population, were calculated using population statistics from the FSO. Confidence intervals for crude rates were calculated using the package propCIs using the function exactci to apply the Clopper-Pearson exact CI approach. Confidence intervals for adjusted rates have been calculated using the package dsrTest to apply the Gamma Method proposed by Fay and Feuer (1997) (Fay and Feuer, 1997).

2.5.2. Interrupted time series analysis

To address the second aim, we used an interrupted time series analysis approach as outlined by Bernal, Cummins and Gasparrini to estimate the effect of selected measures on the legionellosis case numbers (Bernal et al., 2017). The selected events were i) the implementation and lifting of travel restrictions, on March 16 (week 12) and June 15 (week 25), and ii) the opening of schools and leisure activity facilities on May 11 (week 20) after almost two months of closure (The Swiss Federal Council, 2020). As there has been stepwise openings, we excluded the data points during the opening phase from week 20 until week 24. We assumed a lagged level change for both events. With count data available, we modelled the weekly number of cases between 2016 and 2020 using a quasi-Poisson regression model with the log-transformed standardised population as the offset. We incorporated harmonic functions to account for seasonality and a lag-time of one week (incubation time) into the model (Berkelman, 2015).

3. Results

3.1. Time trend in legionellosis cases

Fig. 1 shows the increasing weekly case numbers since 2000 until 2018, followed by a small drop in 2019 and 2020. The annual crude notification rate for legionellosis cases ranged from 1.1/100,000 population (CI: 0.9–1.4) in 2000 to 5.6/100,000 population (CI: 5.1–6.1) in 2020. The highest notification rate was recorded in 2018 with 6.7/100,000 population (CI: 6.2–7.3).[1]

There is a strong annual seasonality in the data peaking around calendar week 36 (Fig. 2). The record-high year of 2018 showed a strong summer peak, which however, shifted to June instead of August. Since 2000, the increase of cases in the summer months has been more pronounced than the increase during the winter months. Comparing the period 2010–2015 with the period 2016–2020, the number of cases increased most strongly in spring (Mar–May) by 85.1%. The cases during summer (Jun–Aug) increased by 75.3%, compared to an increase of 53.3% during autumn (Sept–Nov) and 58.7% during winter (Dec–Feb).

3.2. Content of notification

3.2.1. Demographics

Between 2000 and 2020, the database of the NNSID included 5980 legionellosis cases. Table 2 shows a comparison of the key variables across the years.

Cases comprised of 68.9% (N = 4120) men, the median age was 64 years (1st and 3rd quartile: 53–76). The age group of 60 to 69-year olds made up for one quarter of the legionellosis cases (22.7%). The notification rate of the whole period (2000–2020) was highest for the 80–89 years olds (13.3/100,000 population, Table A1). The proportion of men among all cases was high over all years (range: 54.3%–73.6%) and the overall and all period notification rates were more than double than those for women (5.0/100,000 versus 2.2/100,000 population). Over all study years, the canton of Ticino accounted for 15.0% of all cases, followed by the cantons of Zurich (14.0%) and Berne (10.2%). Yet, the notification rate in Ticino was found to be three to four times higher than the average of the other greater regions (Table A1 and Figure A1). In 2020, fewer cases were reported from the cantons of Geneva (3.4%) and Neuchatel (1.5%) compared to their overall means (7.0% and 2.6%). In contrast, the canton of Valais reported more cases in 2020 (6.1%) than its overall mean (3.5%).

3.2.2. Notification process

Of all cases, 91.9% (N = 5494) were classified as confirmed cases of LD, 1.3% (N = 80) as probable and 6.8% (N = 406) as possible cases. Congruently, 93.5% (N = 5574) of all cases had both, a notification from the physician and from the diagnostic laboratory; 3.8% (N = 227) had only a laboratory notification and 0.1% (N = 4) were recorded with a clinical notification only. This proportion remained largely stable, however, in 2020, 8.1% (N = 39) of all cases were notified to the FOPH without a clinical notification form. This is in line with only 89.6% clinically confirmed LD cases in 2020, the lowest since 2000 (mean 2000–2020: 93.7%); and the highest number of cases classified as probable (11.2%, mean: 6.7%).

[1] The annual crude notification rate for LD cases (legionellosis cases with confirmed pneumonia) ranged from 0.9/100,000 population (CI: 0.7–1.1) in 2000 to 4.9/100,000 population (CI: 4.5–5.4) in 2020. The highest notification rate was recorded in 2018 with 6.3/100,000 population (CI: 5.8–6.9).

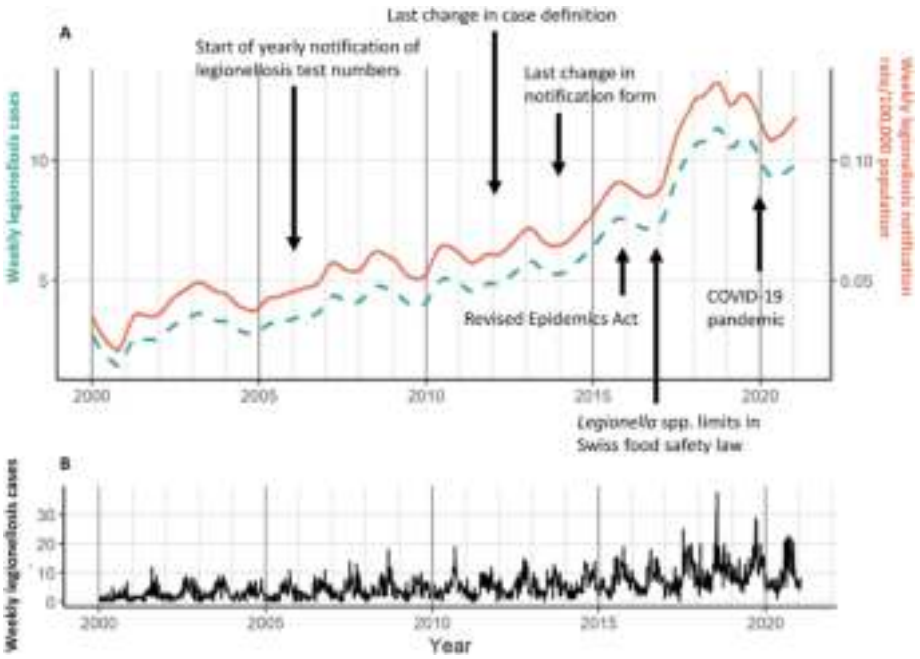

Fig. 1. Time trend of legionellosis cases in Switzerland, 2000–2020. **a** Time trend (without seasonality and randomness). **b** Complete times series of legionellosis cases including trend, seasonality and randomness.

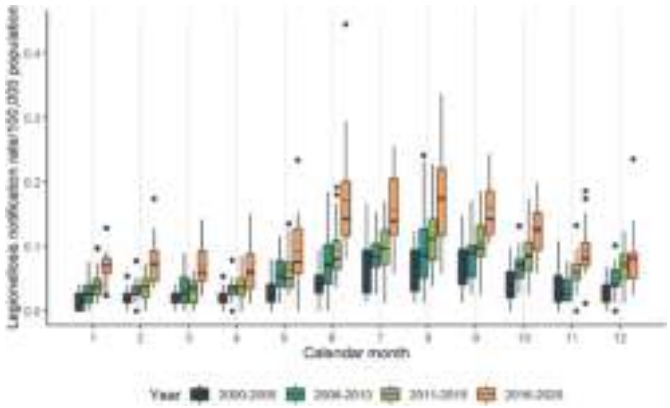

Fig. 2. Seasonality of legionellosis cases in Switzerland, 2000–2020. The red line in the boxplot denotes the mean, the black line the median. The black dots denote outliers. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.2.3. Clinical information

Among all cases with a clinical notification form (N = 5753), 85.8% were hospitalised in 2020 and in 2019 (mean: 89.9%). The median number of days from case date to hospitalisation was 3 days. The overall case fatality rate (CFR) was 5.2% (N = 300). The annual CFR decreased from 7.7% (CI: 2.5–17.0%) in 2000 to 3.6% (CI: 2.1–5.8%) in 2020. The CFR was highest in 2001 (10.2%, CI: 5.6–16.9%) and lowest in 2016 (2.8%, CI: 1.4–5.1%). The median duration from the reported case date to death was 7 days (10th and 90th percentile: 2–24 days). On average 97.4% of cases with a clinical report form were diagnosed with a pneumonia, thereby fulfilling the clinical criteria for diagnosing a LD.

3.2.4. Exposure

If clinical reports were available, the highest proportions of reported risk factors for LD were tobacco smoking (39.6%), age 80 and over (17.7%) and diabetes (13.8%). These proportions remained stable over the years after 2005. Most cases were classified as community-acquired (77.4%, N = 4454) followed by travel-associated LD (13.8%, N = 793), nosocomial (4.0%, N = 231), related to a retirement home (2.9%, N = 169) and occupation-related (1.8%, N = 106). All exposure classifications except retirement home-related cases exhibited a comparable relative seasonality with most cases occurring in summer. Travel-associated cases peaked in August and September. Among all travel-associated cases, the majority was traveling abroad (78.1%).

The proportion of travel-associated legionellosis cases most prominently decreased in 2020 (8.3%, mean: 13.8%), while the number of occupation-associated cases increased to 3.6% (mean: 1.8%). Further, the proportion of travels abroad decreased to 64.9% (mean: 78.1%).

3.2.5. Diagnostics

Most cases were diagnosed using an urine sample (89.5%); sputum (6.4%), bronchoalveolar lavage fluid (6.5%) and serum (2.2%) were significantly less often used. Consequently, the urinary antigen test (UAT) was used for most diagnostics (91.2%), followed by PCR (9.4%) and culture-based diagnostics (7.1%), and serological testing (3.2%). The proportion of PCR tests used increased continuously over the years. Of all 5927 cases with the test specified, 642 (9.2%) had at least two different kinds of tests; the combinations of an UAT with a culture (N = 315) and an UAT with a PCR test (N = 281) were most frequently recorded.

3.2.6. Legionella species

Among all cases, *Legionella pneumophila* has been indicated as the causative agent for 95.5% (N = 5712). This proportion remained high across all years. If a culture or a PCR was indicated in the records, the species could be identified for 82.3% (730 out of 887). Of these, a significant proportion was identified as *Legionella pneumophila* (87.4%), among which serogroup 1 accounted for 21.5%. Only 7 cases of *L. bozemanii*, 4 cases of *L. longbeachae*, 3 cases of *L. micdadei* and 1 case of *L. brunensis* infection were recorded.

3.3. Data quality of the NNSID database

3.3.1. Completeness

The data between 2000 and 2020 was generally complete. In 2020, due to the reduced reporting of the clinical notification form, more

Table 2
Key variables across the years for notification of legionellosis in Switzerland, 2000–2020.

	2000–2005		2006–2010		2011–2015		2016–2020		Overall	
	[%]	*N*	[%]	*N*	[%]	*N*	[%]	*N*	[%]	*N*
Notification										
Confirmed case of LD	89.0	*784*	87.0	*978*	90.9	*1353*	92.1	*2288*	90.4	*5404*
Probable case of LD	4.5	*40*	3.9	*44*	4.3	*64*	1.0	*26*	2.9	*174*
Possible case of LD	6.5	*57*	9.1	*102*	4.8	*72*	6.9	*171*	6.7	*402*
Clinical criteria for case definition fulfilled	93.6	*825*	91.7	*1031*	95.5	*1421*	93.6	*2325*	93.7	*5603*
Laboratory criteria fulfilled	100.0	*881*	99.8	*1122*	99.7	*1484*	99.1	*2462*	99.5	*5950*
Demographics										
Median age (1st - 3rd quartile)	63	*(51–75)*	63	*(51–75)*	64	*(53–75)*	65	*(54–77)*	64	*(63–76)*
Female	32.5	*286*	29.4	*330*	30.4	*452*	31.8	*791*	31.1	*1858*
Swiss nationality	72.2	*636*	65.2	*733*	71.8	*1068*	69.7	*1733*	69.8	*4171*
Seasonality										
Spring (Mar, Apr, May)	14.2	*125*	18.4	*207*	14.9	*222*	16.6	*411*	16.1	*965*
Summer (Jun, Jul, Aug)	37.6	*331*	37.2	*419*	35.7	*531*	37.5	*931*	37.0	*2212*
Autumn (Sep, Oct, Nov)	34.5	*304*	28.8	*324*	31.2	*465*	28.7	*713*	30.2	*1806*
Winter (Jan, Feb, Dec)	13.7	*121*	15.6	*174*	18.1	*270*	17.2	*428*	16.6	*993*
Region										
Central Switzerland	4.2	*37*	6.3	*71*	6.8	*101*	7.6	*189*	6.7	*398*
Eastern Switzerland	8.4	*74*	6.9	*78*	11.4	*169*	9.5	*237*	9.3	*557*
Espace Mittelland	22.2	*196*	20.8	*234*	22.3	*332*	20.4	*506*	21.2	*1268*
Lake Geneva	20.3	*179*	19.7	*221*	21.6	*321*	18.5	*459*	19.7	*1180*
Northwestern Switzerland	14.6	*129*	16.3	*183*	11.1	*165*	13.9	*346*	13.8	*823*
Ticino	14.9	*131*	15.7	*176*	13.9	*207*	15.3	*379*	14.9	*894*
Zurich	15.0	*132*	14.0	*157*	12.6	*188*	14.5	*360*	14.0	*838*
Clinic										
Hospitalisations	86.5	*762*	86.9	*977*	89.3	*1329*	84.7	*2104*	86.5	*5173*
Deaths	6.4	*56*	6.9	*78*	4.4	*66*	4.0	*100*	5.0	*300*
Exposition										
Old-age home	2.7	*24*	2.8	*32*	3.3	*49*	2.6	*64*	2.8	*169*
Community-acquired	72.5	*639*	80.9	*909*	76.9	*1144*	80.0	*1987*	78.3	*4681*
Nosocomial	6.0	*53*	3.8	*43*	3.5	*52*	3.4	*84*	3.9	*231*
Occupational	1.7	*15*	1.4	*16*	1.6	*24*	2.1	*51*	1.8	*106*
Travel-associated	17.0	*150*	11.0	*124*	14.7	*219*	12.0	*299*	13.3	*793*
Risk factors for LD										
Reported as 'No risk'	29.5	*260*	10.5	*118*	14.9	*221*	14.9	*371*	16.2	*971*
Tobacco smoking	17.1	*151*	40.7	*458*	44.9	*669*	40.2	*998*	38.1	*2276*
Alcohol consumption	4.0	*35*	3.1	*35*	2.5	*37*	1.4	*35*	2.4	*142*
Immune suppression	8.5	*75*	13.7	*154*	12.0	*178*	12.2	*304*	11.9	*710*
Diabetes	8.1	*71*	13.1	*147*	14.9	*222*	14.3	*356*	13.3	*796*
Cancer	5.6	*49*	11.7	*131*	9.1	*135*	10.3	*255*	9.5	*570*
Pneumopathy	2.3	*20*	2.8	*31*	2.4	*35*	0.4	*11*	1.6	*97*
Nephropathy	0.2	*2*	1.5	*17*	0.9	*14*	0.4	*10*	0.7	*43*
Cardiopathy	1.0	*9*	2.8	*31*	1.0	*15*	1.4	*34*	1.5	*89*
Age 80+ years	15.0	*132*	16.0	*180*	17.5	*261*	19.4	*482*	17.7	*1055*
Diagnosis										
Urinary antigen test	86.4	*761*	83.2	*935*	85.6	*1274*	82.0	*2036*	83.8	*5010*
Culture	7.3	*64*	6.0	*67*	4.7	*70*	4.6	*114*	5.3	*314*
PCR	3.3	*29*	7.4	*83*	8.6	*128*	10.6	*263*	8.4	*503*
Serology	4.7	*41*	2.9	*33*	2.2	*32*	0.7	*17*	2.1	*123*
Strains										
L. pneumophila	95.5	*840*	95.2	*1072*	96.0	*1429*	95.4	*2371*	95.5	*5712*

LD: Legionnaires' disease; CI: Confidence interval.

clinical information was missing compared to previous years: the hospitalisation status was given only for 89.9% of all cases and the manifestation date (i.e., the date of disease onset) for 82.0%. A detailed overview is provided in Table A2.

3.3.2. Internal validity

Overall, the internal validity of the data was high and only a few inconsistencies were found. In 37 records (0.6%), the case classification and the entries of the clinical and laboratory criteria were discordant. From the cases with known disease onset date (N = 5111), 102 (2%) records indicated an onset date after the notification date. Similarly, in a few cases, the entries of date of death preceded the date of testing. We could not evaluate the indicated exposure classification (e.g., travel-associated) in relation to the incubation timeline.

3.3.3. Timeliness

The median number of days between the case date to the hospitalisation date was 2 days (10 and 90 percentiles: 0–7 days). The median number of days between hospitalisation and reception of the notification at the FOPH was 5 days (10 and 90 percentiles: 1–16 days). On average, there was no delay between reception and data entry at the FOPH (0 days; 10 and 90 percentiles: 0–1 days).

In 2020, the median days between events has remained stable, however, the spread, i.e. the 90% percentile, increased, particularly during the peaks of the pandemic (spring and autumn 2020). Table A3 in the appendix shows the overall median number of days from case date to notification entry at the FOPH.

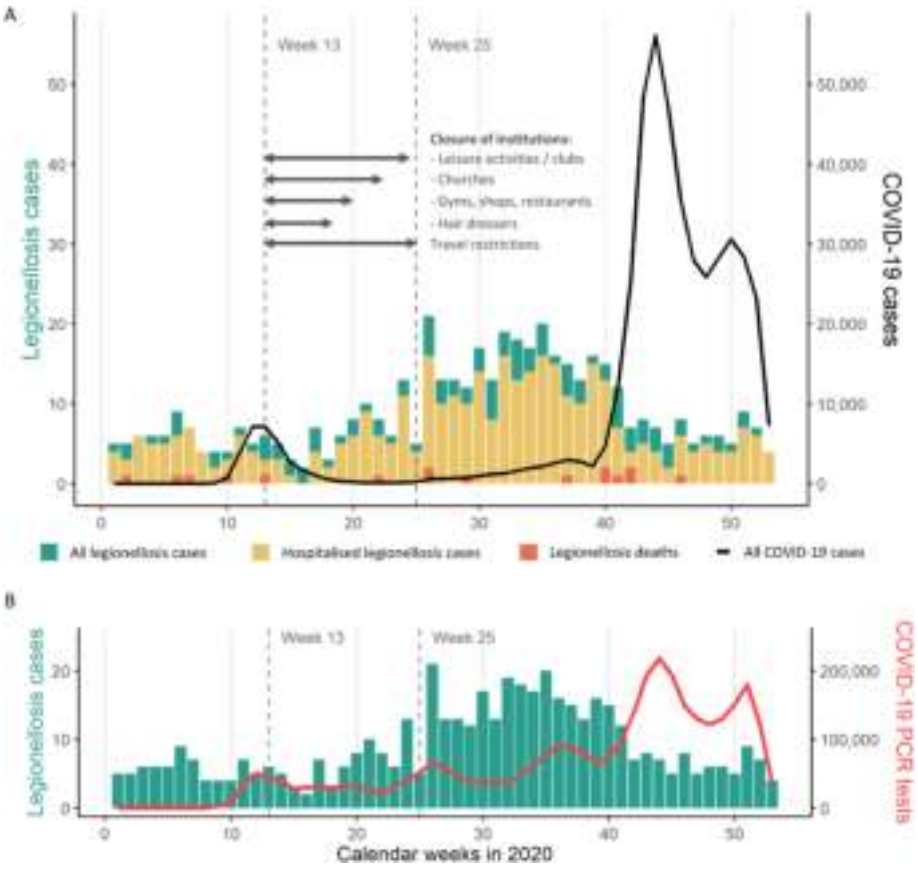

Fig. 3. Legionellosis cases in the context of the COVID-19 pandemic in 2020. **a** Weekly number of legionellosis cases (left y-axis, scale 0–50) and COVID-19 cases (right y-axis, 0–50,000), Switzerland. **b** Weekly number of legionellosis cases (left y-axis, scale 0–20) and COVID-19 PCR tests (right y-axis, scale 0–200,000), Switzerland.

3.4. Legionellosis notifications during 2020

The first cases of COVID-19 were identified in Switzerland in week 8 of 2020 (Fig. 3a). The first wave of the pandemic peaked in week 12 with 7118 cases and the second wave in week 44 with 56,093 cases. The most stringent non-pharmaceuticals measures (closure of schools, shops, sport centres and travel-restrictions) were set in place on March 16 (week 13) and were then gradually removed until week 25. However, daily life was not resumed to levels before the pandemic between the first and second wave as some measures, such as quarantining if traveling from "risk countries" or limiting capacities at certain venues, persisted.

In total 483 legionellosis cases (among them 429 LD cases) were reported in 2020. In week 26 an early peak in legionellosis cases could be seen (21 cases), followed by the expected seasonal increase in cases by week 30/32. The number of legionellosis cases followed the usual seasonality with more cases occurring in summer than in winter. This contrasted with the period of relatively low COVID-19 incidence before the surge of the second wave.

Fig. 3b illustrates the number of legionellosis cases and the frequency of COVID-19 PCR tests performed, which are weakly correlated (Spearman's rank correlation = 0.38, $p < 0.01$).

Fig. 4 shows the results from the interrupted time series analysis. The time of the implementation of travel restrictions is associated with a decrease in notification rate of 35% (95% CI: 0.47–0.90; $p < 0.01$), the re-usage of buildings such as gyms, shops and restaurants (week 20) is statistically non-significantly associated with an 11% increase in notification rate (95% CI: 0.85–1.46; $p = 0.44$). Also all other opening steps were not associated with an increase in cases.

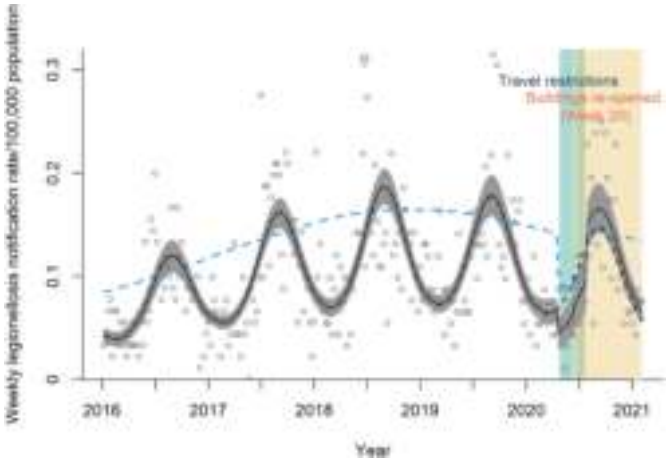

Fig. 4. Interrupted time series analysis using Quasi-Poisson regression model on the number of weekly cases of legionellosis in Switzerland, 2016–2020. The blue line denotes the deseasonalised trend. The dotted black line represents the counterfactual if no interventions took place. The grey arrow denotes a point out of bounds (week 25, weekly notification rate per 100,000 population = 0.42). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

4.1. Interruption of the upwards disease trend since 2018

We evaluated the Swiss legionellosis notification data over two decades. The upward trend since 2000 peaked in 2018 and plateaued thereafter. In 2018, the summer peak was also particularly strong and shifted into June instead of late summer time. This shift was most notably visible in Central Switzerland, Espace Mittelland, Northwestern Switzerland and to a lesser extent in the southern Swiss canton of Ticino. Therefore, this seasonal shift is unlikely driven by a cross-regional outbreak of legionellosis.

Comparing the most recent published European estimates on LD from 2019, Switzerland has one of the highest notification rates; Only Slovenia (9.4/100,000) reported higher rates (European Centre for Disease Prevention and Control (ECDC), 2021). While in about half of the European countries the upward trend in case notification after 2018 persisted, the strong and early summer peak in 2018 could be observed across all the EU/EEA and has been unmatched in 2019. The fact that also the US reported a similar high notification rate in 2018, suggest larger-scale (such as weather and climate) effects impacted LD occurrence (Han, 2021). The impact of climate, weather, relative humidity, and rainfall events in particular promoting LD infection and rising incidences has been highlighted before (Gleason et al., 2016; Sakamoto, 2015; Walker, 2018).

The latest published data from the FOPH show that legionellosis case numbers in 2021 exceeded those of 2018 with a notification rate of 7.8 per 100,000 inhabitants compared to 6.7 (Bundesamt für Gesundheit (BAG), 2022b). As such, data following the post-pandemic years with their extraordinary circumstances need to be closely monitored.

4.2. Stable risk groups and high level of data quality

There has been no remarkable shift in legionellosis case demographics and risk groups across the years. The CFR for LD has been fluctuating throughout the years, but has been lower in recent years than at the start of the century. The overall CFR of 5% calculated from the NNSID data in our study is slightly lower than the average in the EU/EEA of 7% (European Centre for Disease Prevention and Control (ECDC), 2021). However, this figure needs to be interpreted with care: mandatory notification requires the information on the diagnostic (laboratory) findings and a report on clinical findings including exposure data and condition at time of reporting, but a follow-up reporting of the disease outcome including death is not mandatory. Given that notification often occurs early in the disease progression the LD-related CFR of 5% from the NNSID data may be underestimated. Vital statistics are consistently collected at the FSO. The ICD-10 code A481 “Legionnaires’ disease” has been reported as primary or secondary cause of death for on average 23 cases per year (range 12–35 cases) in the decade from 2008 to 2018; (data provided by the FSO to the FOPH). Because the death reports do not always provide the underlying disease leading to respiratory or cardiovascular failure, they tend to underestimate the importance of infectious diseases as cause of death. Still, based on these estimates and for the reasons above, the number of deaths in the NNSID was generally underestimated by an average of 30% (range 1%–58%).

Overall, the extent of data incongruities and missing data in the NNSID database is low, and notifications and data entry are made in a timely manner. Similar to the death status, other post-notification information on the development of the cases, such as the discharge date cannot be universally captured in the surveillance system. As a result, e.g. discharge date was removed from the reporting form in 2014. The median duration from requesting a diagnostic test for *Legionella* infection and legionellosis notification to the FOPH is 5 days and in due-time of the one week time limit for legionellosis notifications (The Federal Assembly of the Swiss Confederation, 2016) and comparable to the Norwegian timeliness (Wolff et al., 2019). The variable “case date”, which fixes the case on the timeline does hamper the interpretation slightly as it can relate to various dates that were recorded within the disease progression. Finally, the current structure of the database is in part marginally user-friendly and/or has been changed (with little readily available documentation) over the years, impeding access to the information. For some reported information (e.g., exposure classification), the database does not allow automatic verification. Electronic reporting could support this process and facilitate data evaluation in the long term. Additionally, some of the incongruities might be avoidable if automated data checks would be included in such an electronic system at entry points with the laboratories and the physicians.

Lastly, the amount of information on each case has been decreasing in recent years with the omission of variables of the clinic progression and risk factors (e.g. occupation). Decreasing the requested information and streamlining the notification process to the data that is essential for the purpose of the surveillance, lowers the workload on the notifying physicians and might further improve (the already high) adherence and quality of the information provided.

4.3. The impact of COVID-19 on LD case numbers

In 2020, the first year of the SARS-CoV-2 pandemic, the number of reported legionellosis cases was similar to 2017 (see Figure A2). A recent report from the FOPH noted a decline of LD cases of 32% compared to the expected case numbers based on the years 2015–2019 (Bundesamt für Gesundheit (BAG), 2021). In our model, starting in 2016, the expected case numbers without the containment measures (the counterfactual) was lower than the actual case numbers. Forward prediction was dependent on the inclusion of years; however, the estimated effect of the investigated measures remained stable. The CFR was lowest in 2020, and a temporal pattern within 2020 could not be observed. We found a weak correlation between the number of COVID-19 tests performed and the number of LD cases identified.

It is difficult to disentangle the effects of the pandemic on legionellosis notification rates. The pandemic itself had an influence on a multitude of aspects of our life and the main causes of LD are not well understood yet. A notable difference in 2020, however, was a 4-percentage-point reduction of clinical notification forms submitted to the NNSID. The clinical notification is sent by the treating physician to the cantonal physicians, who processes and forwards the notification to the FOPH (Schmutz, 2018). In case, the cantonal physician receives a laboratory but no clinical notification, they request a clinical notification from the treating physician. These clinical notification forms are most prominently missing in April and October 2020, suggesting (hospital) physicians and/or cantonal authorities were preoccupied with the consequences and the control of the COVID-19 pandemic. As cases without clinically confirmed pneumonia are counted as legionellosis cases, but not as LD, this leads to an underestimation of LD cases.

The number of both domestic and international travel-associated cases decreased during the pandemic (Steffen et al., 2020). Concurrently, the interrupted time series analysis showed a marked drop in legionellosis cases at the implementation of travel restrictions; and a corresponding increase in cases after they were lifted. Yet, on average only 13.3% of all cases were travel-associated, indicating that either this number is underrepresented or the effect of the travel-restrictions is confounded. We saw only a small effect associated with the reopening of buildings and the presumed exposure to higher concentrations of *Legionella* spp. from extended water stagnation in the buildings’ pipes and plumbing. According to a recent publication, water stagnation-related issues following closure of buildings might have overstated the respective risk for LD (Rhoads and Hammes, 2021). Yet, there is no concluding evidence for either side. The lack of effect could also be due to staggered re-opening of buildings, spreading the new cases and diluting the effect, or flushing recommendations in anticipation of the risk through stagnation have been taken seriously by buildings owners/management and cases were successfully prevented.

5. Conclusion

In Switzerland, the notification rate of LD continuously increased since 2000 to one of the highest rates in Europe, yet the upwards trends was interrupted in 2018, the reason remains unclear. The COVID-19 pandemic seemed to have affected the case numbers mainly through the travel restrictions, which has notably decreased the number of travel-associated cases. Additionally, while physicians seemed to lack resources to keep up with their obligations to notify, the notifications were reported through the diagnostic laboratories in similar frequency and quality compared to previous years, suggesting a robust surveillance system.

Limitations

As this study was based on information from passive disease surveillance, we were limited to cases that were reported. Therefore, we could only approximate the true incidence of the disease. Further, the main drawback on studies involving surveillance data is the lack of denominator data. However, a study on this additional data for the years 2007–2016 has been published previously (Fischer et al., 2020).

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Author contributions

Fabienne B. Fischer and Daniel Mäusezahl conceived and designed the study. Material preparation and data collection were performed by Fabienne B. Fischer and Monica N. Wymann. Analysis and interpretation was performed by Fabienne B. Fischer with support of Daniel Mäusezahl and Monica N. Wymann. The first draft of the manuscript was written by Fabienne B. Fischer and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

The study was conducted under the Epidemics Act (SR 818.101) [35]. The study team received the legionellosis notification data from the FOPH. Other data (COVID-19 cases, non-pharmaceutical measures, and population statistics) are publicly available from the FOPH, the FSO or third parties.

Declaration of competing interest

The authors have no conflicts of interest to declare that are relevant to the content of this article. Monica N. Wymann is staff of the FOPH and participated in her capacities as public health specialist and her function as scientific collaborator within the organisation.

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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.2022.113970.

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Water, sanitation and hygiene (WASH) in schools in Brazil pre-and peri-COVID-19 pandemic: Are schools making any progress?

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ABSTRACT

The previous paucity of data and research on water, sanitation and hygiene (WASH) in schools in Brazil have been preventing an assessment of how safe and healthy schools are to reopen during the COVID-19 pandemic. This study aimed first to assess the current situation of WASH in schools in Brazil and, second, to evaluate to what extent Brazilian schools have been making any progress in providing WASH since the beginning of the COVID-19 pandemic. Data on WASH conditions in schools in Brazil was retrieved from the 2020 and 2021 Brazilian National School Census (BNSC). For the first objective, frequencies of 31 variables were calculated for the whole country and regions, considering all 173,700 schools from BNSC of 2021. Five main variables were considered as indicators of adequate WASH infrastructure in schools. T-test and ANOVA were used to assess differences in these five variables according to the locality, management model and regions. For the second objective only schools presented in both datasets (n = 170,422) were considered to compare WASH in schools pre- and peri-COVID-19 pandemic. Frequencies of 31 variables were calculated for the whole country and regions before and during the pandemic. Paired t-tests were conducted when differences in variables across the years were observed. At the present moment, the majority of schools in Brazil have bathrooms (97%), drinking water with quality suitable for human consumption (95%), improved sanitation facilities (78%) and solid waste collection (70%). Between 2020 and 2021, there was a mix of improvements and deterioration in the school's WASH infrastructure in all regions of the country. Overall, solely considering the WASH infrastructure, schools in the South and Southeast regions of the country are better prepared for the safe reopening. Nevertheless, public schools, schools located in rural areas and the North and Northeast regions of the country, are more in need of WASH interventions. Results indicate that little progress was achieved, and schools in Brazil are still in need of improvements.

1. Introduction

The COVID-19 pandemic revealed that many schools struggle to provide basic water, sanitation and hygiene (WASH). In 2019, 818 million children lacked basic hygiene services at their schools (WHO UNICEF, 2020). That number included 355 million children whose schools had handwashing facilities with water but no soap and 462 million whose schools still had no hygiene service (no handwashing facility or water available) (WHO UNICEF, 2020).

Handwashing has been playing a crucial role in the ongoing COVID-19 pandemic. It is one of the fundamental measures to combat the spread of the new coronavirus, is a cheap, easy, and simple solution, and the public is already quite familiar with the practice (Roy et al., 2020; WHO, 2020). It relies on the presence of sufficient, accessible and functional handwashing facilities, water and soap. Beyond the prevention of COVID-19, handwashing interrupts the transmission cycle of a series of illnesses associated with the lack of available water for personal hygiene called water-washed diseases (Bartram et al., 2021).

Besides expanding the handwashing infrastructure and providing adequate and sufficient supplies for hygiene, the World Health Organization (WHO) checklist to support schools reopening and preparation for COVID-19 also recommends that schools guarantee that water and sanitation facilities are operational, regularly cleaned and disinfected (Benzian et al., 2020). As has been emphasized in the literature, the adoption of hygiene practices in schools, such as handwashing, is not only influenced by the presence of handwashing infrastructure and

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supplies but also by the existence of adequate water and sanitation facilities and by training and health-related knowledge (Cronk et al., 2021; Lopez-Quintero et al., 2009; Shehmolo et al., 2021). Reversely, the availability and quality of water and sanitation in schools are also affected by existing hand hygiene facilities and hygiene materials provided in the school environment (Cronk et al., 2021; Morgan et al., 2021). The relevance of WASH in the context of the COVID-19 pandemic is not restricted to the school environment. However, the transmission of the new coronavirus among children is of special concern taking into account the associated increased incidence of Kawasaki disease and Multisystem inflammatory syndrome cases in children (MIS-C) (Dufort et al., 2020; Godfred-cato et al., 2020; Okarska-Napierała et al., 2020; Verdoni et al., 2020) and the emergence of the new pediatric hepatitis with unknown cause (Brodin and Arditi, 2022; Kendall et al., 2022.; The Lancet Infectious Diseases, 2022). Moreover, children can also experience the manifestation of persistent long-term COVID-19 symptoms after the infection (long COVID), which can ultimately result in their cognitive development impairment (Asadi-Pooya et al., 2021; Borch et al., 2022).

Apart from the prevention of COVID-19 and water-borne and water-washed diseases, the several benefits of the access to WASH in school include: i) decrease in school absenteeism among females (Alam et al., 2017; Montgomery et al., 2016) and among both girls and boys (Lopez-Quintero et al., 2009; Vally et al., 2019); ii) reduction in diarrheal diseases (Jasper et al., 2012; McMichael, 2019; Sangalang et al., 2020; Vally et al., 2019), gastrointestinal symptoms (Lopez-Quintero et al., 2009), soil-transmitted helminthiases (Jasper et al., 2012; McMichael, 2019; Sangalang et al., 2020) and respiratory illness (Jasper et al., 2012; McMichael, 2019); iii) increase in girl's academic achievement (Bergenfeld et al., 2021), and adequate menstrual hygiene management (MHM) practices in the school environment (Bulto, 2021; Korir et al., 2018). The adherence to WASH practices (attitudes and behaviours) is associated with the student's level of knowledge on that topic (Aschale et al., 2021; Assefa and Kumie, 2014; Shehmolo et al., 2021). On that note, as learning environments, schools have the potential to enhance children's teaching and training of WASH practices (Anthonj et al., 2021).

In 2010, access to safe drinking water and sanitation was formally recognized by the United Nations General Assembly as a human right, essential to the full enjoyment of life and the realization of all other human rights (United Nations, 2015a). The Human Right to Water and Sanitation (HRTWS) is directly addressed in the 2030 Agenda for Sustainable Development by the Sustainable Development Goal (SDG) 6, which aims to *ensure availability and sustainable management of water and sanitation for all* (United Nations, 2015b). Notwithstanding that the agenda has a specific goal for WASH and that the HRTWS is explicitly reaffirmed in paragraph 7 of the Agenda's declaration, WASH in schools is included in SDG 4, aiming *to ensure inclusive and equitable quality education and promote lifelong learning opportunities for all* (United Nations, 2015b). Target 4.a seeks to *"build and upgrade education facilities that are child, disability and gender sensitive and provide safe, non-violent, inclusive and effective learning environments for all."* (United Nations, 2015b).

Despite all of the WHO/UNICEF Joint Monitoring Programme's efforts to globally monitor WASH in schools and evaluate the progress to achieving the SDG 4 objective 4.a, there is a noticeable paucity of data on WASH in schools in Brazil. In Brazil, up to 6 million (12%) school-aged population lack proper WASH services (with 15% of schools lacking water service and 5% of schools lacking sanitation services) (WHO UNICEF, 2020). These statistics, however, only capture the reality of a small portion of schools in Brazil. The current state of water services in 85% and sanitation services in 95% of the Brazilian institutions could not be evaluated due to insufficient data (WHO UNICEF, 2020). A recent systematic review performed by Poague et al. (2022) on WASH in schools in low- and middle-income (LMICs) countries also indicated limited data and research on WASH in schools in Brazil and Latin America as well. Out of 65 studies included in the review, only three were conducted in Central America, one in South America and none in Brazil (Poague et al., 2022). According to the WHO/UNICEF report on WASH in schools from 2020, Latin America and the Caribbean were the only regions to record a decrease in data availability on WASH in schools from 2000 to 2019 (WHO UNICEF, 2020). As has been highlighted by Chatterley et al. (2018), harmonized nationally-representative data on WASH in schools is rarely available and, when provided, existing data are often not fully utilized. Since 2014 nationwide data on WASH in schools in Brazil have been publicly available by the Brazilian government as part of the information collected through the Brazilian National School Census (BSNC) (INEP, 2021). Hitherto, evaluation and dissemination of these data have not been done, yet they offer a robust foundation for improving WASH in the Brazilian context. Furthermore, the HRTWS is acknowledged as an unwritten and implicit fundamental right in Brazilian constitutionalism, derived from its connection with other domains and fundamental rights, especially health and dignity (Santiago and Vieira, 2021). In summary, in spite of the relevance of WASH in schools, the availability of data, and the urgent need to provide safe educational environments in the ongoing COVID-19 pandemic, the previous paucity of research on WASH in schools in Brazil have been preventing an assessment of how safe and healthy schools are to reopen during the COVID-19 pandemic.

Thus, the purpose of this study was to first (i) assess the current situation of WASH in schools in Brazil and, second, (ii) to evaluate to what extent Brazilian schools have been making any progress in providing WASH since the beginning of the COVID-19 pandemic.

2. Methods

2.1. Country context

Schools in Brazil can be either private or government-owned and administrated by the municipality (public municipal school), state (public state school) or the Federal Government (or public federal school) (INEP, 2021). The basic education in Brazil is divided into five sequential levels: i) daycares (students aged 0–3 years old); ii) preschool (students aged 3–5 years old); iii) primary first cycle (students aged 6–10 years old); iv) primary second cycle (students aged 11–14 years old); v) secondary school (students aged 15–18 years old) (INEP, 2021). Hitherto, as of June 08, 2022, Brazil is the third nation with the maximum number of accumulative cases of COVID-19 and the second in the number of deaths (WHO, 2022). Due to the pandemic, schools in Brazil have been closed since March 12, 2020 (INEP, 2021). At the present moment, schools are already resuming on-site classes. In some regions, schools operate in a hybrid model, while in others, schools remain temporarily closed and with remote activities (UNESCO, 2022). For this study, we consider the geopolitical division of the Brazilian territory into five major geographic regions, as shown in Fig. 1.

2.2. Data collection and cleaning

Secondary data of schools were retrieved from the 2020 and 2021 Brazilian National School Census (BNSC) provided by the *Instituto National de Estudos e Pesquisas Educacionais Anísio Teixeira* (INEP) (INEP, 2021). The available data in Portuguese was accessed and downloaded in CSV format from the INEP website (INEP, 2022). Since 2014 INEP, the Brazilian government entity responsible for the surveillance of schools, has been collecting and releasing information on an annual basis on the infrastructure of all basic education institutions in the country, regardless of the level of education, locality (rural vs urban) and management model (private or public) (INEP, 2021). Every year, the school's principals, headteachers, or the person in charge must reply to a self-reported survey sent by the INEP. The questionnaire contains 62 questions and should be filled out between June and August, considering the last Wednesday of May as the reference date for data collection (INEP, 2021). The final results are released at the end of January and the

Fig. 1. Administrative divisions of Brazil (states and regions).
*__Midwest__: MT- Mato Grosso, MS – Mato Grosso do Sul, GO – Góias, DF – Distrito Federal; **Northeast:** MA – Maranhão, PI – Piauí, CE – Ceará, PE – Pernambuco, RN – Rio Grande do Norte, - PB – Paraíba, AL – Alagoas, SE – Sergipe, BA – Bahia; **North**: RO – Rondônia, AC – Acre, AM – Amapá, PA – Pará, AM – Amazonas, RR – Roraima; **Southeast**: MG – Minas Gerais, SP – São Paulo, ES – Espírito Santo, RJ – Rio de Janeiro; **South:** PR – Paraná, SC – Santa Catarina, RS – Rio Grande do Sul.

beginning of February from the following year. For the year 2020, due to the extraordinary situation of the COVID-19 pandemic, the reference date for collecting the data was postponed to March 11, 2020, one day before the national closure of schools was established. Hence, the 2020 BNSC dataset describes the state of schools' infrastructure in Brazil right before they were closed. For 2021, the reference date for data collection returned to the original standard (last Wednesday of May of 2021). Thus, the 2021 BNSC, which was released on February 18, 2022, reflects the impacts of the pandemic and the school closure on the state of schools' infrastructure in Brazil. We adopted the word "pre" to designate the state of schools before the beginning of the pandemic (2020 BNSC) and "peri" to refer to the state of schools during the pandemic (2021 BNSC).

Data cleaning and analysis phases, and how each phase is correlated to the research objectives is presented by Fig. 2. The initial databases were first manually exported into Microsoft Excel (2016) by removing the schools that were inactivated or extinct before the pandemic. Inactivated schools are the institutions that are temporarily suspended from school activities, and extinct schools are those that are permanently closed from school activities due to reasons not related to COVID-19 (INEP, 2021). Keeping in mind that, compared to other age groups, children are differently affected by COVID-19 (Cao et al., 2020; Mansourian et al., 2021; Mendoza-Torres et al., 2021), we decided to only include schools that offer one or more levels of basic education and, therefore, attend students aged 0–18 years old (daycare, preschool, primary and secondary schools). In order to allow comparison between datasets (2020 vs 2021), a data quality assessment was conducted to identify schools that were present in both databases.

Data from the BNSC regarding general characteristics of the school and availability of water, sanitation, waste services, and sanitary facilities (bathrooms) were transformed into 31 variables for further analysis (described in Table 1). Binary variables were not exclusionary (i.e., schools can mark more than one option, for instance, schools can declare more than one water supply or more than one waste management pathway). More information about the transformations and compatibility of variables can be found in the Supplementary Material.

2.3. Data analysis

All analyses were conducted using Stata software, version 14 and ArcGIS Pro® software.

2.3.1. Profile of schools in Brazil and in Brazilian regions

For the first objective of this research, the frequencies of the 31 variables were calculated for the whole country and regions, considering all 173,700 schools from BNSC of 2021. Due to the large number of variables available, five main variables were chosen to perform further

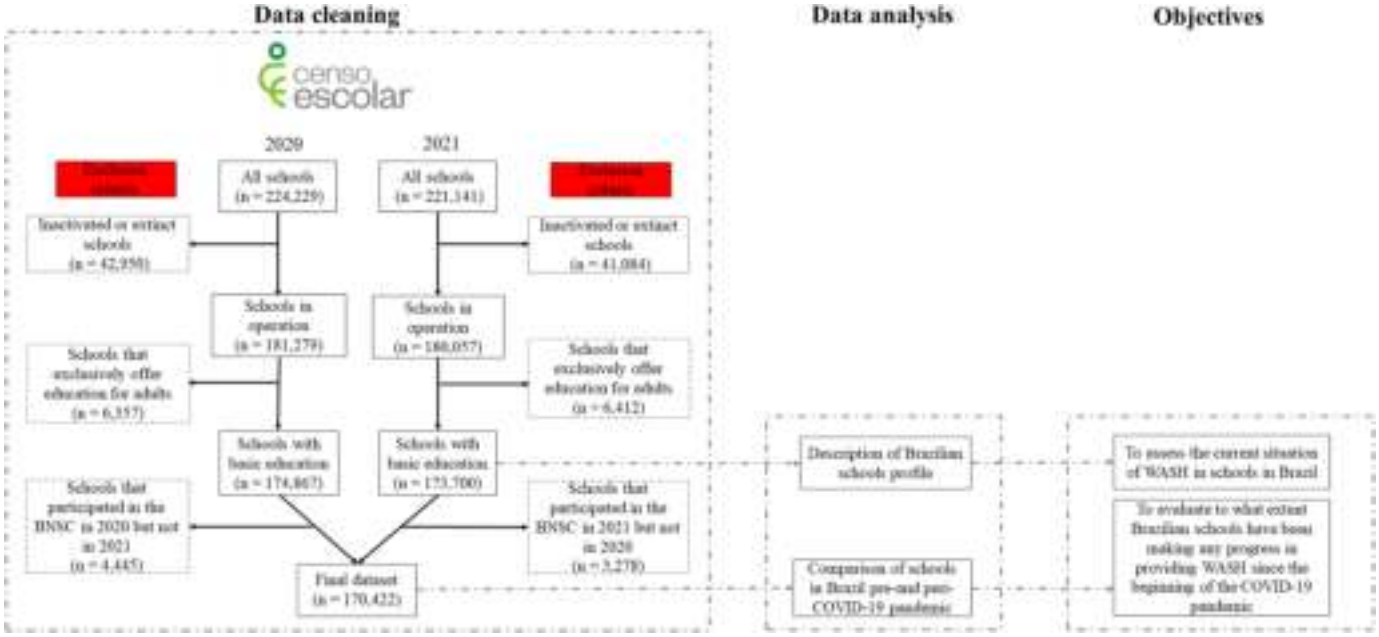

Fig. 2. Flowchart of the data cleaning and analysis steps.

Table 1
Description of WASH and school demographic variables.

Variable	Description
WASH Variables	
Schools with drinking water	The school provides drinking water with quality suitable for human consumption (i.e., ingestion, preparation, and production of food) according to the Brazilian national water quality standards (former *Portaria* nº 2.914/2011 now *Portaria de Consolidação* nº5/2017) (1 – Yes; 0 – No)
Schools with water from public network	The water in the school is supplied by a public network (1 – Yes; 0 – No).
Schools with water from borehole	The water in the school is supplied by a borehole (1 – Yes; 0 – No)
Schools with bathrooms	The school is equipped with sanitary facilities for personal hygiene/physiological needs (1 – Yes; 0 – No)
Schools with bathrooms for youngest children	The school is equipped with sanitary facilities for children 0–5 years old (1 – Yes; 0 – No)
Schools with disability-friendly bathroom	The school is equipped with disability-friendly sanitary facilities following the national guidelines (ABNT - NBR 9050) (1 – Yes; 0 – No)
Schools with bathrooms exclusively for staff	The school is equipped with sanitary facilities for personal hygiene/physiological needs exclusively for staff (1 – Yes; 0 – No)
Schools with bathrooms with showers for students	The school is equipped with sanitary facilities or changing room or washing room with appropriate equipment (shower) for bathing, exclusively for students (1 – Yes; 0 – No)
Schools with water from *cacimba*/cistern/well	The water in the school is supplied by a *cacimba*, cistern, or well (1 – Yes; 0 – No)
Schools with surface water	The water in the school is supplied by surface water source (1 – Yes; 0 – No)
Schools with no water source	There is no water supply in the school (1 – Yes; 0 – No)
Schools with more than one water source	The school has more than one water supply (considering public network, borehole, *cacimba*/cistern/well, and surface water as possible options) (1 – Yes; 0 – No)
Schools connected to a public sewerage system	The school dispose their sewage into a public sewerage system (1 – Yes; 0 – No)
Schools with septic tank	The school dispose their sewage into septic tank (1 – Yes; 0 – No)
Schools with improved sanitation facilities	The school dispose their sewage into a public sewerage system or into a septic tank (1 – Yes; 0 – No).
Schools with unimproved sanitation facilities	The school dispose their sewage into an inadequate facility such as rudimentary cesspit/pit/latrine (1 – Yes; 0 – No)
Schools without sewage collection/treatment	The school has no sewage disposal (1 – Yes; 0 – No)
Schools with solid waste collection	The solid waste in the school is regularly collected by the public cleaning service (1 – Yes; 0 – No)
Schools with solid waste disposal in public destination	The solid waste in the school is disposed in an area licensed by environmental agencies, intended to receive solid waste in a planned manner (e.g., landfills) (1 – Yes; 0 – No)
Schools with burned waste	The solid waste in the school is burned or incinerated (1 – Yes; 0 – No)
Schools with buried waste	The solid waste in the school is buried (1 – Yes; 0 – No)
Schools with waste disposal in another area	The solid waste in the school is disposed in another area (none of the other options) (1 – Yes; 0 – No)
School demographic variables	
Locality of Schools	The school is located in an urban (1) or rural area (2)
Schools' management model	The administration of the school is federal (1); state (2); municipal (3), or private (4). Federal, state, and municipal schools are considered public.
Schools with basic education	The institution offers one or more levels of basic education for children aged 0–18 years old (daycare, preschool, primary first and second cycle, and secondary education) (1 – Yes, 0 – No)
Schools with daycare	The institution offers daycare for children aged 0–3 years old (1 – Yes, 0 – No)
Schools with preschool	The institution offers preschool for children aged 4 and 5 years old (1 – Yes, 0 – No)
Schools with primary education first cycle	The institution offers primary education first cycle for children aged 6 - 10 years old (1 – Yes, 0 – No)
Schools with primary education second cycle	The institution offers primary education second cycle for children aged 11 - 14 years old (1 – Yes, 0 – No)
Schools with secondary education	The institution offers any type of secondary education, including regular high education, high education with technical school, and propaedeutic high education, for children aged 15 - 18 years old (1 – Yes, 0 – No)
Schools with more than one level of education	The institution offers more than one level of education (1 – Yes, 0 – No)

Source: Adapted from INEP (2021).
*Classification of improved and unimproved sanitation facilities followed the definitions of the Joint Monitoring Programme (JMP) (WHO UNICEF, 2020).

tests, namely: i) schools with drinking water; ii) schools with bathrooms; iii) schools with improved sanitation facilities; iv) schools with solid waste collection; v) schools with solid waste disposal in public destination . These five variables can be considered indicators of adequate WASH infrastructure in the schools. The existence of differences according to locality (urban x rural) and management model (public x private) were assessed using *t*-test, while for regions (North, Northeast, Southeast, South and Midwest) by ANOVA test. The significance level was set as 5%.

2.3.2. Comparison of WASH conditions in schools in Brazil and in Brazilian regions before and during the COVID-19 pandemic

For the second objective, the frequencies of the 31 variables for the whole country and all regions were calculated for 2020 (pre-COVID-19) and 2021 (peri-COVID-19), considering only schools presented in both datasets (n = 170,422). Paired t-tests were conducted when differences in variables considering the whole country were observed between both years. The significance level was set as 5%.

3. Results

3.1. WASH in schools in Brazil

In total, 173,700 schools serving 45,305,359 students were evaluated throughout the Brazilian territory (Table 2). The majority of the schools in Brazil are public institutions (78%), located in urban areas (69%). Most of the institutions are administered by municipal governments (61%), while the states, the federal government and the private sector play only a marginal role. Concerning the level of education, approximately 71% of the schools offer more than one level of basic education (e.g., schools offer primary and secondary education, daycare and preschool, or all the possible five levels of education, etc). The most frequent basic education levels offered in the institutions are primary education first cycle (students aged 6–10 years old) (61%) followed by preschool (students aged 3–5 years old) (58%).

Considering the whole country, the majority of the schools have their water supplied by a public network system (75%), followed by boreholes (16%). Only 2% of the schools reported not having water. Sixty-three percent of the schools that reported not having access to any of the water supplies (public network, borehole, *cacimba*/cistern/well and surface water) also declared to provide drinking water for their students. It is noteworthy that the categories are not exclusionary. Approximately 6% of the schools in the country have access to water from more than one water supply (considering as options public network, borehole, *cacimba/cistern/*well and surface water).

Table 2
Characteristics of schools in Brazil and by geographical region in 2021.

	Brazil	North	Northeast	Southeast	South	Midwest
Number and percentage of Schools (n/%)	173,700 (100)	21,620 (12)	59,503 (34)	57,950 (33)	24,587 (14)	10,040 (6)
Number of students enrolled	45,305,359	4,777,381	13,415,129	17,628,109	5,961,399	3,523,341
School's Administration model						
Public schools (%)	78	93	83	68	78	76
Federal schools (%)	0	0	0	0	0	1
State schools (%)	16	17	10	18	22	26
Municipal schools (%)	61	75	72	49	56	49
Private schools	22	7	17	32	22	24
Locality						
Urban Schools (%)	69	38	54	89	83	85
Educational Level*						
Schools with daycare (%)	40	25	44	43	41	33
Schools with preschool (%)	58	60	66	49	58	54
Schools with primary education first cycle (%)	61	79	72	49	51	59
Schools with primary education second cycle education (%)	36	42	33	33	37	44
Schools with secondary education (%)	17	12	12	21	19	24
Schools with more than one level of education (%)	71	74	75	63	77	74
Water						
Schools with drinking water (%)	95	82	95	99	97	98
Schools with water from public network (%)	75	32	68	92	90	86
Schools with water from borehole (%)	16	37	18	7	11	19
Schools with water from *cacimba*/cistern/well (%)	9	12	18	3	2	3
Schools with surface water (%)	4	20	2	2	1	2
Schools with no water source (%)	2	7	3	0	0	0
Schools with more than one water source (%)	6	8	9	4	4	10
Bathroom						
Schools with bathrooms (%)	97	85	97	99	100	99
Schools with bathrooms for youngest children (%)*	60	29	43	74	90	80
Schools with disability-friendly bathrooms (%)	49	31	41	53	62	73
Schools with bathrooms exclusively for staff (%)	54	33	38	70	69	66
Schools with bathrooms with shower for students (%)	44	30	40	47	51	62
Sanitation						
Schools connected to a public sewerage system (%)	56	13	38	88	63	54
Schools with septic tank (%)	23	33	31	8	27	28
Schools with improved sanitation facilities (%)	78	45	68	96	87	80
Schools with unimproved sanitation facilities (%)	18	36	28	4	14	20
Schools without sewage collection/treatment (%)	4	19	5	0	0	1
Solid Waste						
Schools with solid waste collection (%)	70	47	71	63	97	93
Schools with solid waste disposal in public destination (%)	13	1	3	36	2	1
Schools with burned waste (%)	19	51	29	4	3	7
Schools with buried waste (%)	2	9	2	0	3	3
Schools with waste disposal in another area (%)	3	16	2	1	0	1

The sum might exceed 100% because the categories are not exclusionary.
*Only schools with daycare (students aged 0–3 years old) or preschools (students aged 3–5 years old) were considered when analyzing bathrooms for the youngest children (sanitary facilities for children 0–5 years old) (n = 112,927).

Ninety-seven percent of the schools in the country reported having bathrooms. However, less than half of the educational intuitions have bathrooms for people with disabilities (49%) and bathrooms with showers for students (44%). The percentage of schools with bathrooms exclusively for staff is 54%. Eight percent of the schools that reported having bathrooms for the youngest children (0–5 years old, daycare and preschool age) do not attend students from that age group.

Seventy-eight percent of the schools in the country have improved sanitation facilities. Eighteen percent of the schools have unimproved sanitation facilities and 4% of the educational institutions have no sewage collection or treatment.

The three most frequent solid waste management pathways in schools in Brazil are to have their solid waste regularly collected (70%), burned (19%) or disposed in a public destination (13%).

Fig. 3 summarizes the differences in the five main WASH variables (schools with drinking water, bathrooms, improved sanitation, solid waste collection, and solid waste disposal in a public destination), across Brazilian regions. Regarding these five chosen WASH variables, Student's t-test showed that a significantly ($p < 0.001$) higher percentage of urban schools had all five indicators compared with rural schools, while a significantly ($p < 0.001$) higher percentage of private schools had all five indicators compared with public institutions (Table 3).

3.2. WASH in schools in Brazil by region

Schools are mostly located in the Northeast (34%) and Southeast (33%) regions of the country (Table 2). The percentage of public schools is higher in the North and Northeast regions of the country. The majority of the institutions continue to be administered by the municipal governments. With regards to location, the educational institutions in the North are mainly located in rural areas (62%). Concerning the educational level, regardless of the region, most schools have a mix of different levels. The frequency of preschool and primary education first cycle institutions follows the same profile for the whole country. There are substantial differences according to the region for the other levels of education (daycare, primary education second cycle and secondary school).

Regarding water supply in schools, the North region differs from the other regions. Most institutions obtain their water from boreholes (37%) followed by a public network system (32%). The North region is also the one with the lowest percentage of schools with drinking water (82%) and with the highest frequency of schools with surface water (20%). Schools without water are mainly concentrated in the North and Northeast regions (representing 7% and 3% of the schools in the region, respectively). Lack of sanitary facilities was observed in schools in all

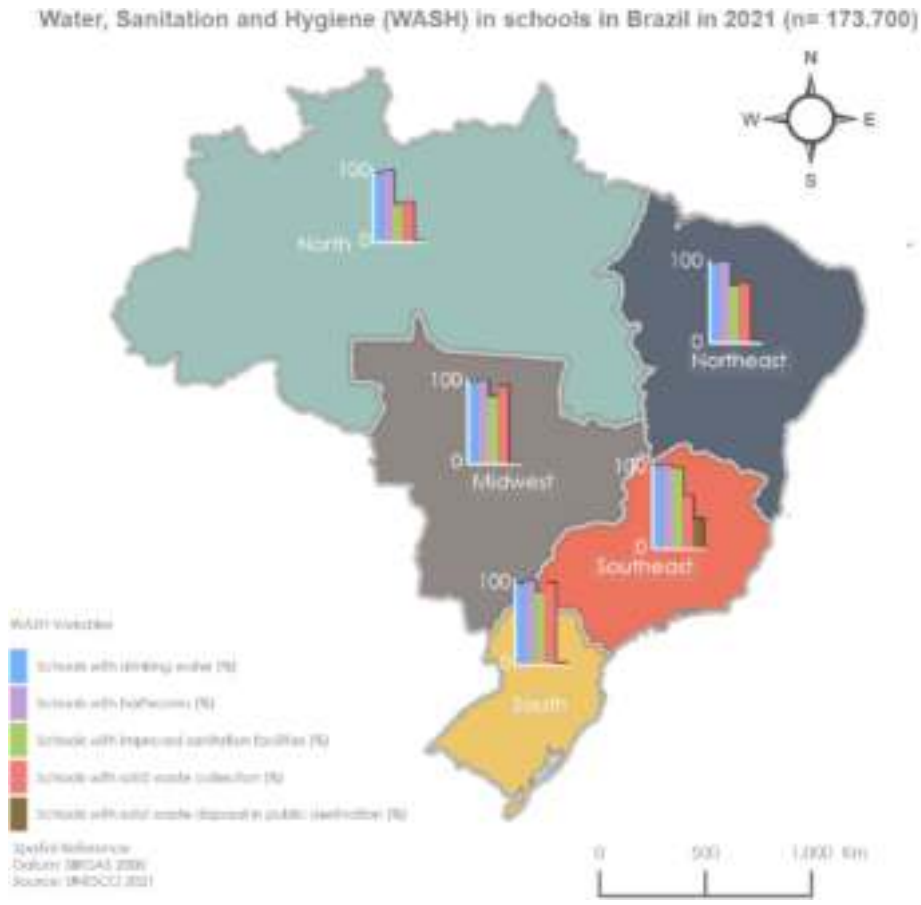

Fig. 3. Water sanitation and hygiene (WASH) in schools in Brazil in 2021 (n = 173,700).

Table 3
Proportion of groups for t-Student tests.

	N	Schools with drinking water (%)	95% CI	Schools with bathrooms (%/CI)	95% CI	Schools with improved sanitation facilities (%)	95% CI	Schools with solid waste collection (%)	95% CI	Schools with solid waste disposal in public destination (%/CI)	95% CI
Locality (Rural x Urban)											
Rural	53,021	88	87.5–88.1	92	91.5–92.0	46	45.9–46.8	39	38.4–39.3	3	3.1–3.4
Urban	120,679	99	98.4–98.6	99	98.8–98.9	91	91.2–91.5	84	83.8–84.3	18	17.6–18.0
Schools' Administration Model (Public x Private)											
Public	135,785	94	94.0–94.3	96	96.1–96.3	72	72.2–72.7	67	66.6–67.1	12	11.8–12.1
Private	37,915	99	99.0–99.2	99	98.4–98.6	96	96.1–96.4	82	82.1–82.8	18	17.8–18.6

All p-values <0.001. CI: Confidence Interval.

regions. The institutions in the North and Northeast regions are the most deficient in terms of available restrooms (the lowest frequency of schools with all types of bathrooms). Except for the North region, most schools in the country have improved sanitation facilities, with the Northeast region having the lowest frequency (68%) and the Southeast the highest (96%). Thirty-six percent of schools in the North region reported having unimproved sanitation facilities, while 19% of schools reported not having sewage collection or treatment. The practice of burning solid waste is widespread in schools in the North and Northeast region, being the most frequent management pathway for solid waste in schools in the North region (51%). For all the other regions of the country, most of the institutions have their solid waste regularly collected.

The ANOVA followed by Tukey post-hoc test indicated differences in the percentage of schools with drinking water, improved sanitation facilities, and solid waste collection according to the region. The test also indicated no significant difference in the percentage of schools with solid waste disposal in public destinations in the North and Midwest regions (1% vs 1%), and between Northeast and South regions (3% vs 2%). Additionally, there was no difference in the percentage of schools with bathrooms between the South and Midwest regions (100% vs 99%). More information can be found in the Supplementary Material.

3.3. Comparison of WASH in schools in Brazil pre- and peri-COVID-19 pandemic

3.3.1. Brazil

Table 4 compares the WASH conditions in schools in Brazil pre- (2020) and peri- (2021) COVID-19 pandemic. The entries in bold represent all variables that presented changes between the two years. Paired t-tests indicated that, for these variables, all differences were statistically significant ($p < 0.001$) (more information can be found in

Table 4
Comparison of WASH conditions in schools in Brazil pre- (2020) and peri- (2021) COVID-19 pandemic (n = 170,422).

	Brazil		North		Northeast		Southeast		South		Midwest	
N	170,422		9785		58,333		21,274		56,832		24,198	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Water												
Schools with drinking water (%)	94	95	82	82	95	95	99	99	90	97	98	98
Schools with water from public network (%)	75	75	32	32	67	67	92	92	89	90	86	86
Schools with water from borehole (%)	16	16	38	38	18	18	7	7	11	11	19	19
Schools with water from cacimba/*cistern*/well (%)	9	9	12	12	18	18	3	3	2	2	3	3
Schools with surface water (%)	4	4	20	20	2	2	2	2	2	1	2	2
Schools with no water source (%)	2	2	6	7	3	3	0	0	0	0	0	0
Schools with more than one water source (%)	6	7	8	8	9	9	4	4	4	4	10	10
Bathroom												
Schools with bathrooms (%)	96	97	84	85	97	97	99	99	99	100	99	99
Schools with bathrooms for youngest children (%)*	59	60	29	29	41	42	73	74	88	90	78	79
Schools with disability-friendly bathrooms (%)	47	48	30	31	39	41	51	53	59	61	71	73
Schools with bathrooms exclusively for staff (%)	49	54	29	33	33	37	66	70	63	68	58	65
Schools with bathrooms with shower for students (%)	44	44	31	30	41	40	47	47	52	51	62	62
Sanitation												
Schools connected to a public sewerage system (%)	56	56	13	12	37	37	88	88	63	63	53	53
Schools with septic tank (%)	23	23	33	33	31	31	8	8	26	27	28	28
Schools with improved sanitation facilities (%)	78	77	45	45	68	67	96	96	86	86	80	80
Schools with unimproved sanitation facilities (%)	18	19	35	36	27	28	4	4	14	14	19	20
Schools without sewage collection/treatment (%)	5	4	21	19	6	5	0	0	0	0	2	1
Solid Waste												
Schools with solid waste collection (%)	81	70	46	47	70	71	96	63	97	97	92	92
Schools with solid waste disposal in public destination (%)	2	13	8	1	2	3	0	36	3	2	3	1
Schools with burned waste (%)	19	19	52	51	30	29	4	4	4	3	7	7
Schools with buried waste (%)	2	2	1	9	2	2	1	0	2	3	1	3
Schools with waste disposal in another area (%)	3	3	15	16	2	2	1	1	0	0	1	1

The sum might exceed 100% because the categories are not exclusionary.
*Only schools with daycare (students aged 0–3 years old) or preschools (students aged 3–5 years old) were considered when analyzing bathrooms for the youngest children (sanitary facilities for children 0–5 years old) (n = 110,251). In bold are all variables that presented changes between 2020 and 2021, considering the whole country, in which paired T-tests were conducted and all differences were statistically significant ($p < 0.001$).

the Supplementary Material). It is noteworthy that the percentage of schools in Brazil that have their solid waste regularly collected by a public cleaning service dropped from 81% in 2020 to 70% in 2021. In contrast, the percentage of schools with solid waste disposal in a public destination (such as landfills) in Brazil increased by 11 percentage points in 2021 compared to the previous year. Moreover, the percentage of schools in Brazil with bathrooms exclusively for staff increased from 49% to 54% between 2020 and 2021.

3.3.2. *Brazilian regions*

The water component was the one with fewer changes from 2020 to 2021, mainly in schools in the South region. The percentage of schools with drinking water in the South region increased from 90 to 97%. Furthermore, there was an increase of 1 percentage point in the frequency of schools with water from public networks and a decrease of 1% in the frequency of schools with surface water in the South region. Results, therefore, indicate improvements from 2020 to 2021 regarding the water conditions in schools in that region. However, there was also an increase of 1% in the percentage of schools with no water source in the North region.

There was an increase of 1% in the percentage of schools with bathroom in the North (from 84 to 85%) and in Southeast region (from 99 to 100%). The frequency of schools with bathroom for youngest children increased in the Northeast (from 41 to 42%), Southeast (from 73 to 74%), South (from 88 to 90%), and Midwest regions (from 78 to 79%). The frequency of schools with disability-friendly bathrooms and bathrooms exclusively for staff increased in schools in all country regions. Regarding the presence of disability-friendly bathrooms in schools, there was an increase of 1% in the North (from 30 to 31%), 2% in the Northeast (from 39 to 41%), in the Southeast (from 51% to 53%), in the South (from 59 to 61%) and in the Midwest region (from 71 to 73%). The percentage of schools with bathrooms exclusively for staff was the variable with the biggest variation from 2020 to 2021. There was an increase of 4% in the North (from 29 to 33%), 4% in the Northeast (from 33 to 37%) and in the Southeast (from 66 to 70%), 5% in the South (from 63 to 68%) and 7% in the Midwest region (from 58 to 65%). The percentage of schools with bathrooms with showers for students, however, decreased by 1% in the North (from 31 to 30%), Northeast (from 41 to 40%) and Southeast regions (from 52 to 51%).

There was an increase of 1% in the frequency of schools with septic tank in the South region (from 26% in 2020 to 27% in 2021) but also in the frequency of schools with unimproved sanitation facilities in the North (35% vs 36%), Northeast (27% vs 28%) and Midwest regions (19% vs 20%). The percentage of schools with improved sanitation facilities in the Northeast region decreased from 68% to 67% in the analyzed period. The frequency of schools with no sewage collection or treatment, however, dropped in the North (21% vs 19%), Northeast (6% vs 5%) and Midwest regions (2% vs 1%).

Results concerning the solid waste management and disposal in schools were very mixed, and the North region was the one with the most changes (with variations in all variables). The percentage of schools with solid waste collected increased in the North region (46% vs 47%) and decreased in the Northeast (70% vs 71%) and Southeast regions (96% vs 33%). Similarly, the frequency of schools with solid waste disposal in a public destination decreased in the North (8% vs 1%), South (3% vs 2%) and Midwest regions (3% vs 1%), while it increased in the Northeast (2% vs 3%) and Southeast regions (0% vs 36%). The percentage of schools that burned their waste dropped in 1 percentage point in the North (52% vs 51%), Northeast (30% vs 29%) and South regions (4% vs 3%). The frequency of schools that buried their solid waste, however, increased in the North (1% vs 9%), South (2% vs 3%) and Midwest regions (1% vs 3%). Moreover, there was also an increase of 1% in the percentage of schools with waste disposal in another area in the North region (15% vs 16%).

4. Discussion

Evaluating 173,700 schools, this study is one of the largest studies to evaluate WASH in schools in Brazil through the COVID-19 pandemic. The present study had two main objectives. First, to describe the current situation of WASH in schools in Brazil, and second, to compare the availability of WASH in schools in Brazil before and during the ongoing COVID-19. To our knowledge, this is the first analysis using the robust 2021 BSNC dataset on WASH in schools.

4.1. The current state of WASH in schools in Brazil

The results revealed that the majority of schools in Brazil have bathrooms (97%), drinking water with quality suitable for human consumption (95%), improved sanitation facilities (78%) and solid waste collection (70%). Results also indicated significant rural-urban and public-private disparities in access to WASH in schools, with government-owned schools and schools located in rural areas presenting greater WASH deficiencies. As has been highlighted before in the literature, in LMICs, rural schools are more likely to lack WASH conditions and facilities than urban schools (Adams et al., 2014; Jordanova et al., 2015; WHO UNICEF, 2020). This result is of particular concern considering that in Brazil, the highest out-of-school rates (children and adolescents aged 4–17 years old) are documented in rural locations (UNICEF, 2021). As part of the institutions' infrastructure, poor WASH conditions contribute to a less appealing learning environment. Improved school WASH conditions have been reported to reduce student absence (Jasper et al., 2012; McMichael, 2019) and school evasion (with higher impact on girls) (Agol and Harvey, 2018; Bergenfeld et al., 2021) by providing services and reducing disease transmission.

With regards to regional differences, the North is the region with schools with the highest lack of appropriate WASH conditions across all domains (lowest frequency of schools with bathrooms, drinking water, improved sanitation facilities, solid waste collection and the highest percentage of schools with surface water and burning waste). The Northeast region follows as the second region with schools presenting the worst panorama. Results also evidenced significant inequalities in the access to WASH in schools according to the region where they are located. The North region is the one with the highest percentage of the out-of-school population aged 4–17 years old, while the Northeast region has in absolute number the majority of the out-of-school population in the country (UNICEF, 2021). In summary, the locations where the school-aged population are more in risk of school absenteeism and dropout (rural areas, North and Northeast regions) are also the ones with the less appropriate school environment in terms of WASH.

Due to the incompatibility of the terms and definitions used in the census and the JMP (WHO UNICEF, 2020), schools in Brazil could not be classified as having basic, limited, no water service and with improved or unimproved water sources. This, however, is not an issue restricted to Brazil. As with WASH in schools monitoring, indicators vary between sources and countries, which hampers the comparison of WASH in schools across locations (Chatterley et al., 2018). According to the systematic review on WASH in schools in LMICs conducted by Poague et al. (2022) considering 18,465 schools described in 65 studies across 30 different countries, the water source in 6% of schools (n = 1118) was classified as "unknown" because the terms and indicators used in the studies did not fit the JMP classification. In Brazil, for instance, the standard when working with WASH is to use the definitions and indicators of the *Plano Nacional de Saneamento Básico* (Brazil, 2014). Based on the PLANSAB guidelines, water, sanitation and waste services can be classified into three categories: i) adequate; ii) poor; iii) or with no service. One of the main premises for the classification of water and sanitation services into the "adequate" category is the quality of the water (potable, safe and with quality suitable for human consumption) and the treatment of the wastewater. Although the JMP concept of "improved" water source involves the potential to deliver safe water, the classification itself does not guarantee that the quality of the drinking water provided by the schools is suitable and safe for human consumption. The same discussion also applies to the sanitation domain. The JMP concept of "improved" sanitation facilities, which considers the existence of an infrastructure designed to hygienically separate excreta from human contact, however, does not guarantee that the sewage, after being collected and separated, is proceeded by treatment. Moreover, packaged and delivered water are also considered improved water sources, whereas according to the PLANSAB, delivered water by water trunks can be classified as poor service. More information about the classification and definitions of the PLANSAB in English can be found in the Supplementary Material.

With regards to sanitation, however, our study indicates a worst scenario compared to the 2020 WHO/UNICEF report on the progress on WASH in schools (WHO UNICEF, 2020). While the JMP estimates that 5% of schools in Brazil have no sanitation service, which represents 2 million students without this resource in the school environment, our findings indicated that 22% of the Brazilian schools could be classified as with no sanitation service, ultimately impacting over 9 million of students. Nonetheless, the percentage of schools with improved sanitation facilities found in this study is higher than what was reported by Poague et al. (2022) in LMICS (31%) and in Central America (64%, though this estimate was based on data from 412 schools included in only three studies).

Nevertheless, the frequency of schools with toilets accessible for students with disabilities in our study was approximately 10 percentage points higher (49%) than what was reported in the WHO/UNICEF report (38%) (WHO UNICEF, 2020). Additionally, the percentage of schools with piped water and toilets (in our study the equivalent to schools with water from public networks and schools with bathrooms) was similar. It draws our attention that 87% of the schools assessed by the WHO/UNICEF (WHO UNICEF, 2020) were located in urban areas, in contrast to 69% in this study. It is not clear what were the inclusion criteria, the total number of schools evaluated in the report and what were the main data sources from Brazil.

4.2. WASH in schools in Brazil pre- and peri-COVID-19

Results indicated improvements in the percentage of schools with drinking water, solid waste disposal in public destinations, bathrooms, bathrooms for the youngest people, disability-friendly bathrooms, and bathrooms exclusively for staff. However, findings also revealed a decrease in the percentage of schools with solid waste collection and improved sanitation facilities (consequently, an increase in the frequency of schools with unimproved or without sanitation facilities). At first sight, changes of one percentage point observed in most of the variables might seem too small to matter, and its statistical significance might be attributed to the large size of the datasets. However, when taking into account the total number of schools (170,422), we can observe that those are relevant variances. For instance, approximately 1704 schools that did not have bathrooms in 2020 incorporated the sanitary infrastructure in the next year.

Simultaneously mix of improvements and deterioration in the variables were observed in schools in all regions of the country. Improvements in water indicators were observed in schools in the South region, while deterioration was noticed in schools in the North region. As for bathrooms, improvements were observed in all regions and in all variables, with the exception of the frequency of schools with bathrooms with showers that reduced in the North, Northeast and South regions. Regarding sanitation, findings indicate a mix of improvements and deterioration of the school's infrastructure in the North, Northeast and Midwest regions. Changes in the solid waste variables followed the same trend as sanitation, with variations in all regions and with changes of most concern in the North region.

4.3. Schools reopening during the COVID-19 pandemic

Overall, solely considering the WASH infrastructure, schools in the South and Southeast regions of the country are better prepared for the safe reopening. At the present moment, schools in the North and Northeast regions are the ones more in need of changes in the WASH infrastructure. Approximately 4768 schools (684 in the North and 4083 in the Northeast region) do not have a water source and, therefore, cannot provide the adequate infrastructure for handwashing and cleaning of facilities.

Additionally, there is a need for improvements in the sanitation domain in schools in all regions. Despite the fact that most schools have bathrooms, the wastewater puts the students and staff at risk of faecal-oral diseases if not properly managed and disposed. Our study shows that 97% of schools reported having bathrooms, but only 77% have sanitation facilities designed to hygienically separate excreta from human contact. Moreover, the presence of the virus and its genetic material in faeces of COVID-19 patients (Chen et al., 2020; Wu et al., 2020) and in the sewage (Chavarria-Miró et al., 2020; Fongaro et al., 2020; Medema et al., 2020) suggests that COVID-19 might also be transmitted through faecal-oral routes (such as by the ingestion of contaminated water or by touching mouths, noses or eyes with hands that had been in direct contact with faeces). On that note, providing adequate and improved sanitation facilities should be a major concern in the school environment, considering that gastrointestinal symptoms of COVID-19 infection (such as diarrhoea and vomiting) are more frequent in children than in adults (Mendoza-Torres et al., 2021). Due to the fact that children have smaller body fluid reserves than adults, they are more at risk of rapidly dehydrating and developing shock when infected by gastrointestinal diseases (Aronson and Shope, 2020).

Among infants, children under three years old are more likely to be infected with SARS-CoV-2 (Mendoza-Torres et al., 2021). Children are still learning the basic principles of hygiene at that age and their developmentally appropriate behaviours include self-soothing by putting their hands and other objects in their mouths (Aronson and Shope, 2020). In addition, in their first three years of life, some children are still learning how to walk, spending much of their time closer to the ground and constantly using (and touching) surfaces to support their balance and movements (Aronson and Shope, 2020). That said, special attention should be given to providing adequate WASH infrastructure in daycares and preschools. However, according to the results, 40% of schools that attend children aged 0–5 years old in Brazil are not equipped with sanitary facilities for children of that age. From 2020 to 2021, there was no change in the frequency of schools with bathrooms for the youngest children in the North region, where the lowest proportion of restroom availability for this age group was reported.

The low frequency of schools in the country equipped with disability-friendly sanitary facilities (60%) also deserves attention. In schools without those facilities, students have to crawl and touch the floor to access the bathrooms (Erhard et al., 2013; Zaunda et al., 2018). When facing these obstacles, students with disabilities also avoid using the facilities during their time in the schools or practising open defecation (Erhard et al., 2013; Zaunda et al., 2018). Therefore, the lack of adequate WASH infrastructure in the school environment results in students adopting non-hygiene practices, which puts them at risk of COVID-19 and other hygiene-related diseases. Despite the improvement in the percentage of schools with disability-friendly bathrooms from 2020 to 2021, less than 50% of schools in the North and Northeast regions have these facilities.

4.4. Limitations and strengths of the Brazilian National School Census

Some limitations of the BNSC have to be highlighted. As a self-reported survey, the information provided by the schools might not be truly accurate and representative of the reality. Despite completion required by law, which means that all schools in Brazil must reply every year to the survey, the differences between the schools that participated across the years (2020 and 2021) indicated that the census has not been able to capture all the educational institutions in the country. Additionally, a series of inconsistencies in the dataset were observed during analysis. Schools that do not have a water supply should not be able to provide drinking water, and only schools with students aged 0–5 years old (with daycare and preschool) should have bathrooms for the youngest children. However, more than half of the schools that do not have a water supply reported having drinking water and 8% of the schools that reported having bathrooms for youngest children do not have students of that age. These results might indicate that the census is being answered without full comprehension of its concepts and the definition of the variables. This possibility is similarly observed in the change in solid waste management in schools in the Southeast region. In 2020, 96% of the schools in that region had their solid waste regularly collected, and 0% of the schools had their solid waste disposed in public destinations. The next year, however, the percentage of schools with solid waste collection in that region dropped to 63%, and the frequency of schools with waste disposal in public destination rose to 36%. Most likely, that happened because the differences between both categories were not clear to respondents. These inconsistencies may also be the consequence of the fact that categories are not exclusionary. For instance, schools can report having all the possible solid waste management choices. We should also consider the possibility that schools without a water supply are able to provide drinking water by purchasing delivered or packaged water, such as water supplied by water trunks or bottled water (Ribeiro et al., 2018). It is also possible that respondents tend to choose the “desirable” answer of the survey (e.g. that the school had drinking water when they did not) because of social desirability bias (Hawthorne effect).

Furthermore, even though the BNSC provides an extensive dataset on WASH in schools in Brazil, it lacks essential information for the assessment of the safety of the school’s infrastructure for its community against COVID-19 and other diseases. The census does not provide any information on the presence of handwashing stations, soap, student to toilet ratio and drinking fountains in schools. The Brazilian Ministry of Education recommends a student to toilet ratio of 20:1 and the placement of drinking fountains in daycares and preschools (Brazil, 2006). The manual for School Building Performance, also elaborated by the Brazilian Ministry of Education suggests a student to toilet ratio of 40:1 and one handwashing station for every 30 students (Brazil, 2005). Additionally, the manual also specifies the placement of one soap dish for every two toilets and that at least 5% of a school’s toilets must be suitable for persons with physical disabilities (Brazil, 2005). However, no information about these infrastructures is collected through the BNSC. Hence, it is not possible to assess if the schools are following the national guidelines. Regardless of the type of water supply (public network, boreholes, wells, etc.), schools in Brazil lack cups for students at drinking fountains (Borges-pedro et al., 2018; Pereira and Sorlini, 2019). When cups are not made available in sufficient quantities in schools, they are shared between students (Borges-Pedro et al., 2018; Pereira and Sorlini, 2019), which is a practice of high risk and concern for the transmission of COVID-19. Schools in Brazil are also known for not having toilets in enough quantities (Coswosk et al., 2019). The lack of cups and toilets in sufficient quantity in schools might lead to students queuing to use the facilities and, thus, disrespecting the social distancing measures. No data is also supplied on the functionality of the infrastructure and on the normative contents of the HRTWS – availability, accessibility, quality and safety, acceptability, privacy and dignity (United Nations, 2015a).

Despite these limitations the BSNC is the main available and public up-to-date source of data on WASH in schools in Brazil. Through a collaborative network coordinated by INEP and composed of municipal, state and federal educational entities of the Brazilian government, the BSNC provides a vast dataset on Brazilian schools, which is essential for the formulation of public policies in education. The consistency seen in

the frequency of the majority of the WASH variables, which is evidenced by the small changes in the variables across the years (especially variables that are of easy comprehension and interpretation by the survey's respondents such as the presence of bathroom and the type of water supply), attest for the reliability of BNSC as research tool. Even though the first nationwide BNSC dates from 2014, the WASH information available in datasets has been rarely used and the BNSC hardly cited in scientific publications. The data provided by the BSNC can and should be used for research and public policy purposes, but with parsimony, keeping in mind its areas of improvement and integrating it with other methods and sources.

The BNSC defined bathrooms as "sanitary facilities for personal hygiene/physiological" needs. Thus, it was not clear if handwashing stations were included as part of the sanitary facilities a bathroom should have. If so, the presence of bathrooms in schools could have been used as a proxy for the assessment of handwashing infrastructure in schools in Brazil. However, we chose not to do so based on our understanding that the existence of bathrooms in school does not imply the existence of handwashing and that, most likely, the respondents of the survey also had that understanding. Moreover, even though the authors recognize that schools that are currently inactivated might be activated once again in the future, these educational institutions were not part of the scope of this research. Due to the urgency in providing safe educational environments in the ongoing COVID-19 pandemic, we prioritized assessing the state of the schools that are currently providing services in Brazil (remotely, in hybrid mode or with face-to-face activities).

5. Conclusion and future research

Results of this study on WASH in schools in Brazil, considering 173,700 schools, indicate that most of the schools in Brazil have bathrooms, drinking water with quality suitable for human consumption, improved sanitation facilities and solid waste collection. Nonetheless, results point out the urgent need for improvements in public schools, schools located in rural areas, and in the North and Northeast regions of the country. Within WASH domains, schools are more in need of changes in the sanitation infrastructure and solid waste management. As for the comparison of WASH in schools pre-and peri-COVID-19 pandemic, 170,422 schools were analyzed. Mixed changes in the variables, with both improvements and deterioration, were observed in schools in all regions of the country. Schools in the South and Southeast regions presented the best WASH infrastructure for the safe reopening, whereas schools in the North and Northeast regions of the country were the least prepared.

Furthermore, it is also important to highlight that WASH infrastructure interventions are time-consuming. Hence, the study might not have been able to capture more changes in the school's infrastructure due to the short time of comparison (2020 and 2021). On that note, we suggest developing a new similar study once the data from the BNSC from 2022 to 2023 are available. Based on the results of this study we also recommend that further research should be conducted to: i) cross-check the reliability of the data from the BNSC, and if the data provided by this dataset really corresponds to the reality of schools; ii) complete the assessment of WASH in schools in Brazil with information regarding the fulfilment of the premises of the HRTWS and the presence of handwashing stations, water and soap available for handwashing, number of handwashing facilities, water drinking fountains, toilets and disability-friendly bathrooms; iii) investigate the origins of the inconsistencies reported in the data provided by the BNSC and the existence of an association between WASH in schools and school absence/dropout rates in Brazil; iv) assess the compatibility of WASH indicators adopted in Brazil (e.g., PLANSAB) and in other Latin American countries and the JMP service ladder and indicators;

Author contributions

KIHMP: conception of the research, data collection, data cleaning, analysis and writing; CA, JIB and JAM: overall design and revision.

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.2022.114069.

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