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A review of latrine front-end characteristics associated with microbial infection risk; reveals a lack of pathogen density data

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ABSTRACT

Unsafe sanitation accounts for an estimated 898,000 global deaths annually. The faecal pathogen transmission pathway is complex with several possible routes. Latrine front-end characteristics and usage behaviours are one key transmission pathway for microbial pathogens, however, there has not yet been a synthesis of the available research. This review aims to compare the microbial infection risks with latrine front-end components including any quantified microbial densities within the household latrines. This review was conducted with no restriction on the geographical location of the research. Of 118 studies reviewed, only ten (8%) have quantified the microbial density inside the household latrines compared to 109 (92%) measuring the infection risks. The reported risks were most frequent for specific bacterial (n = 34), and helminths infections (n = 32) compared to diarrhoea (n = 23), combined (n = 15), protozoan (n = 4), and viral (n = 4) infections. The infections risk decreased for using latrines lying at a higher position on the sanitation ladder (for example flush latrines) compared to those lying lower (for example pit latrines). The trend was similar for using floor materials that were easier to clean and less favourable for pathogen survival inside the latrines (for example, concrete as opposed to earth). Faecal coliforms were reported highest on the surface of the squat pan (743 CFU/cm^2) of pour-flush latrines and helminths on earth floors of pit latrines (1.5 eggs and larvae per gram of soil). Irrespective of latrine type and its position on the sanitation ladder, a dirty latrine, evidenced by a visible lack of cleanliness, significantly increased the risk for all infections. This study recommends that effective microbial infection risk reduction in latrines can be gained efficiently by ensuring washable surfaces and consistent cleaning practices. Future studies should include more rigorous measurements of microbial densities in various latrine types incorporating the different front-end components and usage behaviours.

1. Introduction

Unsafe sanitation is directly related to the transmission of infectious diseases, such as typhoid, and polio and diarrhoeal diseases such as cholera and dysentery (WHO, 2022). The global burden of disease study in 2016 estimated that unsafe sanitation alone accounted for 898,000 annual deaths and 41 million disability-adjusted life-years (DALYs) (Gakidou et al., 2017). While unsafe sanitation persists as a global problem, the highest disease burden is found in regions such as Sub-Saharan Africa and South-east Asia where only 31% and 51% of the population have basic sanitation services, respectively (Prüss-Ustün et al., 2019). The overall risk of diarrhoeal diseases and deaths related to sanitation is generally higher in low to medium Human Development Index (HDI) countries (Fagbamigbe et al., 2021; Riahi et al., 2018). Also, several neglected tropical diseases (NTDs) persist due to unsafe sanitation such as soil-transmitted helminths (STHs), trachoma, and schistosomiasis (WHO, 2022). The transmission of these infectious diseases occurs via faecal-oral routes (for example typhoid), poor hygiene (for example trachoma), and skin contact with contaminated soil or water (for example schistosomiasis). The recent global COVID-19 pandemic has further highlighted the need for safe sanitation systems, as the likelihood of COVID-19 infection increases via contaminated surfaces, aerosols, and faecal residue in sanitation facilities (Amoah et al., 2021; Sun and Han, 2021a). Thus, the microbial infection risk resulting from

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unsafe sanitation occurs due to several microorganisms or pathogens through multiple routes, extending beyond the faecal-oral route alone.

Under Sustainable Development Goal (SDG) 6, Clean Water and Sanitation, targets have been set under SDG 6.2 to provide adequate and equitable sanitation for all (United Nations, 2015b). The WHO/UNICEF Joint Monitoring Program (JMP) has categorised sanitation service levels as open defecation at the bottom, to unimproved, limited, basic and safely managed sanitation services at the top of the ladder (WHO and UNICEF, 2022). While improved sanitation is defined as a sanitation system that hygienically separates human excreta from human contact, safely managed sanitation is defined as using private improved sanitation facilities where human excreta is treated safely onsite or safely transported and treated offsite (WHO and UNICEF, 2022). Thus, SDG 6 has recognised safely managed sanitation which underlines the importance of safe disposal of excreta to reduce the risk of sanitation-related infections (WHO and UNICEF, 2015).

The transmission of sanitation-related pathogens can occur through multiple exposure pathways. Pathways for faecal-oral infections are described by the F diagram: fingers, food, fluids, fields and flies (Wagner and Lanoix, 1958). Contaminated fomites (inanimate objects and frequent human contact surfaces) are included as an additional transmission pathway in F-diagram due to their ability to transfer pathogens to other surfaces or environmental reservoirs (Julian, 2016; Stephens et al., 2019). An increase in access to improved sanitation facilities does not always guarantee the reduction in all sanitation-related infections as these interventions might not adequately break all pathogen transmission pathways (Odagiri et al., 2016). Although the safe disposal of faeces has been demonstrated to reduce the transmission of infectious diseases, it is also essential to assess the changes in faecal contamination of the surrounding environment (Berendes et al., 2017; Wagner and Lanoix, 1958). Several reviews have been conducted to evaluate and associate the impact of overall sanitation interventions with changes in infectious disease rates (Cairncross et al., 2010; Esrey et al., 1991; Stocks et al., 2014; Strunz et al., 2014). Similarly, SaniPath Exposure Assessment Tool, developed by Raj et al. (2020) is one of the largest tools to provide valuable data on the identification and quantification of human exposure to faecal contamination from multiple pathways. The SaniPath tool primarily focuses on nine environmental faecal exposure pathways in public domains such as drinking water, bathing water, surface water, ocean water, flood water, open drains, raw produce, street foods and public or shared latrines. However, it does not provide detailed information on specific sanitation characteristics and does not include exposure pathways in private spaces. Furthermore, there is a lack of comprehensive studies that quantify microbial infection risks with the actual microbial pathogen densities associated with specific sanitation hardware or characteristics. To our knowledge, no review has been conducted that links latrine front-end characteristics and usage behaviours with sanitation-related infections in household latrines. Also, the available literature on microbial densities of pathogens in latrines, including frequent touch surfaces, has not been collated.

The overall aim of this review was to examine the front-end characteristics of private and shared household latrines associated with human microbial infection risks. The specific objectives were a) to determine the state of knowledge of infection risk by microorganism type and measurable microbial densities within the latrine, b) to understand the relationship between different microbial infection risks and latrine characteristics, including latrine front-end components and usage behaviours, and c) to make recommendations on which latrine front-end characteristics can be modified to efficiently reduce the microbial infection risks.

2. Material and methods

2.1. Search strategy

This literature review was conducted using the Web of Science ™ database as the search engine. Only journal articles with full text in English were selected. The search was performed without any restriction on the geographical location of the research and the publication date till 12th January 2023. Keywords were set using the Boolean operators of the advanced search in the Web of Science. The keyword comprised three groups: the first included terms related to the latrine (toilet* OR sanitation* OR latrine*), the second included terms related to disease (disease* OR health OR risk OR infection* OR prevalence*) and the third excluded food.

In this review, the initial search identified 2077 articles in Web of Science all databases (Fig. 1). A total of 1351 articles were selected after discarding duplicate and non-relevant articles based on titles in preliminary screening. Primary screening was completed based on the relevance of the abstract, returning a total of 408 articles. Of these, 110 articles were selected after the secondary screening which involved reading the full text of the articles fulfilling the selection criteria detailed below. Additional articles relating to microbial loads within latrines (eight articles) were identified from screening the reference lists of journal articles identified in secondary screening. Finally, 118 articles were selected for analysis as per the study criteria mentioned below.

2.2. Selection criteria

The final selection criteria were studies that aimed at either quantifying the association of microbial infection risks with latrine characteristics (effect measured as ratios) or those that measured the concentration of microorganisms inside the private or shared household latrines. The selection of household latrines is driven by the lack of a comprehensive compilation of associated microbial infection risks specifically related to household latrines in the sanitation literature. This is particularly significant as household latrines, given their frequent use, provide an optimal environment for risk microbial infections within households. This review includes the key sanitation-related symptomatic infections such as diarrhoea, specific bacterial infections, viral infections, and protozoan infections. It further includes studies that have measured the risks of combined infections or health conditions such as acute respiratory infections, gastrointestinal diseases, parasitic infections, reproductive tract infections and ulcerative colitis. For microbial densities, the focus of this study was to identify research reporting the concentrations of microorganisms explicitly inside the front-end of household latrines or their associated components and surfaces, while excluding those only reporting the presence or absence of microorganisms. Therefore, studies measuring the concentrations of microorganisms on fomites other than household latrine surfaces, as well as on stool samples, faecal sludge and air samples inside latrines were excluded. Only studies that quantify faecal indicator bacteria (faecal coliforms and *Escherichia coli*), helminths, and bacterial pathogens were included. Also, the studies that only measured the infection risks with latrine availability or access were excluded as the risks based on the mere presence or absence of latrines did not provide sufficient depth for analysis. In addition, studies only comparing the microbial infection risks for open defecation practices, child faeces disposal, presence of flies and mosquitos in the latrine, personal hygiene and household hygiene have been excluded as they did not establish any direct association with specific latrine hardware or characteristics. Studies assessing the risks of nutritional outcomes such as stunting, underweight, overweight and shortness were outside the scope of this review as these health outcomes can have several other confounding factors (Momberg et al., 2021; Vilcins et al., 2018). Summarising the overall morbidity and mortality through meta-data analysis was not attempted in this review as published data was limited.

2.3. Data extraction

The data was collated in tables in Microsoft Excel under two broad categories and five sub-categories. The two broad categories were based

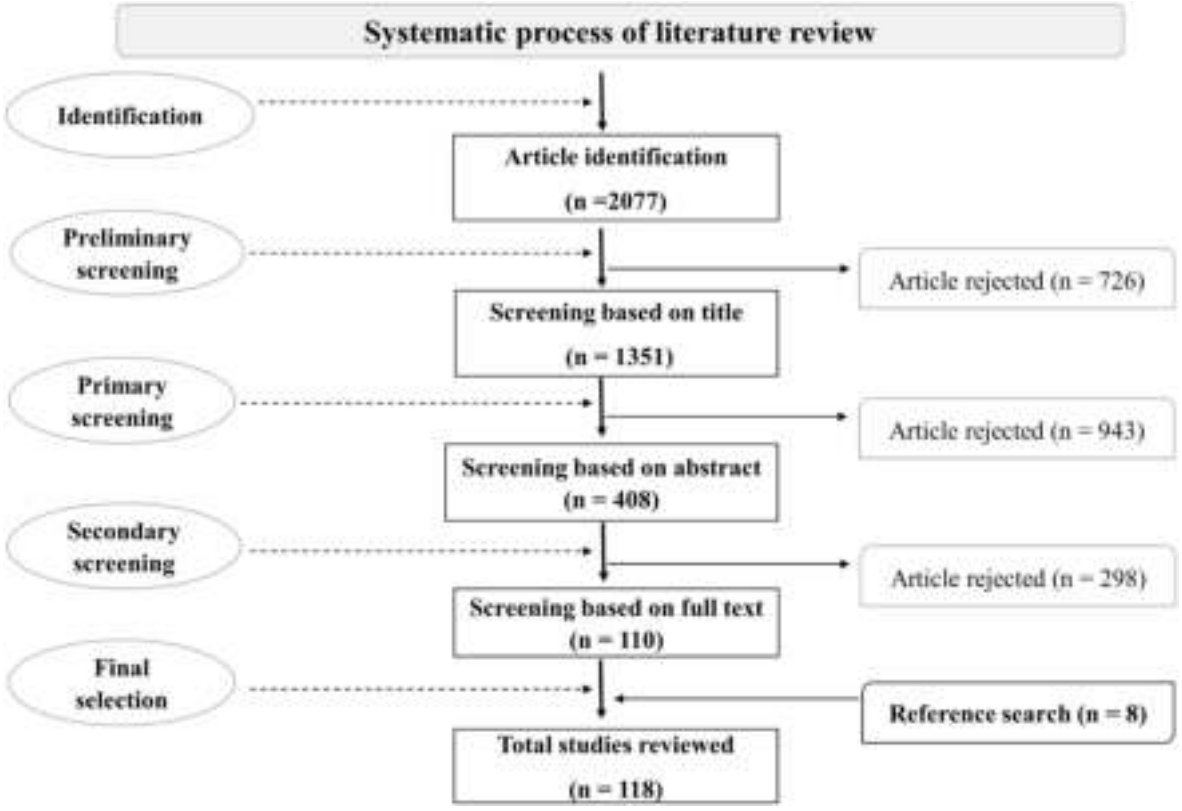


Fig. 1. Schematic of the stepwise process followed for literature review with a total of 118 articles reviewed.

on whether articles had quantified the association of microbial infections (with effect measure presented as ratios) or articles with measure of microbial densities inside the latrine. Additionally, six subcategories included sanitation service types, latrine types, front-end components, latrine usage behaviours, latrine ownership and latrine distance from the household. The sanitation service types include categorisation based on overall unimproved or improved latrines. Latrine types include categorisation based on both front and back-end types reported in the literature. The front-end system or user interface comprises the place where human excretion occurs (Fig. 2). The choice of technologies used in the latrine front-end in any given context is influenced by factors such as water availability for flushing, users' preferences and behaviours, local materials availability and compatibility with the subsequent back-end systems (Tilley et al., 2014). Front-end systems are categorised into two main types based on flush systems: water-based systems (such as cistern-flush or pour-flush) requiring a regular supply of water, and dry or non-flush systems (such as pit latrines with holes in the ground connected directly to the

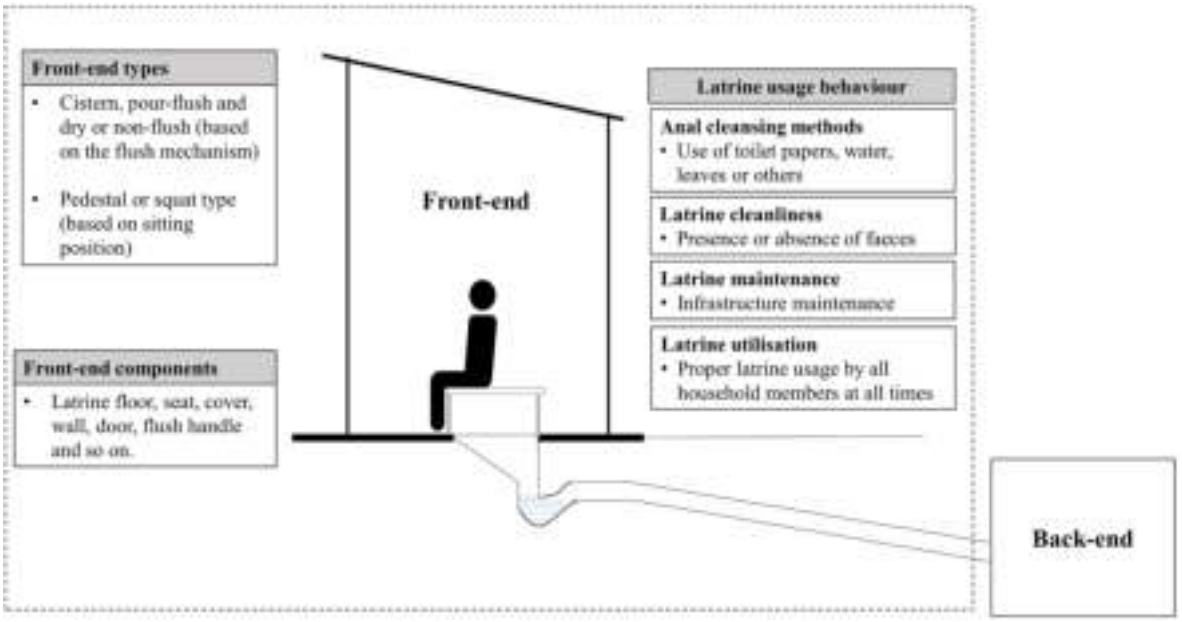


Fig. 2. Schematic diagram of latrine front-end (dotted box) indicating the front-end types, front-end components, and latrine usage behaviours.

pit) which operate without the water. Water-based systems can be further classified as cistern-flush systems (with automated flush) and pour-flush systems (requiring manual flush by pouring water from buckets). Depending on the user's sitting preference, front-ends include options such as a pedestal-type (with a raised pedestal on which the user can sit) or a squat-type (with a squat pan or holes in the ground over which the user can squat) (Thomas and Gold, 2020). The back-end system consists of components that contain, treat, and dispose of human excreta such as simple pits and septic tanks. Front-end components include all hardware components the users interact with during latrine use such as the latrine floor, superstructure, anal cleansing, and flushing hardware. Information on latrine usage behaviours was further categorised as anal cleansing methods, latrine cleanliness, latrine maintenance, and frequency of latrine utilisation.

To compare the state of the literature on quantifying the association of microbial infections with latrines, standardisation in classification terminology was needed. The associations between microbial infections and sanitation systems were reported primarily in terms of odds ratios (OR) or risk ratios (RR) in the literature. Data on both unadjusted (unadj) and adjusted (adj) ratios with confidence interval (CI) were assembled from the studies reviewed, irrespective of their statistical significance as reporting non-significant results is important (Halsey, 2019), particularly while looking at the overall trend between microbial infection risks and latrine characteristics. The microbial infection risks corresponding to the sub-categories were then presented as the increased or decreased risk. In addition to summarising risk ratios in tables, a forest plot was created for those studies assessing the risk of microbial infections for improved or unimproved latrines. The improved and unimproved sanitation, if not explicitly mentioned in the literature, is considered using the SDGs or Millennium Development Goals (MDGs) definition, depending on the time frame of the study. MDG had categorised sanitation service level as improved if the facility hygienically separated faeces from human contact and is not shared with other households and the rest was categorised as unimproved (United Nations, 2015a). However, SDG has categorised sanitation services into open defecation, unimproved, shared, basic and safely managed sanitation (United Nations, 2021). For the SDGs basic and safely managed are both considered improved. Due to the inconsistency in terminologies for describing the latrine types and the lack of explicit definitions for latrine cleanliness, maintenance and utilisation, these terms had to be interpreted and were matched to appropriate categories based on the available information. Specifically, how studies reported the latrine usage frequency by households was not consistent, hence analysis was not performed for the number of users or usage rates per day. Furthermore, the HDI index as defined by UNDP (2022) has been used in this review for comparing the countries in terms of development. Countries are categorised based on HDI scores as low (< 0.550), medium (< 0.550 to 0.699), high (0.700 to 0.799) and very high (> 0.800) (UNDP, 2022).

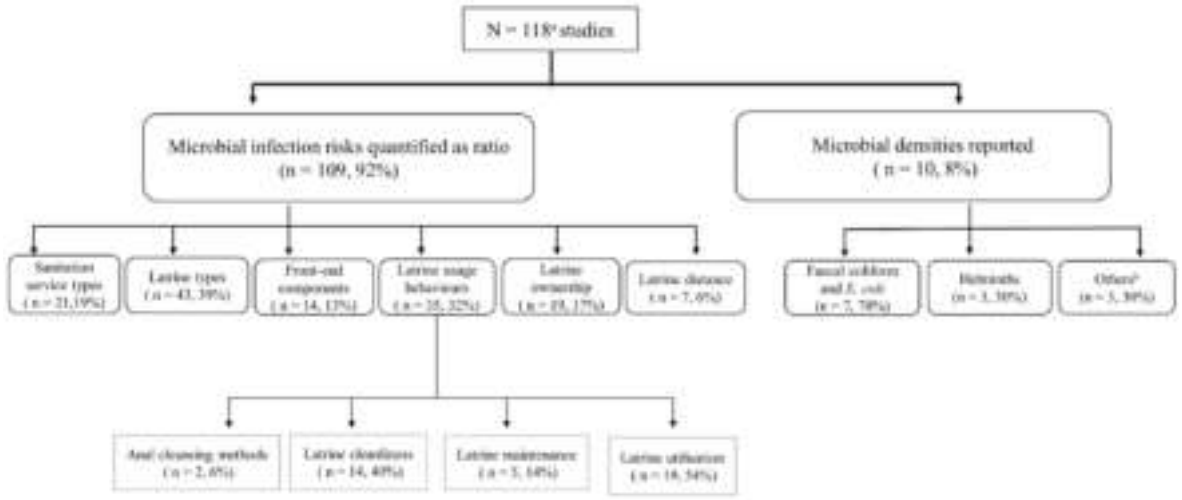
3. Results

3.1. Characteristics of the studies

Out of 118 studies, the majority of them are from countries with low to medium HDI (n = 97, 82%) compared to countries with high (n = 16, 14%) and very high HDI (n = 3, 3%) (UNDP, 2022). Among these 69 were from the African continent, with more than half from Ethiopia (n = 36). Others were conducted in Asia (n = 35), South America (n = 7), Central America (n = 2), Pacific Islands (n = 2) and North America (n = 1). This indicates a geographical bias towards studies in Africa and especially Ethiopia which received distinct attention.

Of 118 studies, 109 (92%) reported the latrine-associated microbial infections quantified in terms of odds or risk ratios, compared to only 10 studies (8%) quantifying the density of the microorganisms actually sampled from the latrines (Fig. 3). This indicates a significant information gap in the quantification of microbial densities inside the household latrine. Of 109 studies reporting risks, information on latrine types (n = 43) and latrine usage behaviours (n = 35) were more numerous as compared to sanitation service types (n = 21), latrine ownership (n = 19), front-end components (n = 14), and latrine distance from the household (n = 7). Furthermore, of the 35 studies with latrine usage behaviours, only two reported anal cleansing methods and five reported latrine maintenance compared to latrine utilisation (n = 19) and latrine cleanliness (n = 14). This shows a further gap in studies assessing the microbial infection risks with the choice of anal cleansing methods and latrine maintenance.

The type of microbial infections reported in the studies (with risk) ranged from symptomatic infections such as diarrhoea to helminths,



[a] Sum of the categories or sub-categories does not add up to the total number of studies as one study has data on multiple categories or subcategories.
[b] *Listeria monocytogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Fig. 3. Characteristics of the 118 studies included in this review with two broad categories based on the quantified microbial infection risks as ratios and microbial densities reported inside the front-end of household latrines.

other bacterial, protozoan, viral and combined infections. Of 109 studies with risks, the most explored were bacterial infections (n = 34, 31%), with half of them for trachoma (n = 17). Studies also reported helminths (n = 33, 30%), diarrhoea (n = 23, 21%), combined infections (n = 15, 14%), protozoa (n = 4, 4%) and viral infections (n = 4, 4%) (Table 1). For studies measuring the actual concentration of microorganisms inside latrines (10 studies), faecal indicator bacteria (faecal coliforms and *E. coli*) were reported the most (seven studies) compared to helminths (three studies) and other causative agents of bacterial infections (three studies) such as *Listeria monocytogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

3.2. Risk of microbial infections with unimproved and improved sanitation

In general, compared to not having any facilities having unimproved sanitation was associated with a reduced risk of infections such as diarrhoea (Soboksa, 2021) and schistosomiasis (Cha et al., 2019) (Table 2). Stepping up the sanitation ladder, unimproved sanitation compared with improved sanitation is associated with higher odds of infections such as diarrhoea (Getachew et al., 2018; Getahun and Adane, 2021; Saha et al., 2022), hookworm (Phillips et al., 2022), schistosomiasis (Murenjekwa et al., 2021), and trachoma (Abdala et al., 2018; Mengistu et al., 2022; Seyum et al., 2022; Shimelash et al., 2022; Taleo et al., 2017). These trends can be seen on the forest plot (Fig. 4) where having unimproved latrines (compared to improved latrines) increase the risk of infections while improved latrines have protective effects compared to the unimproved ones. For instance, Getahun and Adane (2021) reported the likelihood of acute diarrhoea was around four times (adj OR = 3.57, 95% CI = 1.64 to 6.51) more for unimproved household latrines compared to an improved one. Further, the odds of trachoma for households with unimproved latrines, as opposed to improved ones, were reported as high as five times (adj OR = 5.18, 95% CI = 1.95 to 13.69) (Shimelash et al., 2022). This is consistent with the overall protective effect of improved latrines (compared to unimproved or no latrines) for *Campylobacter* spp. (Amour et al., 2016), diarrhoea (Akter et al., 2022), schistosomiasis (Cha et al., 2019), STHs (Benjamin-Chung et al., 2015) and trachoma (Garn et al., 2018; Muhammad et al., 2014). This highlights the effectiveness of improved sanitation in separating faeces from human contact, thereby decreasing the risk of exposure to various microbial infections. Two studies did not follow this trend; Brainard et al. (2018) reported having improved sanitation (as opposed to unimproved) with higher odds of typhoid and Alemayehu et al. (2020a) reported unimproved sanitation (compared to improved) with a protective effect for diarrhoea. While no detailed explanation of this effect has been provided by the authors, this might be related to latrine usage behaviours as the likelihood of exposure to faeces increases if the latrine is not hygienic even if it is improved.

3.3. Risk of microbial infections with latrine types and front-end components

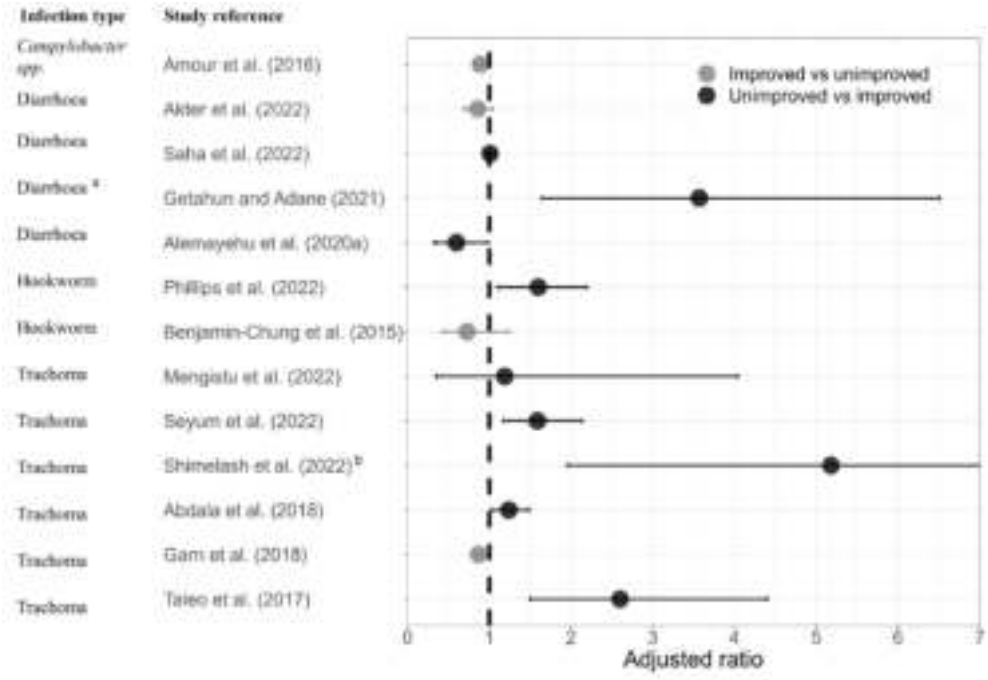
The risk of microbial infections with different latrine types was quantified in 43 studies with the highest number of studies reporting helminth infections (17 studies) (Table 1). There are consistent results for nearly all studies reporting the infection risks for latrine types. Latrine types that have a higher position on the sanitation ladder (compared to those lying lower) were protective against several infections such as diarrhoea (Table 3), other specific bacterial, helminths, protozoan, viral, and combined infections (Table S1 – S5). For instance, overall having a flush latrine (as opposed to a pit latrine or no latrine) was protective against diarrhoea (Iwashita et al., 2022; Nasir et al., 2020), STHs (Anunobi et al., 2019; Asaolu et al., 2002; Opara et al., 2021; Riess et al., 2013; Ster et al., 2021), typhoid (Brainard et al., 2018) and ulcerative colitis (Sood et al., 2014). Households with flush latrines were reported to have a 34% (adj IRR = 0.66, 95% CI = 0.49 to 0.88) lower risk of diarrhoea as compared to those having pit latrines (Iwashita et al., 2022). This trend complemented studies that found an increased risk for households using latrine types lying lower on the sanitation ladder as opposed to ones lying higher. For example, the risk of STHs infection was around three times (adj OR = 2.92, 95% CI = 1.75 to 4.88) (Table S2) for households with pit latrines but was protective for households using flush latrines (adj OR = 0.43, 95% CI = 0.25 to 0.73) (Opara et al., 2021). Similarly, Hlaing et al. (2016) reported the odds of having gastrointestinal diseases (combination of diarrhoea, dysentery and other related diseases) was about nine times (adj OR = 8.66, 95% CI = 4.03 to 18.59) (Table S5) for households with non-water seal latrines (compared to water seal latrines).

Regarding the front-end components, the presence of the latrine superstructures, roofs, slabs, drop-hole covers, and vent pipes was overall found to be protective against nearly all microbial infections reported across studies. For instance, the presence of a drop-hole cover in the pit latrine was found to be protective against bacterial infections (Knee et al., 2018) and STHs (Steinbaum et al., 2019), which aligns with an increased risk of not having a drop-hole cover for diarrhoea (Getahun and Adane, 2021), protozoan infections (Dagne and Alelign, 2021) and STHs (Alemu et al., 2022; Goshu et al., 2021). Similarly, household latrines with superstructure and roof were found to have a reduced risk for diarrhoea (Cha et al., 2017; Soboksa et al., 2019), other bacterial, protozoan and viral infections (Knee et al., 2018) as compared to not having superstructure. The presence of latrine slabs (as opposed to the absence of latrine slabs) was protective against several infections such as diarrhoea (Adane et al., 2017), protozoan infections (Knee et al., 2018), schistosomiasis (Olamiju et al., 2022), STHs (Steinbaum et al., 2019), and viral infections (Knee et al., 2018). For example, Exley et al. (2015) found the presence of slab (compared to the absence of slab) in household latrines associated with 82% (adj OR = 0.18, 95% CI = 0.10 to

Table 1
Summary of the number of studies that have quantified the microbial infection risk based on sub-categories with types of infections. Full details of the collated studies are provided in Tables 2 – 3 and Tables S1–S5.

Sub-categories	No. of studies within each sub-category (n)	Diarrhoea (n)	Bacterial infections (n)	Helminth infections (n)	Protozoan infections (n)	Viral infections (n)	Combined infections (n)
Improved and unimproved sanitation	21	9	10	5	0	0	0
Latrine types	43	7	11	17	5	4	5
Front-end components	14	4	2	6	2	1	0
Latrine usage behaviours	35	10	11	8	0	0	0
Latrine ownership	19	7	8	2	1	0	1
Latrine distance from the household	7	4	4	1	1	0	3
Sub-total	109[a]	23[a] (21%)[b]	34[a] (31%)[b]	33[a] (30%)[b]	4[a] (4%)[b]	4[a] (4%)[b]	15[a] (14%)[b]

[a] Sum of the number of studies with information on sub-categories and infection types does not add up to the total number of studies measuring risk and infection types reported. This is because some studies have information on multiple sub-categories and infections.
[b] Percentage calculated for a total of 109 studies measuring microbial infection risk.



[a] acute diarrhoea.
[b] the maximum upper 95% confidence interval is equal to 13.69, while the current plot only displays till 7.

Fig. 4. Forest plot showing the microbial infection risk for improved and unimproved latrines. The grey colour represents the ratio estimated for improved as compared to unimproved latrines, while the black colour represents the ratio for unimproved compared to improved latrines. Only the adjusted ratios are plotted. The horizontal bar represents the corresponding 95% confidence interval of the ratios. The vertical dashed line represents the adjusted ratio of one (no effect). Further details of the studies are provided in Table 2.

0.34) lower odds of *E. coli* (Table S1). Further, washable, and quick-drying materials were found to lower the risk of infections. For example, the risk of helminth infections was four times (adj OR = 3.97, 95% CI = 1.01 to 7.25) higher for households using latrines built with earth or mud as opposed to those with concrete (Nath et al., 2022) (Table S2). Further, Dumba et al. (2008) reported an overall higher risk of all STHs for latrines with earthen or wooden slabs (adj OR = 1.32, 95% CI = 0.76 to 2.32) compared to concrete slabs.

On the other side, few studies have reported a reverse trend of increased microbial infection risks for having certain types of latrines compared to not having any latrines at all. For instance, households with hanging latrines were found to have an increased risk of Shigellosis infections compared to those without a latrine (Ahmed et al., 1994). Similarly, Corrales et al. (2006) reported significantly elevated risks for *Ascaris lumbricoides* (adj OR = 15.50, 95% CI = 3.30 to 74.80) (Table S2) and *Trichuris trichiura* (adj OR = 7.10, 95% CI = 3.00 to 17.10) in households using double-vault desiccating eco-san latrines as compared to those without any latrine. Further, some exceptions were observed where latrines lying at lower positions on sanitation ladder had a lower risk of microbial infections compared to those lying higher. For example, Nasir et al. (2020) reported a reduced risk of diarrhoea associated with the use of traditional dry latrines (adj OR = 0.83, 95% CI = 0.78 to 0.89) (Table 3) compared to pit latrines in Afghanistan. This was explained by the improved design of the traditional dry latrines which were constructed with an adequate distance from the residence and water source, unlike the pit latrines that were close to the households and posed a risk for contamination of water sources. Similarly, flush latrines with septic systems were associated with an increased risk of typhoid as compared to pit latrines (Brainard et al., 2018). However, no further details were provided to contextualise this result.

3.4. Risk of microbial infections with latrine usage behaviours

The microbial infection risks associated with various latrine usage behaviour were reported in 35 studies with bacterial infections most commonly reported (Table 1). Latrine usage behaviours included information on anal cleansing methods, latrine cleanliness, latrine maintenance and latrine utilisation. For the anal cleansing method, the odds of having diarrhoea were elevated by three times (adj OR = 3.02, 95% CI = 1.19 to 7.64) for using leaves and two times (adj OR = 2.08, 95% CI = 0.96 to 4.51) (Table 3) for using paper as compared to water (Soboksa et al., 2019). It was attributed to poor storage and management of the toilet papers and leaves after use, increasing the risk of contact with faeces. Furthermore, the presence of toilet paper and water for flush had reduced the risk to *Shigella* spp. compared to not having them, which highlights the importance of the presence of a sufficient quantity of water and cleansing material in the latrine (Chompook et al., 2006) (Table S1).

Latrine cleanliness, especially a dirty latrine with the presence of faeces in or around the latrine, is identified as a significant risk factor for several infections namely, diarrhoea (Adane et al., 2017; Alemayehu et al., 2020b; Baye et al., 2021; Getachew et al., 2018; Getahun and Adane, 2021; Mekonnen et al., 2019; Natnael et al., 2021), trachoma (Last et al., 2014), typhoid (Brainard et al., 2018), STHs (Asfaw et al., 2020; Fenta et al., 2022; Steinbaum et al., 2019), and reproductive tract infection (Ademas et al., 2020). Out of 15 studies assessing the risk of latrine cleanliness, seven studies alone have identified dirty latrines (compared to clean latrines) as associated with higher odds of getting diarrhoea. For instance, Adane et al. (2017) reported the odds of diarrhoea four times (adj OR = 3.90, 95% CI = 1.50 to 10.30) (Table 3) higher for households with dirty latrines as compared to the clean ones. This is also consistent with studies finding reduced risk infections such as diarrhoea (Soboksa et al., 2019) and STHs (Fenta et al., 2022) for using regularly cleaned latrines as compared to dirty ones. The higher odds for several infections for the visible presence of faeces in or around the latrines is likely due to increased exposure to faeces directly while visiting the latrine and more chances of contamination of food, water, and the surrounding areas in households through mediums such as flies (Alemayehu et al., 2020b; Baye et al., 2021).

In addressing latrine maintenance, only six studies have assessed microbial infection risks associated with infrastructural up-keep such as proper sub and superstructures, intact roofs, and overall functionality of

Table 2
Summary of studies assessing the risk of microbial infections with unimproved and improved sanitation.

Name of infections or microorganisms	Sanitation type	Risk in reference to	Study settings	Location	Estimate type	Unadjusted (unadj) ratio (95% CI)	Adjusted (adj) ratio (95% CI)	Study Reference
Increased risk								
Diarrhoea	Unimproved	Improved	Rural	India	OR	1.13 (1.1 – 1.17) **	1.01 (0.96 – 1.07)	Saha et al. (2022)
Diarrhoea[a]	Unimproved	Improved	Peri-urban	Ethiopia	OR	4.93 (1.94 – 9.50) **	3.57 (1.64 – 6.51) **	Getahun and Adane (2021)
Diarrhoea	Unimproved	Improved	Rural	Ethiopia	OR	1.20 (0.66 – 2.17)		Getachew et al. (2018)
Hookworm	Unimproved		Rural	Ethiopia	OR	1.60 (1.10 – 2.20) **	1.60 (1.10 – 2.20) **	Phillips et al. (2022)
Schistosomiasis[b]	No improved latrine	Improved	Rural	Zimbabwe	OR	1.13 (0.91 – 1.40)		Murenjekwa et al. (2021)
Trachoma	Unimproved	Improved	Rural	Ethiopia	OR	1.11 (0.33 – 3.68) ***	1.19 (0.35 – 4.04)	Mengistu et al. (2022)
Trachoma	Unimproved	Improved	Urban, rural	Afghanistan	OR	1.03 (0.76 – 1.39)		Salam et al. (2022)
Trachoma	Unimproved	Improved		Ethiopia	OR	2.12 (1.59 – 2.82) ***	1.59 (1.17 – 2.14) ***	Seyum et al. (2022)
Trachoma	Unimproved	Improved		Ethiopia	OR	7.86 (3.60 – 17.30)	5.18 (1.95 – 13.69) **	Shimelash et al. (2022)
Trachoma	Unimproved	Improved	Rural	Mozambique	OR	1.21 (1.07 – 1.37) ***	1.24 (1.03 – 1.49) ***	Abdala et al. (2018)
Trachoma	Unimproved	Improved		Vanuatu	OR	2.40 (1.40 – 3.90) **	2.60 (1.50 – 4.40) **	Taleo et al. (2017)
Trichuris trichiura	Improved	Unimproved	Rural	Bangladesh	PR	0.93 (0.75 – 1.14)	1.03 (0.84 – 1.27)	Benjamin-Chung et al. (2015)
Typhoid	Improved	Unimproved[c]	Urban	DR Congo	OR	3.85 (0.36 – 44.23)		Brainard et al. (2018)
Decreased risk								
Ascaris lumbriscoides	Improved	Unimproved	Rural	Bangladesh	PR	0.78 (0.59 – 1.04)	0.91 (0.67 – 1.24)	Benjamin-Chung et al. (2015)
Campylobacter spp.	Improved			Multiple countries[d]	RR		0.89 (0.81 – 0.97)	Amour et al. (2016)
Diarrhoea	Improved	Unimproved	Urban, rural	Bangladesh	PR	0.89 (0.73 – 1.09)	0.86 (0.69 – 1.05)	Akter et al. (2022)
Diarrhoea[a]	Unimproved	Improved	Peri-urban	Ethiopia	OR	0.98 (0.39 – 2.48)		Natnael et al. (2021)
Diarrhoea	Improved	No latrine	Urban, rural	Ethiopia	OR	1.10 (0.93 – 1.36)	0.92 (0.72 – 1.18)	Soboksa (2021)
Diarrhoea	Unimproved	No latrine	Urban, rural	Ethiopia	OR	0.86 (0.75 – 0.98) *	0.91 (0.69 – 1.19)	Soboksa (2021)
Diarrhoea	Unimproved	Improved	Rural	Ethiopia	OR		0.60 (0.33 – 0.99) *	Alemayehu et al. (2020a)
Hookworm	Improved	Unimproved	Rural	Bangladesh	PR	0.60 (0.37 – 0.97)	0.73 (0.43 – 1.24)	Benjamin-Chung et al. (2015)
Schistosomiasis[e]	Unimproved	No latrine	National survey	Sudan	OR		0.88 (0.82 – 0.93) ***	Cha et al. (2019)
Schistosomiasis[e]	Improved	No latrine	National survey	Sudan	OR		0.45 (0.41 – 0.51) ***	Cha et al. (2019)
Trachoma	Improved	Unimproved		Multiple countries[f]	PR		0.87 (0.83 – 0.91) **	Garn et al. (2018)
Trachoma	Improved	Unimproved		Nigeria	OR	0.62 (0.55 – 0.70) ***		Muhammad et al. (2014)

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001,
OR = Odds Ratio, PR = Prevalence Ratio, RR = Risk Ratio.
[a] Acute diarrhoea,
[b] *Schistosoma haematobium*,
[c] Pit latrine,
[d] Bangladesh, India, Nepal, Pakisthan, South Africa, Tanzania, Brazil, and Peru,
[e] *S. haematobium* or *S. mansoni*,
[f] 13 countries: Cote d'Ivoire, Egypt, Guinea, Malawi, Yemen, Nigeria, Vanuatu, Ethiopia, Lao People's Democratic Republic, Solomon Islands, Democratic Republic of Congo, Mozambique, and Benin.

Table 3
Summary of studies assessing the risk of diarrhoea with latrine types, front-end components, usage behaviour, ownership, and distance from the household.

Information sub-categories	Latrine characteristics	Risk in reference	Study settings	Location	Effect estimate type	Unadjusted (unadj) ratio (95% CI)	Adjusted (adj) ratio (95%CI)	Study Reference
Increased risk								
Latrine types	Pit latrine	Flush latrine	Urban	Kenya	HR	2.44 (2.22 – 2.69)	1.44 (1.19 – 1.74) ***	Deichsel et al. (2020)
	Dry latrine	Septic tank	Urban	Cote d'Ivoire	OR	1.80 (1.20 – 2.70) **		Kouame et al. (2014)
	Latrine with septic tank	Connected to sewer	Urban	Mexico	OR	1.70 (1.00 – 2.93) *		Cifuentes et al. (2002)
	Sanitary latrine[a]	Non-sanitary latrine	Rural	Bangladesh	OR	1.59 (1.34 – 1.88) **		Myaux et al. (1997)
Latrine front-end components	Cover absent in drop-hole[b]	Cover present	Peri-urban	Ethiopia	OR	1.78 (0.76 – 3.50)		Getahun and Adane (2021)
Latrine usage behaviours: anal cleansing	Anal cleanse with paper	Anal cleanse with water		Ethiopia	OR		2.08 (0.96 – 4.51)	Soboksa et al. (2019)
	Anal cleanse with leaf	Anal cleanse with water		Ethiopia	OR		3.02 (1.19 – 7.64) *	Soboksa et al. (2019)
Latrine usage behaviours: cleanliness	Dirty latrine[b]	Clean latrine	Urban	Ethiopia	OR	2.46 (1.84 – 3.93) ***	1.37 (1.21 – 3.50) ***	Baye et al. (2021)
	Dirty latrine[b]	Clean latrine	Peri-urban	Ethiopia	OR	2.12 (1.13 – 4.95) **		Getahun and Adane (2021)
	Dirty latrine[b]	Clean latrine	Peri-urban	Ethiopia	OR	2.72 (1.35 – 5.52) **	3.34 (1.34 – 8.31) **	Natnael et al. (2021)
	Dirty latrine	Clean latrine	Urban, rural	Ethiopia	OR	4.45 (2.45 – 8.10) *	2.92 (1.38 – 6.19) *	Alemayehu et al. (2020b)
	Dirty latrine[b]	Clean latrine	Urban, rural	Ethiopia	OR	1.27 (0.98 – 1.64)	1.09 (0.82 – 1.46)	Mekonnen et al. (2019)
	Dirty latrine	Clean latrine	Urban, rural	Ethiopia	OR	1.96 (1.07 – 3.60) *	2.46 (1.20 – 5.04) *	Getachew et al. (2018)
	Dirty latrine[b]	Clean latrine	Urban	Ethiopia	OR	7.20 (3.10 – 16.91) *	3.90 (1.50 – 10.30) *	Adane et al. (2017)
	Clean latrine	Dirty latrine	Rural	DR Congo	OR	1.08 (0.67 – 1.74)	1.09 (0.63 – 1.89)	Cha et al. (2017)
Latrine usage behaviours: utilisation	Improper latrine utilisation	Proper latrine utilisation	Rural	Ethiopia	OR	2.13 (0.99 – 4.57)	2.01 (0.89 – 4.52)	Degebasa et al. (2018)
Latrine ownership	Shared latrine	Private latrine	Rural	India	OR	1.29 (1.21 – 1.37) **		Saha et al. (2022)
	Shared latrine[b]	Private latrine	Urban	Ethiopia	OR	1.54 (0.95 – 2.50)		Baye et al. (2021)
	Shared latrine[b]	Private latrine	Peri-urban	Ethiopia	OR	1.01 (0.39 – 2.28)		Natnael et al. (2021)
	Shared latrine	Private latrine	Urban	Kenya	HR	2.15 (1.99 – 2.33)	1.30 (0.88 – 1.91)	Deichsel et al. (2020)
	Shared latrine	Private latrine	Urban, rural	Ethiopia	OR	0.61 (0.34 – 1.08)	1.09 (0.58 – 2.07)	Getachew et al. (2018)
	Shared latrine (≥ six households)[b]	Shared latrine (one to five households)	Urban	Ethiopia	OR	4.10 (2.30 – 7.30)	4.70 (2.40 – 9.40)	Adane et al. (2017)

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Table 3 (*continued*)

Information sub-categories	Latrine characteristics	Risk in reference	Study settings	Location	Effect estimate type	Unadjusted (unadj) ratio (95% CI)	Adjusted (adj) ratio (95%CI)	Study Reference
Latrine distance from the household	Latrine distance within 500 m	Latrine distance <500 m	Rural	India	OR		0.57 (0.23 – 1.37)	Giri et al. (2022)
	Latrine distance <15 m[b]	Latrine distance >15 m	Peri-urban	Ethiopia	OR	1.18 (0.64 – 1.68)		Getahun and Adane (2021)
	Latrine distance <15 m[b]	Latrine distance >15 m	Urban	Ethiopia	OR	6.20 (2.70 – 14.70)		Adane et al. (2017)
Decreased risk								
Latrine types	Pit latrine	Open defecation	Rural	India	OR		0.75 (0.33 – 1.68)	Giri et al. (2022)
	Pour-flush latrine	Open defecation	Rural	India	OR		0.91 (0.46 – 1.79)	Giri et al. (2022)
	Flush latrine	Pit latrine	Rural	Vietnam	IRR	0.69 (0.51 – 0.92) **	0.66 (0.49 – 0.88) **	Iwashita et al. (2022)
	Traditional dry latrine	Pit latrine	Urban, rural	Afghanistan	OR	0.81 (0.75 – 0.87) ***	0.83 (0.78 – 0.89) ***	Nasir et al. (2020)
	Flush latrine	Pit latrine	Urban, rural	Afghanistan	OR	0.87 (0.79 – 0.97) *	0.84 (0.75 – 0.94) **	Nasir et al. (2020)
Front-end components	Slab absent[b,c]	Slab present	Urban	Ethiopia	OR	0.90 (0.40 – 1.90)		Adane et al. (2017)
	Superstructure present	Superstructure absent	Rural	DR Congo	OR	0.30 (0.13 – 0.66) *	0.35 (0.14 – 0.88) *	Cha et al. (2017)
	Superstructure present[c]	Superstructure absent[c]		Ethiopia	OR		0.97 (0.59 – 1.59)	Soboksa et al. (2019)
Latrine usage behaviours: cleanliness	Latrine cleaned regularly	Latrine not cleaned regularly		Ethiopia	OR		0.36 (0.19 – 0.66) *	Soboksa et al. (2019)
Latrine usage behaviours: maintenance	Functional latrine[d]	Non–functional latrine	Rural	Ethiopia	OR	0.37 (0.15 – 0.89) *	0.69 (0.23 – 2.07)	Anteneh and Kumie (2010)
Latrine usage behaviours: utilisation	Proper latrine utilisation[e]	Improper latrine utilisation	Rural	Ethiopia	OR	0.38 (0.17 – 0.87) *	0.63 (0.22 – 1.81)	Anteneh and Kumie (2010)
Latrine ownership	Private latrine	Shared latrine	Peri-urban	Ethiopia	OR	0.64 (0.42 – 1.02)		Getahun and Adane (2021)
Latrine distance from the households	Latrine distant less than 15 m from home[b]	Latrine distant 15 m or more from home	Peri-urban	Ethiopia	OR	0.97 (0.48 – 1.96)		Natnael et al. (2021)

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

HR = Hazard Ratio, OR = Odds Ratio, IRR = Incidence Risk Ratio.

[a] With fence and platform,

[b] Acute diarrhoea,

[c] For pit latrine,

[d] Latrine with sub and superstructures providing services even if it required maintenance,

[e] Households with functional latrines and child faeces disposed in a latrine, no observable faeces in and/or latrine and the compound and the presence of clear footpath to the latrine is uncovered with grasses or other barriers of walking.

Table 4
Concentration of faecal coliforms and helminths reported inside the latrines and their components.

Microorganisms	Sanitation service types	Latrine types	Sample location	Study settings	Location	Analysis method	Media used	Concentrations reported in studies (Converted unit)	Study Reference
Faecal coliforms[a]									
E. coli	Improved	Cistern, Pour-flush latrine	Slab and seat	Urban	Nepal	Membrane filtration	Brilliance™[b]	0.2 CFU/cm^{2}[i]	McGinnis et al. (2019)
E. coli	Improved	Cistern, Pour-flush latrine	Spray and bucket handle	Urban	Nepal	Membrane filtration	Brilliance™[b]	0.4 CFU/cm^{2}[i]	McGinnis et al. (2019)
E. coli	Improved	Cistern, Pour-flush latrine	Door handle	Urban	Nepal	Membrane filtration	Brilliance™[b]	0 CFU/cm^{2}[i]	McGinnis et al. (2019)
E. coli	Improved	Cistern flush latrine	Flush handle	Urban	Nepal	Membrane filtration	Brilliance™[b]	0.08 CFU/cm^{2}[i]	McGinnis et al. (2019)
E. coli	Unimproved		Hand contact points	Urban, rural	Tanzania	Membrane filtration	m-coliblue24[c]	137.1 CFU/100 ml[j]	Exley et al. (2015)
E. coli	Improved		Hand contact points	Urban, rural	Tanzania	Membrane filtration	m-coliblue24[c]	17.7 CFU/100 ml[j]	Exley et al. (2015)
E. coli	Unimproved	Pit latrine without a slab	Hand contact points	Urban, rural	Tanzania	Membrane filtration	m-coliblue24[c]	154 CFU/100 ml[j]	Exley et al. (2015)
E. coli	Improved	Pit latrine with slab	Hand contact points	Urban, rural	Tanzania	Membrane filtration	m-coliblue24[c]	7.6 CFU/100 ml[j]	Exley et al. (2015)
E. coli	Improved	Pour-flush latrine	Hand contact points	Urban, Rural	Tanzania	Membrane filtration	m-coliblue24[c]	17 CFU/100 ml[j]	Exley et al. (2015)
E. coli	Improved	Pit latrine	Concrete Slab	Peri-urban	Tanzania	Membrane filtration	MI agar[d]	1.5 log CFU/g[h] (31.6 CFU/g)	Pickering et al. (2012)
E. coli	Unimproved	Pit latrine	Earthen Slab	Peri-urban	Tanzania	Membrane filtration	MI agar[d]	2.3 log CFU/g[h] (199.5 CFU/g)	Pickering et al. (2012)
E. coli	Unimproved	Pit latrine	Latrine wall	Peri-urban	Tanzania	Membrane filtration	MI agar[d]	0.2 log CFU/cm^{2}[h] (1.6 CFU/cm^{2})	Pickering et al. (2012)
E. coli	Improved	Pour-flush latrine	Squat pan	Rural	Cambodia	Petri film Plate	3M Petrifilm plate[e]	3 CFU/cm^{2}[h]	Sinclair and Gerba (2011)
E. coli	Improved	Pour-flush latrine	Latrine floor	Rural	Cambodia	Petri film Plate	3M Petrifilm plate[e]	2 CFU/cm^{2}[h]	Sinclair and Gerba (2011)
E. coli	Improved	Pour-flush latrine	Ladle handle	Rural	Cambodia	Petri film Plate	3M Petrifilm plate[e]	<1 CFU/cm^{2}[h]	Sinclair and Gerba (2011)
E. coli	Improved	Cistern flush latrine	Doorknob (inside toilet), floor mat, floor, wall, toilet paper holder, hand towel, sink, flush lever, washlet control panel, toilet seat, rim, slippers, diaper pail	Urban	Japan	Agar stamp method	XM-G agar medium[f]	0 CFU/10 cm^{2} (0 CFU/cm^{2})	Ojima et al. (2002a)
E. coli	Improved	Cistern flush latrine	Toilet mat, paper holder and sink	Urban	Japan	Agar stamp method	XM-G Agar medium[f]	1 – 9 CFU/10 cm^{2} (0.1 – 0.9 CFU/cm^{2})[k]	Ojima et al. (2002b)
Faecal coliform	Improved	Pour-flush latrine	Ladle handle	Rural	Cambodia	Spot plate	Difco mFC agar	114 CFU/cm^{2}[h]	Sinclair and Gerba (2011)
Faecal coliform	Improved	Pour-flush latrine	Squat pan	Rural	Cambodia	Spot plate	Difco mFC agar	743 CFU/cm^{2}[h]	Sinclair and Gerba (2011)
Faecal coliform	Improved	Pour-flush latrine	Latrine floor	Rural	Cambodia	Spot plate	Difco mFC agar	395 CFU/cm^{2}[h]	Sinclair and Gerba (2011)
Faecal coliform	Improved	Cistern flush latrine	Latrine floor	Urban	United States	Spread plate	Difco mFC agar	−1.33 log CFU/cm^{2}[j] (0.05 CFU/cm^{2})	Rusin et al. (1998)
Faecal coliform	Improved	Cistern flush latrine	Flush handle	Urban	United States	Spread plate	Difco mFC agar	0.27 log CFU/cm^{2}[j] (2 CFU/cm^{2})	Rusin et al. (1998)
Faecal coliform	Improved	Cistern flush latrine	Toilet seat	Urban	United States	Spread plate	Difco mFC agar	−1.55 log CFU/cm^{2}[j] (0.03 CFU/cm^{2})	Rusin et al. (1998)

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Table 4 (*continued*)

Microorganisms	Sanitation service types	Latrine types	Sample location	Study settings	Location	Analysis method	Media used	Concentrations reported in studies (Converted unit)	Study Reference
Helminths [g]									
Ascaris spp. and hookworm	Unimproved	Pit latrine without a slab	Drop hole	Urban, rural	Tanzania	Sedimentation and flotation		0.20 egg/g[i]	Exley et al. (2015)
Ascaris spp. and hookworm	Unimproved	Pit latrine with a broken slab	Drop hole	Urban, rural	Tanzania	Sedimentation and flotation		0.34 egg/g[h]	Exley et al. (2015)
Ascaris spp.	Unimproved	Pit latrine	Drop hole	Rural	Tanzania	Centrifugation and flotation		0 – 0.2 egg/g[k]	Baker and Ensink (2012)
Hookworm	Unimproved	Pit latrine	Drop hole	Rural	Tanzania	Centrifugation and flotation		0 – 38 (egg and larvae)/g[k]	Baker and Ensink (2012)
Overall helminths	Unimproved	Pit latrine	Drop hole	Rural	Tanzania	Centrifugation and flotation		1.5 eggs and larvae/g[h]	Baker and Ensink (2012)
Taenia spp.	Unimproved	Pit latrine	Drop hole	Rural	Tanzania	Centrifugation and flotation		0 – 0.3 egg/g[k]	Baker and Ensink (2012)
Ascaris spp.		Non-flush latrine	Latrine floor	Urban, peri-urban	Brazil	Centrifugation and flotation		0.6 egg/g[i]	Schulz and Kroeger (1992)
Ascaris spp.		Flush latrine	Latrine floor	Urban, peri-urban	Brazil	Centrifugation and flotation		1 egg/g[i]	Schulz and Kroeger (1992)

CFU = colony forming units, mFC = membrane Faecal Coliform, egg/g = eggs per gram of dry soil.

[a] Surface swab samples,

[b] Total Coliforms/*E. coli* Selective Agar, Oxoid Microbiology Products, Cheshire, United Kingdom,

[c] HACH, Loveland, USA,

[d] Two enzyme substrates, the fluorogen 4-Methylumbelliferyl-$-Dgalactopyranoside (MUGal) and a chromogen Indoxyl-$-D-glucuronide (IBDG),

[e] 3M, St Paul, MN, USA,

[f] Nissui Pharmaceuticals Co., Ltd (Tokyo, Japan,

[g] Soil samples,

[h] Arithmetic mean.

[i] Median.

[j] Geometric mean,

[k] Range.

latrines. Overall, having a latrine, irrespective of its maintenance, was found to reduce the risk of microbial infections such as enteric pathogens (Baker et al., 2018), Enterococci (Baker et al., 2018) and trachoma (Montgomery et al., 2010a) as compared to not having latrines at all. Moreover, the magnitude of risk was lower for the latrines that were maintained properly compared to poor ones which indicates the protective effect of well-maintained latrines (Baker et al., 2018; Montgomery et al., 2010a). This is also consistent with increased risk of STHs (adj OR = 1.90, 95% CI = 1.17 to 3.10) (Table S2) reported for using latrines in poor infrastructural condition (compared to larine in good condition) (Dumba et al., 2008). Similarly, the use of a functional latrine was protective against the risk of diarrhoea compared to a non-functional one (Anteneh and Kumie, 2010). This can be due to the lower motivation of household members to use the non-functional latrine with failed infrastructure.

For latrine utilisation, the microbial infection risks were accessed in 19 studies. Overall, households not utilising the latrine properly or not having one (compared to proper latrine utilisation) have an increased risk of infections such as diarrhoea (Anteneh and Kumie, 2010; Degebasa et al., 2018), *Campylobacter* spp. (Lengerh et al., 2013), trachoma (Abdilwohab and Abebo, 2020; Ayelgn et al., 2021; Ketema et al., 2012; Nigusie et al., 2015; WoldeKidan et al., 2019), helminth infections (Alemu et al., 2022; Fetene et al., 2021; Goshu et al., 2021; Khieu et al., 2014; Nigo et al., 2021) and intestinal parasitic infections (Hailu et al., 2020; Tulu et al., 2014). For instance, Nigusie et al. (2015) reported the risk of trachoma as high as 10 times (adj OR = 10.27, 95% CI = 4.28 to 24.99) (Table S1) for households not using latrines properly. Although the definition of proper latrine utilisation was not consistent or not provided in all studies, a logical deduction can be made that the failure to always use the latrine properly, such as excreta not being deposited in a drop-hole or bowl, even by one household member might elevate the microbial infection risks to other members of the family.

3.5. Risk of microbial infections with latrine ownership and distance from the household

Latrine ownership was classified as the individual (private) household latrines or shared with the neighbours. Microbial infection risks with latrine ownership were reported in 18 studies. Overall, sharing a latrine with other households or not having a private latrine (compared to having a private latrine) increased the risk of diarrhoea (Baye et al., 2021; Deichsel et al., 2020; Getachew et al., 2018; Natnael et al., 2021; Saha et al., 2022) (Table 3), bacterial infections such as bacterial vaginosis (Das et al., 2021) and trachoma (Mengistu et al., 2022; Montgomery et al., 2010b; Seyum et al., 2022) (Table S1), helminths (Abebe et al., 2011; Geleto et al., 2022; Hall et al., 1994) (Table S2), and protozoan infections (Matthys et al., 2011) (Table S3). Also, the risk of infections such as diarrhoea (Adane et al., 2017) and trachoma (Montgomery et al., 2010b) was elevated with an increase in the number of households sharing latrines. For instance, the risk of acute diarrhoea was about five times (adj OR = 4.70, 95% CI = 2.40 to 9.40) (Table 3) elevated for households sharing a latrine with six or more households. This might be due to the high chances of shared latrines being unhygienic because of shared ownership and more users per latrine, compared to private household latrines. On the contrary, Hestvik et al. (2010) reported the risk of *Helicobacter pylori* around four times (adj OR = 3.70, 95% CI = 1.50 to 9.30) (Table S1) more for households with private latrines than shared latrines, which was attributed to a spurious association by authors.

The microbial infection risks with latrine distances from the household were reported in only seven studies. Two studies, Giri et al. (2022) and Natnael et al. (2021) have reported a decreased risk of diarrhoea for having latrines less than 15 m and within 500 m from home, respectively. Further, having a latrine inside the household was associated with lower odds of parasitic infections (Palmeirim et al., 2021) as opposed to the one outside. This aligns with the increased risk of intestinal microsporidiosis reported as six times higher (adj OR = 6.20, 95% CI = 1.50 to 25.90) (Table S5) for households without indoor latrines (meaning no latrine at all) compared to having one (Al-Brhami et al., 2022). The authors attributed this increased risk to the absence of latrines, leading households to rely on public latrines that may have potentially contaminated surfaces such as door handles and water taps (Al-Brhami et al., 2022). Further, Coles et al. (2009) reported an increased risk for acute respiratory infections for households whose latrines were distant and not located within their property. On the contrary, Getahun and Adane (2021) and Adane et al. (2017) have reported an increased risk for acute diarrhoea if latrines were located in closer proximity (<15 m) to households. While the details on latrine proximity were not discussed by Getahun and Adane (2021), Adane et al. (2017) have attributed this elevated risk to possible pathogen transmission via vectors such as flies as faeces were observed in over half of the latrines studied.

3.6. Microbial densities within latrines

The microbial densities inside the household latrines were measured only in ten studies (out of 118 studies) (Table 4). The fieldwork for these studies was undertaken in seven countries, with three from Tanzania and one each from Brazil, Cambodia, Japan, Nepal, Peru, and the United States. Out of ten studies, concentrations of faecal coliforms or *E. coli* were reported in seven studies (Exley et al., 2015; McGinnis et al., 2019; Ojima et al., 2002a, 2002b; Pickering et al., 2012; Rusin et al., 1998; Sinclair and Gerba, 2011), while three studies measured concentration of helminth eggs (Baker and Ensink, 2012; Exley et al., 2015; Schulz and Kroeger, 1992). Three studies measured other bacterial loads such as *Listeria monocytogenes* (Canales et al., 2019), *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Ojima et al., 2002a, 2002b). Further, the majority of studies used culture methods for microbial quantification, except for one study by Pickering et al. (2012) which conducted both culture and molecular microbial quantification. Of the ten studies measuring concentration, flush latrines (cistern or pour-flush) were reported in six and pit latrines (with or without slab) were reported in only four studies. In reporting the latrine surfaces for faecal coliform bacteria and *E. coli*, most of them reported frequent touchpoints by users' hands and feet, when seated or squatted during latrine usage. These surfaces include latrine seats, floor, ladle handles, flush handles, and door handles. On the other hand, the latrine floor (where feet are placed) and the surface near the latrine drop hole were reported for helminths.

3.6.1. Faecal coliforms and E. coli

The sample size for all studies measuring the concentration of faecal coliforms was generally small with 20 latrines or less. Two studies had higher sample numbers; Ojima et al. (2002b) researched with 86 latrines and Exley et al. (2015) with 341 latrines. Of seven studies measuring concentrations of faecal coliform or *E. coli*, six measured the concentration in various human contact surfaces of flush (cistern and pour) latrines and two in pit latrines (with or without slab) (Table 4). Surface swab samples from different human contact surfaces of latrines were collected in most of the studies (four studies). On the other hand, two studies (Ojima et al., 2002a, 2002b) used the agar stamp method, where agar plates were directly contacted to various latrine surfaces, whereas one study by Pickering et al. (2012) collected the soil samples from the latrine floor. For the enumeration of *E. coli*, membrane filtration method was used by three studies (Exley et al., 2015; McGinnis et al., 2019; Pickering et al., 2012), the direct stamp method by two studies (Ojima et al., 2002a, 2002b) and 3M Petrifilm plates method by one study (Sinclair and Gerba, 2011). A spot plate method (Sinclair and Gerba, 2011) and a spread plate method (Rusin et al., 1998) were used for two studies measuring faecal coliform bacteria. All studies assessing faecal coliforms or *E. coli* on latrine surfaces typically measured their densities as Colony Forming Units (CFU) per square centimetre (McGinnis et al., 2019; Ojima et al., 2002a, 2002b; Sinclair and Gerba, 2011), with some

studies expressing the density in logarithm scale (Pickering et al., 2012; Rusin et al., 1998). However, Exley et al. (2015), used CFU per 100 ml of eluted sample due to difficulties in swabbing small hand contact points such as door handles. The swabbed area for each surface varied among the studies. McGinnis et al. (2019) averaged from 100 cm^2 for pit latrine walls to 10 cm^2 for flush handles. Smaller surfaces such as handles of doors, flush, and buckets were swabbed through the entire surface, and their average areas were estimated. Sinclair and Gerba (2011) sampled a smaller surface area of 4 cm^2 for all latrine surfaces (ladle handle, squat pan, and latrine floor). Rusin et al. (1998) sampled a larger area of 929 cm^2 for the latrine floor, but the area for latrine seats and flush handles was not specified. Further, both Ojima et al. (2002a, 2002b) measured *E. coli* on various latrine surfaces using a 10 cm^2 stamp agar plate. The microbial density data was reported in Table 4, both in its original unit as the author presented it and was transformed into CFU per cm^2 for standardisation.

The overall concentrations of *E. coli* decreased when the quality of the pit latrines was improved. For instance, Pickering et al. (2012) observed six times lower *E. coli* concentrations on the soil samples collected from on top of or adjacent to the concrete slab of Tanzanian pit latrines (31.6 CFU/g of soil) compared to pit latrines with the earthen floor (199.5 CFU/g of soil), although the difference was not significant. Similar results were reported in Tanzania where, *E. coli* concentrations in frequent hand contact points of the latrines were nine to 20 times higher in pit latrines without slab compared to pit latrines with slab and pour-flush latrines, respectively (Exley et al., 2015). This highlights the importance of washable latrine surfaces in reducing faecal contamination. Regarding flush latrines, the concentrations of *E. coli* and faecal coliform bacteria were found to be generally higher in pour-flush compared to cistern flush latrines. The average concentration of *E. coli* on the slab and the seat of flush (cistern or pour-flush) latrines ranged between 0 and 3 CFU/cm^2, whereas the overall range in surfaces of flush, ladle, bucket or spray bidet handles was reported within 0 to 23 CFU/cm^2 across two studies conducted in Nepal (McGinnis et al., 2019) and Cambodia (Sinclair and Gerba, 2011). Further, in Japanese cistern flush latrines, no *E. coli* was detected on nearly all latrine surfaces (Ojima et al., 2002a) except a lower level (0.1 to 0.9 CFU/cm^2) detected on toilet mats, paper holders and sinks (Ojima et al., 2002b). This lower to no detection of *E. coli*, in this case, was attributed to the common practice of wiping latrine surfaces using cleaning agents in Japan.

For faecal coliform bacteria, the average concentration on the flush latrine (pour-flush and cistern flush) seats and slabs ranged between 0.03 and 743 CFU/cm^2, and 2 to 114 CFU/cm^2 on flush and ladle handles across the latrines in Cambodia (Sinclair and Gerba, 2011) and United States (Rusin et al., 1998). However, the level of faecal coliform bacteria was considerably high in pour-flush (squat) compared to cistern flush latrines (pedestal) within these two studies. However, caution must be taken while comparing these latrines and their surfaces of different study settings and usage behaviour.

3.6.2. Helminth eggs

The sample size for studies measuring the helminth concentrations ranged between 20 and 167 latrines. Of three studies reporting helminth concentrations, two were conducted in Tanzania and one in Brazil (Table 4). Simple pit latrines were reported in all three studies and only one study reported the flush latrines. In all studies, soil samples were taken from the surfaces near drop holes or latrine floors where the feet are placed while using the latrine. The sedimentation and flotation techniques were used for analysis in all studies. In general, the range of helminth concentrations was reported higher with latrines lying lower on the sanitation ladder compared to those lying higher. The range of STHs was 0 to15 eggs/g of soil in Tanzanian pit latrines without a slab, with a mean of 0.2 eggs/g of soil (Exley et al., 2015). Another study from Tanzania in a similar type of pit latrine reported the range of STHs as 0 to 38 eggs and larvae per gram of soil, with an average of 1.5 eggs and larvae per gram of soil (Baker and Ensink, 2012). The higher range of helminth concentrations reported in Baker and Ensink (2012) compared to Exley et al. (2015) is likely due to the inclusion of larvae as well. However, it can be also due to differences in sampling seasons, study settings and user behaviours across the two studies.

The results were consistent for hookworms in two Tanzanian studies, where hookworms were the most frequently reported compared to other helminths such as *Ascaris* spp. and *Taenia* spp. On the other hand, some contradictions of higher helminth densities were reported in latrines with roofs or latrines lying higher on the sanitation ladder compared to those without roofs or lying lower on the sanitation ladder. For instance, a lower density of helminth eggs was reported in pit latrines without a roof compared to ones with a roof, likely due to inactivation and desiccation caused by exposure to sunlight (Baker and Ensink, 2012). Also, a study by Schulz and Kroeger (1992) found the concentration of *Ascaris* spp. higher in the in-house built flush latrines floors (1 egg/g) compared to non-flush ones (0.6 egg/g). This was attributed to the storage of used toilet paper in an open basket in the flush latrines which might increase the risk of exposure to faeces. This emphasises that a latrine can be contaminated even if it is of the highest quality, if not maintained hygienically.

3.6.3. Other bacterial contaminations

Only three studies reported other bacterial densities, aside from faecal coliforms and helminths. From Peru, the concentration *of Listeria monocytogenes* was reported in the range of 0.21 to 260 MPN/10 cm^2 on latrine surfaces (flush handles and toilet surfaces) (Canales et al., 2019). However, further information on the latrine types and associated surfaces were not provided to contextualise this finding. A study conducted by Ojima et al. (2002a) in Japanese cistern flush latrines did not detect *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the various latrine surfaces (doorknob, floor mat, floor, wall, toilet paper holder, sink, flush lever, toilet seat, toilet rim, toilet slipper, washlet control panel). The authors discussed the bias implications of additional cleaning activities by household prior to sampling. Another study, by Ojima et al. (2002b) in a similar setting, detected a higher level (1 to 9.9 CFU/cm^2) of *S. aureus* in latrine surfaces such as toilet paper holders and toilet bowls compared to other surfaces (light switch, doorknob, toilet mat, wall, sink, faucet handle, flush lever, toilet seat and toilet slipper), where concentration ranged from 0.1 to 0.9 CFU/cm^2. In addition, *P. aeruginosa* was only detected in the toilet seat and inner doorknob, in low concentration (0.1 to 0.9 CFU/cm^2) but not detected in other latrine surfaces (light switch, floor, wall, sink faucet handle, flush lever, toilet bowl, toilet slippers). This all shows that the latrine surfaces which are cleaned less frequently (such as the doorknob and toilet paper holder) could be the potential area for contamination.

4. Discussion

4.1. Study summary and geographical distribution

One of the main findings of this review is that studies quantifying the association of microbial infections as odds or risk ratios (n = 109, 92%) were ten times more numerous than studies with field-based measurement of the concentrations of microorganisms within the household latrine (n = 10, 8%). The much higher portion of studies with reported risk ratios is likely due to the epidemiological focus which aims to determine the prevalence and risk factors associated with infectious diseases (outcome variable), where sanitation or latrine type is included as one of many other risk factors. Also, the sanitation research assessing the impact of sanitation intervention has traditionally focused on specific health outcomes rather than pathogen transmission pathways (Sclar et al., 2016). On the other hand, measuring the concentrations of microorganisms is dependent on the availability of laboratory equipment, reagents, and skilled human resources (Chuang et al., 2011; Vujcic et al., 2014). Apart from these resources, the quantification of microbial density is also subjected to considerable time and logistical requirements

for sample collection, transportation, and processing. This presents a barrier to measuring microbial concentrations, particularly in the areas of low or medium HDI countries which have limited laboratory services (Holcomb and Stewart, 2020; Kirschke et al., 2020; Wright et al., 2014).

Of those studies reporting the microbial infection risks with latrines, there was a clear bias towards studies estimating the risks with latrine types (n = 43, 39%) and usage behaviour (n = 35, 32%) compared to latrine ownership (n = 19, 17%), front-end components (n = 14, 13%), and latrine distance from the household (n = 7, 6%). This could be linked to the MDG and SDG definition of improved sanitation which focuses on latrine type rather than the quality of individual components or overall latrine cleanliness and maintenance (Exley et al., 2015). Another plausible explanation for the limited information on latrine ownership and distance from a household might be a smaller body of evidence linking these factors to the microbial infection risks especially compared to not having access to a latrine at all. Another reason may be due to survey data being based only on interviewees' responses and without investigating sanitation facilities by direct field observation and assessing latrine distance or position from the household. A similar limitation was found in the review conducted by Ziegelbauer et al. (2012) which specifically examined the association between sanitation availability and use in STH infections. The review revealed that instead of direct field observations, studies often relied on questionaries to assess the usage and availability of sanitation facilities.

There is a logical skew towards some geographical regions, namely African and Asian countries with low to medium HDI, that still have major challenges in the sanitation sector specifically in achieving SDG 6.2. The high frequency of studies from Ethiopia can be attributed to the strong involvement of Ethiopian universities in research and development in public health. Most studies from Ethiopia included in this review were found to be affiliated with and funded by the Ethiopian university sector. This corroborates the important role of national universities in the acceleration of sanitation research in low and medium HDI countries. Hence, the collaboration between researchers, governments and other stakeholders such as the private sector and donors can enhance the understanding of contextual challenges based on evidence of health outcomes and identify locally sustainable interventions for protecting public health (Sinharoy et al., 2019).

4.2. Latrine types and front-end components

We found a general trend that sanitation-related microbial infections were reduced when using sanitation or latrine types that lie higher on the sanitation ladder (for example flush latrines compared to pit latrines without water seals). This trend was also evident in the reported microbial density data, where the average *E. coli* concentration in hand contact points of pour-flush latrines was found almost an order of magnitude lower compared to pit latrines without slab (Exley et al., 2015). The protective effect of flush latrines is due to the presence of water-seal in the latrine which creates a barrier for vectors such as flies to get in contact with the faeces (Mara, 1985; Stenström et al., 2011). However, having a flush latrine is generally dependent on regular access to water (either stored in containers or running water), which further aids in keeping the latrine hygienic. The availability of latrine types that lie higher on the sanitation ladder, such as a flush latrine, likely reflects higher socioeconomic status compared to a household with a non-flush or pit latrine with limited access to sanitation facilities (Deichsel et al., 2020). This review's findings are in-line with previously conducted systematic reviews and meta-analyses where the overall protective effect of improved sanitation (compared to unimproved or open defecation) was reported for sanitation-related health outcomes (Freeman et al., 2017; Strunz et al., 2014; Wolf et al., 2014). However, these reviews have a different focus from our study as they examined the impact of overall water, sanitation, and hygiene access on STHs, diarrhoeal infections and sanitation impacts on infection diseases and nutrition. On the other hand, Sclar et al. (2016) found little to no effect of sanitation improvement on the overall faecal-oral transmission pathways, suggesting the need for sanitation assessments that investigate the effect of sanitation interventions on multiple transmission pathways such as fingers, food, fluids, fields and fomites.

It is interesting to note that faecal contamination levels were high on the surfaces of pour-flush latrines (squat) latrines in Cambodia, with faecal coliforms level of 743 CFU/cm^2 (Sinclair and Gerba, 2011). This high concentration on the surface of pour-flush squat pans was attributed to grooves present in the foot placing of squat flush latrines, which elevated the chances of small water pool formation and enhanced pathogen persistence and potential transmission. A previous study by Mahdavinejad et al. (2011) also found that the presence of pathogens was more frequent in squat-flush latrines compared to pedestal-flush latrines in Iran. A review by Sun and Han (2021a) has also stated an elevated risk of transmission of faecal pathogens and COVID-19, from squat-flush latrines as opposed to pedestal cistern flush, due to splashes generated during open flushing mechanisms and faecal residual retained on footrest grooves. Luo et al. (2023) have also concluded the high risk of microbial infections from squat-flush latrines located at a public building in China due to flush-generated aerosols deposited on the latrine surfaces. Although squat-flush latrines are widely used by countries in Asia (for example, China, India, and Japan), Central America and Africa (Luo et al., 2023; Sun and Han, 2021a), limited studies have thoroughly assessed or compared the risks of microbial infections associated with such latrines. Thus, there is a need for detailed studies on potential pathogen transmission pathways within squat-flush latrines as these pathways can vary depending on the type of flush mechanisms and sitting configurations used.

The importance of the presence of front-end components such as latrine slabs, cover (drop–hole) and superstructure, in creating the barrier to separating faeces from human contact was underlined in multiple studies (Table 1). Within these front-end components, lower microbial infection risks were reported for using materials that were easier to clean and less favourable for pathogen growth. This trend was also evident in microbial densities where lower concentrations of *E. coli* were reported on latrine surfaces such as latrine walls and floors constructed with brick (compared to ones with earth, palm, or grass) and concrete (compared to earth floor), respectively. Apart from that, having a proper latrine superstructure such as a brick wall or roof and door that gives privacy and security, might encourage the proper and consistent use of latrines by all household members with dignity (Sinha et al., 2017). Washable surfaces facilitate quick drying, minimising the survival and transmission of pathogens, unlike moisture-retaining surfaces such as earth or wood (Dumba et al., 2008). This aspect particularly needs more attention for STHs such as hookworm, as larvae can directly penetrate human skin from contaminated floors (Bethony et al., 2006). In addition, having washable floors allows for convenience in cleaning the latrine surfaces which might be contaminated while usage. Hence, further interventions are required to upgrade latrine floors and promote the importance of having a clean latrine.

As would be expected there were a few anomalies where studies reported an increased risk of microbial infections for using specific latrine types (compared to having no latrines) or for latrines at higher positions on the sanitation ladder (Ahmed et al., 1994; Brainard et al., 2018; Corrales et al., 2006) or protective effect of latrine lying lower on sanitation ladder (Nasir et al., 2020). These variations can be attributed to factors such as unsafe faeces disposal and the reuse of human faeces in agriculture. For example, the use of hanging latrines in Bangladesh posed significant environmental and health hazards as they directly discharge faeces into water bodies and thereby elevating the risk of Shigellosis (Ahmed et al., 1994). Similarly, the use of eco-san latrines in El Salvador was associated with a significant risk of STHs due to inadequate pathogen treatment in the biosolids used for agricultural purposes (Corrales et al., 2006). This highlights the need for effective pathogens inactivation in human faeces before promoting nutrient recycling in agriculture. Therefore, it is essential to consider the overall

sanitation improvements that ensure safe disposal practices and safe methods for nutrient recycling, apart from just focusing on latrine infrastructures along with improved or unimproved classifications.

4.3. Latrine usage behaviour

Dirty latrines, with the visible observation of faeces in or around the latrines, were found to be a major predictor of negative health impacts related to sanitation, regardless of the study setting and latrine type (Table 3). The presence of faeces in or around latrines and households is a long-known predictor for negative health impacts for sanitation-related infectious diseases (Bartlett et al., 1992). The presence of faeces inside the latrines can occur due to lack of adequate water (in flush latrines) (Saxton et al., 2017), improper management of anal cleansing materials (such as disposal of used toilet papers in an open bin) or simply due to user's behaviours (for example, faeces not dropped on latrine hole or bowl). These conditions can create an environment similar to open defecation (Luo et al., 2023), elevating the risk of exposure and spreading faeces to other areas in the household (Steinbaum et al., 2019). Multiple studies have also found that households with clean latrines (compared to dirty latrines) are more likely to use them properly, as cleanliness reduces odour and motivates consistent usage (Omer et al., 2022; Woyessa et al., 2022). This current review also strengthens the statement that even latrines positioned higher on the sanitation ladder may pose the risk of microbial infections if they are not kept clean. As an example, flush latrine floors reported higher concentrations of *Ascaris* spp. compared to non-flush latrine floors in Brazil (Schulz and Kroeger, 1992). The authors attributed this to the practice of storing used toilet paper in open containers within flush latrines, which increases the risk of microbial transmission. Similar practices of disposing of used toilet papers in open containers within flush latrines have also been noted in other studies (Kratzke et al., 2014; Sun and Han, 2021b). These findings highlight the importance of considering latrine usage behaviours in various cultural settings while designing sanitation and hygiene interventions, as they can influence the pathways of pathogen transmission. Also, the lower microbial densities observed on cistern flush latrine surfaces in Japan (Ojima et al., 2002b) and the United States (Rusin et al., 1998) were due to regular cleaning practices involving wiping surfaces with disinfecting agents. These finding emphasises the need to implement interventions that focus on educating users about maintaining latrine cleanliness even for those with upgraded latrine infrastructure.

There is a research gap in incorporating and comparing the risk of various anal cleaning methods in sanitation literature. The choice of anal cleansing materials and methods varies with different contexts, cultures, and sanitation practices around the world (Garg and Singh, 2016). Lack of adequate anal cleansing materials and their proper management can lead to higher faecal contamination on the user's hand as well as on latrine surfaces such as walls and floors (McMahon et al., 2011). This can further impact the contamination of latrine surfaces due to the interaction between the user's hand and latrine hardware during or after the anal cleansing process. This is supported by a few studies that have examined faecal contamination levels on the surfaces of anal cleansing hardware such as spray bidets, bucket handles, ladle handles and toilet paper holders (Table 4). It is interesting to note that in Nepal, higher level of faecal contamination was observed on the anal cleansing hardware (spray bidet and bucket handle) compared to other latrine surfaces such as floors or seats. A plausible explanation is that most people in Nepal cleanse via washing, where one of the user's hands comes in contact with faeces directly during the process of anal cleansing with water. This increases the risk of faecal contamination on these surfaces. This finding concurs with the previous study by Han et al. (1986) in Myanmar where concentration of faecal coliform were reported remarkably higher (range: 0 to 10^7 CFU) and more frequent on the hands of mothers of under-five children who used water as anal cleansing compared to those using papers (range: 0 to 15 CFU). Although handwashing with soap and water is effective for decontaminating hands, a rational deduction can be that the contaminated hand after defecation can contaminate the latrine surfaces such as door handles, flush handles, and others, before any hand washing, especially if the handwashing station is located outside the latrine. A recent study by Abney (2022) has also identified touching the toilet entrance door, seat and exit door without handwashing as the highest risk scenario for the likelihood of viral infections (adenovirus, SARS-CoV-2, and norovirus) in the cistern-flush latrines. Furthermore, unsafe handling of anal cleansing materials by an individual might pose risks to subsequent users (Stenström et al., 2011). Previous studies (Iyo et al., 2016; Kanayama Katsuse et al., 2017; Tsunoda et al., 2019) assessing the microbial contamination on anal cleansing hardware such as the surface of bidet nozzles (automatic nozzle fitted on toilet seat delivering a water jet to clean the anus after defecation) have detected bacteria such as *E. coli* and *P. aeruginosa* and *S. aureus*. However, those studies were mainly conducted in hospital settings in Japan, where automatic bidet toilet seats are common (Tsunoda et al., 2019). Anal cleansing needs are often neglected in sanitation interventions and are incorporated rarely in sanitation literature (McMahon et al., 2011), especially in low to medium-HDI countries. Han et al. (1986) reported around four times higher risk of diarrhoea and dysentery in under-five children in Myanmar whose mothers used water for anal cleansing compared to using paper. Although the association was not statistically significant, it indicates that the choice of anal cleansing materials can play an important role in faeces-to-hand contamination. Thus, incorporating anal cleansing methods is essential to understand the faecal pathogen transmission pathways from different types of latrines.

4.4. Latrine ownership and distance from the household

This review found 19 studies (Table 1) estimating the microbial infection risks with latrine ownership. The findings from this review of increased risk associated with sharing latrines with neighbours shared concur with the previous review by Heijnen et al. (2015). This elevated risk of shared latrines is attributed to dirty latrines compared to privately owned ones as people lack the motivation to use and clean shared latrines responsibly (Oduro-Kwarteng et al., 2009; Taleo et al., 2017). Exley et al. (2015), in contrast, found significantly lower *E. coli* concentration on frequent hand contact surfaces of shared latrines (9 CFU/100 ml) compared to private ones (18 CFU/100 ml) in Tanzania and the contamination levels were observed to decrease further with an increasing number of households and users sharing the latrines. Although Exley et al. (2015) didn't explicitly mention the reasons for this finding, it can be inferred that households sharing the latrines in that particular study context were maintaining the latrines clean. While several other factors can vary with the study setting, caution should be taken while comparing these studies. Apart from that, vulnerable groups such as women and children are less likely to use shared or communal latrines during the night due to safety or violence concerns (Biran et al., 2011; Kwiringira et al., 2014) which limits their sustainable use.

With only seven studies, a gap was articulated in studies assessing the microbial infection risks with latrine distance from the household (Table 1). Limited information on latrine distance from the household might owe to the focus of retrieved studies, which are inclined more to epidemiology compared to sanitation-specific field studies. Overall, indoor latrines or latrines lying in closer proximity to households (within 15 m or 500 m) had a reduced risk of microbial infections compared to those located far from households. Latrines inside the house are more likely to be clean to avoid the odour nuisance (Palmeirim et al., 2021). Also having a latrine within the household compound or in proximity to the household was reported to have a higher likelihood of its usage by all household members in several Ethiopian studies (Ashebir et al., 2013; Omer et al., 2022; Woyessa et al., 2022). Latrines that are far from residence might hinder the proper latrine utilisation for the elderly or people with disabilities (Woyessa et al., 2022) and limit access during

the night for children and women due to security concerns. In contrast, the reverse trend of higher diarrhoeal risks reported for latrines located closer (within 15 m) to households reported in two studies (Adane et al., 2017; Getahun and Adane, 2021) indicates the probability of infections from vectors such as flies if latrines are not maintained clean. The elevated risk of latrine proximity can also be due to the potential contamination from leaching through the latrine back-end to household water sources such as tube well (Kimani-Murage and Ngindu, 2007).

4.5. Implications of microbial contamination inside latrines

This literature review shows a considerable gap in information related to microbial measurement inside the household latrine. The highest contamination surfaces for faecal coliforms and helminths in the latrines were found to vary with latrine types (pit latrines compared to flush latrines) and the presence of front-end components such as latrine slabs. The reported range of concentration of faecal coliform on surfaces of flush latrines (cistern and pour-flush) such as latrine seats (0.03 to 743 CFU/cm^2) and flush handles and anal cleansing hardware (2 to 114 CFU/cm^2) shows that latrine surfaces can be potential pathways for microbial infections. Similarly, frequent hand contact points of the pit and flush latrines in Tanzania were also contaminated with average *E. coli* ranging from 7.6 to 154 CFU/100 ml. The absence of the recommended surface hygiene standards or acceptable upper limit of bacteria on latrine surfaces in the existing literature creates challenges for what to consider as clean and comparing the degree of contaminations on those surfaces (Hambraeus and Malmborg, 1980). Considering these findings highlighting the infection risks associated with microbial contamination on latrine surfaces, it is imperative to adopt robust methods for risk assessment and quantification. Quantitative Microbial Risk Assessment (QMRA) is an approach that offers a framework to integrate information on pathogen occurrence, infection doses, and exposure to determine the health implications related to microbial hazards (Haas et al., 2014; Hamilton and Haas, 2016). However, the complexity of applying QMRA to latrine surfaces is evident from the lack of studies linking microbial pathogen density on latrine surfaces to probabilities of infections. This complexity is further compounded by factors such as variations in latrine usage frequency among household members and the transfer rates of pathogen from surface to hands and subsequently to mouth. Although *E. coli* used as the indicative organisms are not inherently infectious, certain faecal-oral pathogens, such as pathogenic strains of *E. coli* (for example enterohemorrhagic strains) or *Shigella* spp., have very low infective doses (<10 CFU) (Kothary and Babu, 2001; Schmid-Hempel and Frank, 2007). Previous research has detected such pathogens on latrine surfaces, such as door handles, underscoring significant health risks (Abiose, 2019; Hossain et al., 2021). Further, there is evidence of high bacterial transfer rates from hard and non-porous surfaces to hands and hands to lips (Rusin et al., 2002) and the risk can be further exacerbated without proper handwashing after latrine usage. Thus, regular disinfection of these surfaces is essential to reduce the potential negative health impact, especially among vulnerable household members such as children, the elderly or individuals with compromised immune systems (Ojima et al., 2002a), even if the pathogens are present at low levels (Scott et al., 1982). Thus, the design of sanitation and hygiene interventions needs to pay attention to minimising the contamination of these surfaces and breaking the transmission pathways at multiple points.

The higher concentration of *E. coli* (six times elevated) in pit latrines with earthen slabs compared to the concrete slabs in Tanzania (Pickering et al., 2012) indicate the importance of using washable and quickly drying surfaces for latrine construction. Further, the reported range of helminths (0 to 38 ova and larvae per gram of soil) on earthen floors of pit latrines pose an elevated risk of STHs infections such as hookworm, especially for children using the latrine barefooted (Chioma et al., 2015). Also, the increased contamination of STHs in soil samples from latrines with broken slabs indicates the importance of proper maintenance (Exley et al., 2015) as cavities and cracks formed in the broken slab can be difficult to clean and make it favourable for the survival of pathogens. Hence, using materials that facilitate cleaning behaviours to build the latrine and regular maintenance of front-end infrastructures after construction can be important to reduce microbial contaminations on these surfaces.

4.6. Sampling methodologies

Looking at sampling methods, all studies collecting surface swab samples from the latrine surfaces have used the pre-moistened swab immersed in standard neutral solutions. The choice of wet swabbing by all studies for the latrine surfaces allows for ease of data comparison due to higher recovery of microbial density from these surfaces that are expected to be usually dry. Recovery rates from the heterogeneous surfaces of latrines have not yet been quantified. Literature on surface recovery from contaminated food and health-care settings are not directly comparable as surfaces tend to be uniform, non-porous and made from consistent materials (Maunula et al., 2017; Scoullos, 2020). However, the properties of latrine surfaces vary greatly due to heterogeneity within the front-end components, characteristics of materials used and maintenance of latrines. Also, microbial contamination of surfaces can vary largely depending on the surface features, for instance, porosity, roughness, irregularity, or their current conditions such as old or new and dry or clean (Ismaïl et al., 2013). Regarding the swabbing method, variation was observed in swabbing areas, sample collection methods, and diluent used across the studies. Due to the lack of standardised methods, the results from the few studies published that have quantified the microbial loads inside latrines do not lend themselves to ease of comparison. This aligns with the previous studies that have identified the necessity to standardise sampling methods for swab samples from various surfaces that are dusty, porous, and rough (Scoullos, 2020; Verdon et al., 2014). Hence, there is a clear need for an integrated and standard method for measuring microbial density from such surfaces to assess the risk associated with these surfaces.

Nearly all the studies that measured the concentration of faecal coliform or *E. coli* have used traditional culture methods for enumeration. The limitation of using traditional faecal-indicating bacteria such as *E. coli* by culture methods has been pointed out in several studies and they are not capable of tracking the faecal sources directly (Holcomb and Stewart, 2020; Ramírez-Castillo et al., 2015; Solo-Gabriele et al., 2000). Traditional cultural methods, however, continue to be a valuable tool for monitoring faecal contamination as it is affordable and practical, especially in the context of low and medium-HDI countries (Cabral, 2010). Molecular microbiology techniques allow for microbial source tracking via quantitative PCR (qPCR), which can identify the sources of faecal contamination (Farnleitner and Blanch, 2017) and enable us to understand and track the pattern of faecal contamination (McLellan and Eren, 2014). If laboratory resources and technical capacity are present, then expanding testing to include molecular microbiology along with traditional indicators would allow for microbial source tracking. These results could inform the design and implementation of targeted sanitation interventions, effectively breaking the faecal transmission pathways.

4.7. Key gaps in the literature

Overall, a smaller number of studies (10 out of 118 studies) were found reporting the densities of various microbial pathogens inside household latrines. Further, no studies were identified that were designed to link microbial infection instances with the pathogen loads in household latrines. There are publications from public latrine settings where microbial pathogen concentrations inside the latrine were measured primarily for hospitals (Ding et al., 2021; Newsom, 1972; Tsunoda et al., 2019), offices (Carducci et al., 2016), and public toilets (Amoah et al., 2021; Flores et al., 2011; Wogu and Okubotimibi, 2020).

These surfaces from public settings may not reflect the contamination inside household latrines due to the variation in user number, behaviours, and frequency of latrine usage. The modelling of environmental transmission pathways to identify and quantify human exposure to faecal contaminations in public or communal spaces has been conducted in several studies (Amin et al., 2019, 2020; Berendes et al., 2017; Capone et al., 2021; Holcomb et al., 2020; Mills et al., 2018; Raj et al., 2020). Yet limited studies have conducted QMRA with particular attention to assessing the role of contaminated latrine surfaces as a possible route of transmission of infectious diseases. Quantification of pathogens is a key piece of input data needed during the exposure assessment stage of QMRA, where potential pathogen dose distribution is determined for an exposed population (Mills et al., 2018). A recent QMRA conducted by Abney (2022) has also underlined the lack of pathogen density data on various surfaces of latrines and further states that most of the studies assessing microbial contamination inside latrines are limited to the presence or absence of pathogens which makes it difficult in quantifying the exposed health outcome. Hence, a lack of published data on the concentration of pathogens limits the application of QMRAs to link and quantify the risk of transmission of infectious diseases from contaminated latrine surfaces.

To address this gap, more detailed studies are needed to measure pathogen loads and identify high-risk human contact points within latrines across different settings. These contact points can be contextual and vary based on latrine types, front-end characteristics, and usage behaviours. Quantifying the microbial loads on latrine surfaces is even more relevant in countries with low to medium HDI where there are greater challenges in achieving safe sanitation and hygiene. In-depth studies measuring microbial concentrations using standardised cultural and molecular methods on latrine surfaces, especially at frequent human contact points, are recommended.

4.8. Inconsistency in terminologies used for latrine categorisation

An important challenge faced during this review was the lack of consistency around definitions and terminology used for sanitation. For example, Ketema et al. (2012) classified latrines as modern and traditional types, without any further details mentioned on the basis of categorisation. Similarly, the risk of STHs infection was estimated by Ster et al. (2021) for a water closet type compare to a “latrine type”, without any indication of what a latrine type meant. Furthermore, a flush latrine can be pedestal or squat (based on the front-end sitting position) and cistern or pour-flush (based on the flush mechanism). Similarly, flush latrines can be connected to multiple options of back-end systems such as pits, septic tanks, or sewers. Hence, categorising latrines simply as “flush” does not provide a full picture of how the latrine is being used. Similar problems of inconsistency in sanitation terminology have been reflected by previous reviews (Bartram et al., 2014; Heijnen et al., 2015; Nasim et al., 2022; Ziegelbauer et al., 2012) conducted to assess the health outcome associated with the faecal-oral route. For instance, Bartram et al. (2014) have highlighted the extensive problem of heterogenous use of terms such as open pit, pit or traditional latrine to describe the same sanitation type. Although the introduction of the JMP sanitation ladder by WHO and UNICEF (2022) can be regarded as an important step in efforts to standardise categorisation, further work is needed essential to clearly categorise latrines based on front-end and back-end components. The Compendium of Sanitation is one widely used reference where Tilley et al. (2014) present clear information on different configurations of sanitation infrastructure that are designed for the selection of context-specific and compatible sanitation types. However, a wide range of terminologies has still been used to categorise sanitation infrastructure such as dry, pit, open, traditional, non-flush, closed, flush, modern, sanitary, water closet and so on. Also, the pathogen transmission pathways from latrines can largely vary according to the infrastructure used for front-end and back-end systems. Besides latrine infrastructure, inconsistency, and lack of explicit definitions of terminologies were also observed for latrine usage behaviours such as latrine cleanliness, maintenance, and utilisation. For example, latrine conditions have been categorised as poor, fair, and good, without any details of the basis of those categorisations (Dumba et al., 2008). This heterogeneity results in difficulty comparing and interpreting results from various studies and limits the potential knowledge sharing in the sanitation and public health sector. Thus, consistency of sanitation terminology is crucial not only for academic and scientific purposes but also important to translate and communicating the safe use of sanitation systems to the public.

4.9. Limitations of the study

There is some sanitation information and data available in grey literature such as reports from government, non-governmental organisations and agencies which are not included in this study. The reasoning for not including grey literature is that the information is not published via the peer review process, which introduces greater uncertainties in data quality, validation, and credibility. To increase the quantity of peer-reviewed sanitation literature, journal publication support is needed for sanitation-implementing organisations and researchers in lower HDI countries. This review has brought together a wide variety of study types, which has its challenges. While all the studies have quantified the microbial infection risks or concentration of microorganisms with latrines, they vary significantly in several aspects. The studies are diverse in terms of contexts, locations, study settings, weather conditions, targeted populations, study designs, sample sizes, sanitation types, sample collection and analysis methods and depth of exploration. These variabilities between the studies did not enable meta-analysis of the reported odd ratios. Hence, these variances have been considered when comparing the results among the studies.

5. Conclusions

This review identifies a significant research gap in quantifying the microbial densities within household latrines. Latrine types that sit on a higher position on the sanitation ladder reduced the risk of microbial infections, however, this review has revealed the critical importance of maintaining latrine cleanliness, regardless of latrine type. It also provides further evidence that latrine surfaces can be a potential pathway for pathogen transmission, highlighting the need for multilevel barriers to minimise human exposure to pathogens during latrine use. Therefore, to effectively reduce microbial risks in latrines, this study recommends ensuring washable surfaces that enable efficient cleaning and disinfection, as well as consistent cleaning practices.

The findings of this review suggest that sanitation and hygiene interventions should prioritise latrine cleanliness rather than just focusing on handwashing practices or constructing new latrine infrastructure. Interventions should include providing education and guidance to the users on efficient cleaning techniques and recommending cost-effective disinfecting or cleaning products best available such as bleach. Future research should explore the relationship between microbial densities and the properties of different materials used inside latrines. Further work is needed to incorporate latrine usage behaviours such as preferred methods of anal cleansing, latrine cleanliness and maintenance which will enhance our understanding of pathogen transmission pathways and inform the design of context-specific sanitation and hygiene interventions.

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Author's contribution

Sabita Adhikari: Conceptualisation, study methodology design, execution of literature search, reviewing all literature, drafting the manuscript, editing the manuscript for final submission.

Dr Erin Hunter: Critically revising the written work and editing the manuscript for final submission.

Dr Jack van de Vossenberg: Critically revising the written work and editing the manuscript for final submission.

Dr Jacqueline Thomas: Conceptualisation, study methodology design, reviewing key literature, critically revising the written work, editing the manuscript for final submission.

Institutional review board statement

Not applicable.

Informed consent statement

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Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2023.114261.

References

Abdala, M., Singano, C.C., Willis, R., Macleod, C.K., Backers, S., Flueckiger, R.M., Vilanculos, A., Terefe, D., Houane, M., Bikele, F., Kello, A.B., Downs, P., Bay, Z., Senyonjo, L., Solomon, A.W., 2018. The epidemiology of trachoma in Mozambique: results of 96 population-based prevalence surveys. Ophthalmic Epidemiol. 25, 201–210.

Abdilwohab, M.G., Abebo, Z.H., 2020. High prevalence of clinically active trachoma and its associated risk factors among preschool-aged children in Arba Minch health and demographic surveillance site, Southern Ethiopia. Clin. Ophthalmol. 14, 3709–3718.

Abebe, A., Asmamaw, A., Zelalem, A., Yitayal, S., Takele, T., Biniam, M., Wubet, B., Simon, G., Baye, G., 2011. Soil transmitted helminths and Schistosoma mansoni infections among school children in Zarima town, northwest Ethiopia. BMC Infect. Dis. 11 (189).

Abiose, O.F., 2019. Bacterial contamination of selected public toilet door handles within adekunle ajasin university campus, akungba-akoko, ondo state, Nigeria. Int. J. Sci. Basic Appl. Res. 43 (1), 76–86.

Abney, S.E., 2022. Application of Quantitative Microbial Risk Assessment to Domestic Hygiene. PhD Thesis. The University of Arizona.

Adane, M., Mengistie, B., Kloos, H., Medhin, G., Mulat, W., 2017. Sanitation facilities, hygienic conditions, and prevalence of acute diarrhea among under-five children in slums of Addis Ababa, Ethiopia: baseline survey of a longitudinal study. PLoS One 12 (8).

Ademas, A., Adane, M., Sisay, T., Kloos, H., Eneyew, B., Keleb, A., Lingerew, M., Derso, A., Alemu, K., 2020. Does menstrual hygiene management and water, sanitation, and hygiene predict reproductive tract infections among reproductive women in urban areas in Ethiopia? PLoS One 15 (8).

Ahmed, F., Clemens, J.D., Rao, M.R., Banik, A.K., 1994. Family latrines and pediatric shigellosis in rural Bangladesh - benefit or risk. Int. J. Epidemiol. 23 (4), 856–862.

Akter, J., Islam, M.R., Akter, S., Rahman, M.M., Hossain, F., Anam, M.R., Alam, M.A., Sultana, P., Rashid, S., 2022. Equity in access to safely managed sanitation and prevalence of diarrheal diseases in Bangladesh: a national and sub-national analysis. BMC Infect. Dis. 22 (1).

Al-Brhami, K.A.R., Abdul-Ghani, R., Al-Qobati, S.A., 2022. Intestinal microsporidiosis among HIV/AIDS patients receiving antiretroviral therapy in Sana'a city, Yemen: first report on prevalence and predictors. BMC Infect. Dis. 22 (1).

Alemayehu, B., Ayele, B.T., Kloos, H., Ambelu, A., 2020a. Individual and community-level risk factors in under-five children diarrhea among agro-ecological zones in southwestern Ethiopia. Int. J. Hyg Environ. Health 224.

Alemayehu, M., Alemu, T., Astatkie, A., 2020b. Prevalence and determinants of diarrhea among under-five children in Benna Tsemay district, South Omo Zone, southern Ethiopia: a community-based cross-sectional study in pastoralist and agropastoralist context. Adv. public health 1–11, 2020.

Alemu, Y., Degefa, T., Bajiro, M., Teshome, G., 2022. Prevalence and intensity of soil-transmitted helminths infection among individuals in model and non-model households, south west Ethiopia: a comparative cross-sectional community based study. PLoS One 17 (10).

Amin, N., Liu, P.B., Foster, T., Rahman, M., Miah, M.R., Ahmed, G.B., Kabir, M., Raj, S., Moe, C.L., Willetts, J., 2020. Pathogen flows from on-site sanitation systems in low-income urban neighborhoods, Dhaka: a quantitative environmental assessment. Int. J. Hyg Environ. Health 230, 12.

Amin, N., Rahman, M., Raj, S., Ali, S., Green, J., Das, S., Doza, S., Mondol, M.H., Wang, Y., Islam, M.A., 2019. Quantitative assessment of fecal contamination in multiple environmental sample types in urban communities in Dhaka, Bangladesh using SaniPath microbial approach. PLoS One 14 (12), e0221193.

Amoah, I.D., Pillay, L., Deepnarian, N., Awolusi, O., Pillay, K., Ramlal, P., Kumari, S., Bux, F., 2021. Detection of SARS-CoV-2 RNA on contact surfaces within shared sanitation facilities. Int. J. Hyg Environ. Health 236, 113807-113807.

Amour, C., Gratz, J., Mduma, E., Svensen, E., Rogawski, E.T., McGrath, M., Seidman, J. C., McCormick, B.J.J., Shrestha, S., Samie, A., Mahfuz, M., Qureshi, S., Hotwani, A., Babji, S., Trigoso, D.R., Lima, A.A.M., Bodhidatta, L., Bessong, P., Ahmed, T., Shakoor, S., Kang, G., Kosek, M., Guerrant, R.L., Lang, D., Gottlieb, M., Houpt, E.R., Platts-Mills, J.A., Etiology Risk Factors, I., 2016. Epidemiology and impact of Campylobacter infection in children in 8 low-resource settings: results from the MAL-ED study. Clin. Infect. Dis. 63 (9), 1171–1179.

Anteneh, A., Kumie, A., 2010. Assessment of the impact of latrine utilization on diarrhoeal diseases in the rural community of hulet ejju enessie woreda, east gojjam zone, amhara region. Ethiop. J. Health Dev. 24 (2), 110–118.

Anunobi, J.T., Okoye, I.C., Aguzie, I.O., Ndukwe, Y.E., Okpasuo, O.J., 2019. Risk of soil transmitted helminthiasis among Agrarian communities of Kogi State, Nigeria. Ann. Glob. Health. 85 (1).

Asaolu, S.O., Ofoezie, I.E., Odumuyiwa, P.A., Sowemimo, O.A., Ogunniyi, T.A.B., 2002. Effect of water supply and sanitation on the prevalence and intensity of Ascaris lumbricoides among pre-school-age children in Ajebandele and Ifewara, Osun State, Nigeria. Trans. R. Soc. Trop. Med. Hyg. 96 (6), 600–604.

Asfaw, M.A., Wegayehu, T., Gezmu, T., Bekele, A., Hailemariam, Z., Gebre, T., 2020. Determinants of soil-transmitted helminth infections among pre-school-aged children in Gamo Gofa zone, southern Ethiopia: a case-control study. PLoS One 15 (12).

Ashebir, Y., Rai Sharma, H., Alemu, K., Kebede, G., 2013. Latrine use among rural households in northern Ethiopia: a case study in Hawzien district, Tigray. Int. J. Environ. Stud. 70 (4), 629–636.

Ayelgn, K., Guadu, T., Getachew, A., 2021. Low prevalence of active trachoma and associated factors among children aged 1-9 years in rural communities of Metema district, northwest Ethiopia: a community based cross-sectional study. Ital. J. Pediatr. 47 (1).

Baker, K.K., Senesac, R., Sewell, D., Sen Gupta, A., Cumming, O., Mumma, J., 2018. Fecal fingerprints of enteric pathogen contamination in public environments of Kisumu, Kenya, associated with human sanitation conditions and domestic animals. Environ. Sci. Technol. 52 (18), 10263–10274.

Baker, S.M., Ensink, J.H.J., 2012. Helminth transmission in simple pit latrines. Trans. R. Soc. Trop. Med. Hyg. 106 (11), 709–710.

Bartlett, A.V., Hurtado, E., Schroeder, D.G., Mendez, H., 1992. Association of indicators of hygiene behavior with persistent diarrhea of young children. Acta Paediatr. 81, 66–71.

Bartram, J., Brocklehurst, C., Fisher, M.B., Luyendijk, R., Hossain, R., Wardlaw, T., Gordon, B., 2014. Global monitoring of water supply and sanitation: history, methods and future challenges. Int. J. Environ. Res. Publ. Health 11 (8), 8137–8165.

Baye, A., Adane, M., Sisay, T., Hailemeskel, H.S., 2021. Priorities for intervention to prevent diarrhea among children aged 0-23 months in northeastern Ethiopia: a matched case-control study. BMC Pediatr. 21 (1).

Benjamin-Chung, J., Nazneen, A., Halder, A.K., Haque, R., Siddique, A., Uddin, M.S., Koporc, K., Arnold, B.F., Hubbard, A.E., Unicomb, L., Luby, S.P., Addiss, D.G., Colford, J.M., 2015. The interaction of deworming, improved sanitation, and household flooring with soil-transmitted helminth infection in rural Bangladesh. PLoS Neglected Trop. Dis. 9 (12).

Berendes, D., Kirby, A., Clennon, J.A., Raj, S., Yakubu, H., Leon, J., Robb, K., Kartikeyan, A., Hemavathy, P., Gunasekaran, A., 2017. The influence of household- and community-level sanitation and fecal sludge management on urban fecal contamination in households and drains and enteric infection in children. Am. J. Trop. Med. Hyg. 96 (6), 1404–1414.

Bethony, J., Brooker, S., Albonico, M., Geiger, S.M., Loukas, A., Diemert, D., Hotez, P.J., 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 367 (9521), 1521–1532.

Biran, A., Jenkins, M., Dabrase, P., Bhagwat, I., 2011. Patterns and determinants of communal latrine usage in urban poverty pockets in Bhopal, India. Trop. Med. Int. Health 16 (7), 854–862.

Brainard, J., D'Hondt, R., Ali, E., Van den Bergh, R., De Weggheleire, A., Baudot, Y., Patigny, F., Lambert, V., Zachariah, R., Maes, P., Kenge, D.K.-K., Hunter, P.R., 2018. Typhoid fever outbreak in the Democratic Republic of Congo: case control and ecological study. PLoS Neglected Trop. Dis. 12 (10).

Cabral, J.P., 2010. Water microbiology. Bacterial pathogens and water. Int. J. Environ. Res. Publ. Health 7 (10), 3657–3703.

Cairncross, S., Hunt, C., Boisson, S., Bostoen, K., Curtis, V., Fung, I.C., Schmidt, W.-P., 2010. Water, sanitation and hygiene for the prevention of diarrhoea. Int. J. Epidemiol. 39 (Suppl. 1_1), i193–i205.

Canales, R.A., Wilson, A.M., Sinclair, R.G., Soto-Beltran, M., Pearce-Walker, J., Molina, M., Penny, M., Reynolds, K.A., 2019. Microbial study of household hygiene conditions and associated Listeria monocytogenes infection risks for Peruvian women. Trop. Med. Int. Health 24 (7), 899–921.

Capone, D., Berendes, D., Cumming, O., Holcomb, D., Knee, J., Konstantinidis, K.T., Levy, K., Nala, R., Risk, B.B., Stewart, J., Brown, J., 2021. Impact of an urban sanitation intervention on enteric pathogen detection in soils. Environ. Sci. Technol. 55 (14), 9989–10000.
Carducci, A., Donzelli, G., Cioni, L., Verani, M., 2016. Quantitative microbial risk assessment in occupational settings applied to the airborne human Adenovirus infection. Int. J. Environ. Res. Publ. Health 13 (7), 733.
Cha, S., Elhag, M.S., Lee, Y., Cho, D., Ismail, H.A.H.A., Hong, S., 2019. Epidemiological findings and policy implications from the nationwide schistosomiasis and intestinal helminthiasis survey in Sudan. Parasites Vectors 12 (429).
Cha, S., Lee, J.E., Seo, D.S., Park, B.M., Mansiangi, P., Hwang, J.-S., Lee, J., 2017. Associations between household latrines and the prevalence of diarrhea in Idiofa, Democratic Republic of the Congo: a cross-sectional study. Am. J. Trop. Med. Hyg. 97 (2), 460–468.
Chioma, U., Mbanugo, J., Nwachukwu, E., 2015. Prevalence of intestinal helminthes parasite in stools of nursery and primary schools pupils in Uga, Anambra State, Nigeria. Sky J. Microbiol. Res. 3 (1), 006-010.
Chompook, P., Todd, J., Wheeler, J.G., von Seidlein, L., Clemens, J., Chaicumpa, W., 2006. Risk factors for shigellosis in Thailand. Int. J. Infect. Dis. 10 (6), 425–433.
Chuang, P., Trottier, S., Murcott, S., 2011. Comparison and verification of four field-based microbiological tests: H_2S test, Easygel®, Colilert®, Petrifilm. J. Water, Sanit. Hyg. Dev. 1 (1), 68–85.
Cifuentes, E., Mazari-Hiriart, M., Carneiro, F., Bianchi, F., Gonzalez, D., 2002. The risk of enteric diseases in young children and environmental indicators in sentinel areas of Mexico City. Int. J. Environ. Health Res. 12 (1), 53–62.
Coles, C.L., Sherchand, J.B., Khatry, S.K., Katz, J., LeClerq, S.C., Mullany, L.C., Tielsch, J. M., 2009. Nasopharyngeal carriage of S-pneumoniae among young children in rural Nepal. Trop. Med. Int. Health 14 (9), 1025–1033.
Corrales, L.F., Izurieta, R., Moe, C.L., 2006. Association between intestinal parasitic infections and type of sanitation system in rural El Salvador. Trop. Med. Int. Health 11 (12), 1821–1831.
Dagne, N., Alelign, A., 2021. Prevalence of intestinal protozoan parasites and associated risk factors among school children in Merhabete district, central Ethiopia. J. Parasitol. Res. 2021, 9916456-7.
Das, P., Lisnek, D., Sahoo, K.C., Sinha, S., Mohanty, J., Sahoo, P., Bilung, B., Panda, B., Tanton, C., Torondel, B., 2021. Identifying risk factors for lower reproductive tract infections among women using reusable absorbents in Odisha, India. Int. J. Environ. Res. Publ. Health 18 (9), 15.
Degebasa, M.Z., Weldemichael, D.Z., Marama, M.T., 2018. Diarrhea status and associated factors in under five years old children in relation to implemented and unimplemented community-led total sanitation and hygiene in Yaya Gulele in 2017. Pediatr. Health Med. Therapeut. 9, 109–121.
Deichsel, E.L., Pavlinac, P.B., Mbori-Ngacha, D., Walson, J.L., Maleche-Obimbo, E., Farquhar, C., Bosire, R., John-Stewart, G.C., 2020. Maternal diarrhea and antibiotic use are associated with increased risk of diarrhea among HIV-exposed, uninfected infants in Kenya. Am. J. Trop. Med. Hyg. 102 (5), 1001–1008.
Ding, Z., Qian, H., Xu, B., Huang, Y., Miao, T., Yen, H.-L., Xiao, S., Cui, L., Wu, X., Shao, W., Song, Y., Sha, L., Zhou, L., Xu, Y., Zhu, B., Li, Y., 2021. Toilets dominate environmental detection of severe acute respiratory syndrome coronavirus 2 in a hospital. Sci. Total Environ. 753, 141710-141710.
Dumba, R., Kaddu, J.B., Mangen, F.W., 2008. Intestinal helminths in Luweero district, Uganda. Afr. Health Sci. 8 (2), 90–96.
Esrey, S.A., Potash, J.B., Roberts, L., Shiff, C., 1991. Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis, and trachoma. Bull. World Health Organ. 69 (5), 609.
Exley, J.L.R., Liseka, B., Cumming, O., Ensink, J.H.J., 2015. The sanitation ladder, what constitutes an improved form of sanitation? Environ. Sci. Technol. 49 (2), 1086–1094.
Fagbamigbe, A.F., Uthman, A.O., Ibisomi, L., 2021. Hierarchical disentanglement of contextual from compositional risk factors of diarrhoea among under-five children in low-and middle-income countries. Sci. Rep. 11 (1), 1–17.
Farnleitner, A., Blanch, A., 2017. Microbial Indicators- "Workhorses" in the Field of Health-Related Water Quality Testing, Global Water Pathogen Project- Part 2 Indicators and Microbial Source Tracking Markers. Michigan State University, MI, UNESCO.
Fenta, A., Mesganaw, B., Belachew, F., Teshale, G., Abebaw, G., Tesfa, E., Alemayehu, G., 2022. Prevalence and risks of soil transmitted helminths among Ethiopian school children: a cross-sectional study. Asian Pac. J. Tropical Med. 15 (3), 121–127.
Fetene, Y., Hailu, T., Yimer, M., Alemu, M., 2021. Determinants of helminthic infections and anemia among schoolchildren in Bahir Dar Zuria district, northwest Ethiopia. J. Parasitol. Res. 2021, 9913118-7.
Flores, G.E., Bates, S.T., Knights, D., Lauber, C.L., Stombaugh, J., Knight, R., Fierer, N., 2011. Microbial biogeography of public restroom surfaces. PLoS One 6 (11), e28132.
Freeman, M.C., Garn, J.V., Sclar, G.D., Boisson, S., Medlicott, K., Alexander, K.T., Penakalapati, G., Anderson, D., Mahtani, A.G., Grimes, J.E.T., Rehfuess, E.A., Clasen, T.F., 2017. The impact of sanitation on infectious disease and nutritional status: a systematic review and meta-analysis. Int. J. Hyg Environ. Health 220 (6), 928–949.
Gakidou, E., Afshin, A., Amanuel Alemu, A., Kalkidan Hassen, A., Abbafati, C., Abbas, K. M., Abd-Allah, F., Abdulle, A.M., Semaw Ferede, A., Aboyans, V., Abu-Raddad, L.J., Abu-Rmeileh, N.M.E., Abyu, G.Y., Isaac Akinkunmi, A., Olatunji, A., Afarideh, M., Agrawal, A., Agrawal, S., Ahmadieh, H., Muktar Beshir, A., Miloud Taki Eddine, A., Aichour, A.N., Aichour, I., Akinyemi, R.O., Akseer, N., Fares, A., Al-Aly, Z., Alam, K., Alam, N., Alam, T., Alasfoor, D., Kefyalew Addis, A., Ali, K., Alizadeh-Navaei, R., Alkerwi, A.a., Alla, F., Allebeck, P., Al-Raddadi, R., Alsharif, U., Altirkawi, K.A., Alvis-Guzman, N., Amare, A.T., Amini, E., Ammar, W., Amoako, Y.A., Ansari, H., Antó, J.M., Carl Abelardo, T.A., Anwari, P., Arian, N., Ärnlöv, J., Artaman, A., Aryal, K.K., Asayesh, H., Solomon Weldegebreal, A., Tesfay, M.A., Avila-Burgos, L., Euripide Frinel, G.A.A., Awasthi, A., Azzopardi, P., Bacha, U., Badawi, A., Balakrishnan, K., Ballew, S.H., Barac, A., Barber, R.M., Barker-Collo, S.L., Bärnighausen, T., Barquera, S., Barregard, L., Barrero, L.H., Batis, C., Battle, K.E., Baumgarner, B.R., Baune, B.T., Beardsley, J., Bedi, N., Beghi, E., Bell, M.L., Bennett, D.A., Bennett, J.R., Bensenor, I.M., Berhane, A., Derbew Fikadu, B., Bernabé, E., Betsu, B.D., Beuran, M., Beyene, A.S., Bhansali, A., Bhutta, Z.A., Burcu Kucuk, B., Bikbov, B., Birungi, C., Biryukov, S., Blosser, C.D., Dube Jara, B., Bou-Orm, I.R., Brauer, M., Breitborde, N.J.K., Brenner, H., et al., 2017. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 390 (10100), 1345–1422.
Garg, P., Singh, P., 2016. Postdefecation cleansing methods: tissue paper or water? an analytical review. Dis. Colon Rectum 59 (7), 696–699.
Garn, J.V., Boisson, S., Willis, R., Bakhtiari, A., al-Khatib, T., Amer, K., Batcho, W., Courtright, P., Dejene, M., Goepogui, A., Kalua, K., Kebede, B., Macleod, C.K., Iiunga, K., Madeleine, M., Mbofana, M.S.A., Mpyet, C., Ndjemba, J., Olobio, N., Pavluck, A.L., Sokana, O., Southisombath, K., Taleo, F., Solomon, A.W., Freeman, M. C., 2018. Sanitation and water supply coverage thresholds associated with active trachoma: modeling cross-sectional data from 13 countries. PLoS Neglected Trop. Dis. 12 (1).
Geleto, G.E., Kassa, T., Erko, B., 2022. Epidemiology of soil-transmitted helminthiasis and associated malnutrition among under-fives in conflict affected areas in southern Ethiopia. Trop. Med. Health 50 (1).
Getachew, A., Tadie, A., Hiwot, M.G., Guadu, T., Haile, D., Cherkos, T.G., Gizaw, Z., Alemayehu, M., 2018. Environmental factors of diarrhea prevalence among under five children in rural area of North Gondar zone, Ethiopia. Ital. J. Pediatr. 44.
Getahun, W., Adane, M., 2021. Prevalence of acute diarrhea and water, sanitation, and hygiene (WASH) associated factors among children under five in Woldia Town, Amhara Region, northeastern Ethiopia. BMC Pediatr. 21 (1).
Giri, M., Behera, M.R., Behera, D., Mishra, B., Jena, D., 2022. Water, sanitation, and hygiene practices and their association with childhood diarrhoea in rural households of Mayurbhanj district, Odisha, India. Cureus Journal of Medical Science 14 (10).
Goshu, A., Alemu, G., Ayehu, A., 2021. Prevalence and intensity of soil-transmitted helminths and associated factors among adolescents and adults in Bibugn woreda, northwest Ethiopia: a community-based cross-sectional study. J. Trop. Med. 2021, 7043881–9.
Haas, C.N., Rose, J.B., Gerba, C.P., 2014. Quantitative Microbial Risk Assessment. John Wiley & Sons.
Hailu, T., Abera, B., Mulu, W., Kassa, S., Genanew, A., Amor, A., 2020. Prevalence and factors associated with intestinal parasitic infections among pregnant women in west Gojjam Zone, northwest Ethiopia. J. Parasitol. Res. 2020, 8855362–6.
Hall, A., Conway, D.J., Anwar, K.S., Rahman, M.L., 1994. Strongyloides stercoralis in an urban slum community in Bangladesh: factors independently associated with infection. Trans. R. Soc. Trop. Med. Hyg. 88 (5), 527–530.
Halsey, L.G., 2019. The reign of the p-value is over: what alternative analyses could we employ to fill the power vacuum? Biol. Lett. 15 (5).
Hambraeus, A., Malmborg, A.S., 1980. Disinfection or cleaning of hospital toilets—an evaluation of different routines. J. Hosp. Infect. 1 (2), 159–163.
Hamilton, K., Haas, C., 2016. Critical review of mathematical approaches for quantitative microbial risk assessment (QMRA) of Legionella in engineered water systems: research gaps and a new framework. Environ. Sci. J. Integr. Environ. Res.: Water Research & Technology 2 (4), 599–613.
Han, A.M., Oo, K.N., Aye, T., Hlaing, T., 1986. Personal toilet after defaecation and the degree of hand contamination according to different methods used. Trop. Med. Int. Health 89 (5), 237–241.
Heijnen, M., Routray, P., Torondel, B., Clasen, T., 2015. Shared sanitation versus individual household latrines in urban slums: a cross-sectional study in Orissa, India. Am. J. Trop. Med. Hyg. 93 (2), 263–268.
Hestvik, E., Tylleskar, T., Kaddu-Mulindwa, D.H., Ndeezi, G., Grahnquist, L., Olafsdottir, E., Tumwine, J.K., 2010. Helicobacter pylori in apparently healthy children aged 0-12 years in urban Kampala, Uganda: a community-based cross sectional survey. BMC Gastroenterol. 10.
Hlaing, Z.N., Mongkolchati, A., Rattanapan, C., 2016. A household level analysis of water sanitation associated with gastrointestinal disease in an urban slum setting of South Okkalapa Township, Myanmar. Environment (Wash. D C) 9 (2), 91–100.
Holcomb, D.A., Knee, J., Sumner, T., Adriano, Z., de Bruijn, E., Nalá, R., Cumming, O., Brown, J., Stewart, J.R., 2020. Human fecal contamination of water, soil, and surfaces in households sharing poor-quality sanitation facilities in Maputo, Mozambique. Int. J. Hyg Environ. Health 226, 113496.
Holcomb, D.A., Stewart, J.R., 2020. Microbial indicators of fecal pollution: recent progress and challenges in assessing water quality. Curr. Environ. Health Rep. 7 (3), 311–324.
Hossain, Z.Z., Sultana, R., Begum, A., Jensen, P.K.M., 2021. Investigation of the domestic reservoirs of diarrheagenic Escherichia coli in diarrhea case households of urban Bangladesh. Curr. Microbiol. 78 (7), 2534–2547.
Ismaïl, R., Aviat, F., Michel, V., Le Bayon, I., Gay-Perret, P., Kutnik, M., Fédérighi, M., 2013. Methods for recovering microorganisms from solid surfaces used in the food industry: a review of the literature. Int. J. Environ. Res. Publ. Health 10 (11), 6169–6183.
Iwashita, H., Tokizawa, A., Thiem, V.D., Takemura, T., Nguyen, T.H., Doan, H.T., Pham, A.H.Q., Tran, N.L., Yamashiro, T., 2022. Risk factors associated with diarrheal episodes in an agricultural community in Nam Dinh province, Vietnam: a prospective cohort study. Int. J. Environ. Res. Publ. Health 19 (4).

Iyo, T., Asakura, K., Nakano, M., Yamada, M., Omae, K., 2016. Bidet toilet seats with warm-water tanks: residual chlorine, microbial community, and structural analyses. J. Water Health 14 (1), 68–80.
Julian, T.R., 2016. Environmental transmission of diarrheal pathogens in low and middle income countries. Environ Sci Process Impacts 18 (8), 944–955.
Kanayama Katsuse, A., Takahashi, H., Yoshizawa, S., Tateda, K., Nakanishi, Y., Kaneko, A., Kobayashi, I., 2017. Public health and healthcare-associated risk of electric, warm-water bidet toilets. J. Hosp. Infect. 97 (3), 296–300.
Ketema, K., Tiruneh, M., Woldeyohannes, D., Muluye, D., 2012. Active trachoma and associated risk factors among children in Baso Liben District of East Gojjam, Ethiopia. BMC Publ. Health 12.
Khieu, V., Schaer, F., Marti, H., Bless, P.J., Char, M.C., Muth, S., Odermatt, P., 2014. Prevalence and risk factors of strongyloides stercoralis in takeo province, Cambodia. Parasites Vectors 7.
Kimani-Murage, E.W., Ngindu, A.M., 2007. Quality of water the slum dwellers use: the case of a Kenyan slum. J. Urban Health 84 (6), 829–838.
Kirschke, S., Avellán, T., Bärlund, I., Bogardi, J.J., Carvalho, L., Chapman, D., Dickens, C. W., Irvine, K., Lee, S., Mehner, T., 2020. Capacity challenges in water quality monitoring: understanding the role of human development. Environ. Monit. Assess. 192 (5), 1–16.
Knee, J., Sumner, T., Adriano, Z., Berendes, D., de Bruijn, E., Schmidt, W.-P., Nala, R., Cumming, O., Brown, J., 2018. Risk factors for childhood enteric infection in urban Maputo, Mozambique: a cross-sectional study. PLoS Neglected Trop. Dis. 12 (11).
Kothary, M.H., Babu, U.S., 2001. Infective dose of foodborne pathogens in volunteers: a review. J. Food Saf. 21 (1), 49–68.
Kouame, P.K., Dongo, K., Hung, N.-V., Zurbruegg, C., Luethi, C., Hattendorf, J., Utzinger, J., Biemi, J., Bonfoh, B., 2014. Ecohealth approach to urban waste management: exposure to environmental pollutants and health risks in Yamoussoukro, Cote d'ivoire. Int. J. Environ. Res. Publ. Health 11 (10), 10292–10309.
Kratzke, C., Short, M., San Filippo, B., 2014. Promoting safe hygiene practices in public restrooms: a pilot study. J. Environ. Health 77 (4), 8–13.
Kwiringira, J., Atekyereza, P., Niwagaba, C., Günther, I., 2014. Gender variations in access, choice to use and cleaning of shared latrines; experiences from Kampala Slums, Uganda. BMC Publ. Health 14 (1), 1–11.
Last, A.R., Burr, S.E., Weiss, H.A., Harding-Esch, E.M., Cassama, E., Nabicassa, M., Mabey, D.C., Holland, M.J., Bailey, R.L., 2014. Risk factors for active trachoma and ocular Chlamydia trachomatis infection in treatment-naive trachoma-hyperendemic communities of the Bijagos Archipelago, Guinea Bissau. PLoS Neglected Trop. Dis. 8 (6).
Lengerh, A., Moges, F., Unakal, C., Anagaw, B., 2013. Prevalence, associated risk factors and antimicrobial susceptibility pattern of Campylobacter species among under five diarrheic children at Gondar University Hospital, northwest Ethiopia. BMC Pediatr. 13.
Luo, D., Huang, J., Zheng, X., Liu, F., Li, Y., Wang, Y., Qian, H., 2023. Spread of flushing-generated fecal aerosols in a squat toilet cubicle: implication for infection risk. Sci. Total Environ. 859, 160212.
Mahdavinejad, M., Bemanian, M., Farahani, S.F., Tajik, A., 2011. Role of toilet type in transmission of infections. Res. Intell. 1 (2), 110–113.
Mara, D.D., 1985. The Design of Pour-Flush Latrines. World Bank, Washington, DC (TAG technical note no. 15).
Matthys, B., Bobieva, M., Karimova, G., Menglibоeva, Z., Jean-Richard, V., Hoimnazarova, M., Kurbonova, M., Lohourignon, L.K., Utzinger, J., Wyss, K., 2011. Prevalence and risk factors of helminths and intestinal protozoa infections among children from primary schools in western Tajikistan. Parasites Vectors 4.
Maunula, L., Rönnqvist, M., Åberg, R., Lunden, J., Nevas, M., 2017. The presence of norovirus and adenovirus on environmental surfaces in relation to the hygienic level in food service operations associated with a suspected gastroenteritis outbreak. Food Environ. Virol. 9 (3), 334–341.
McGinnis, S., Marini, D., Amatya, P., Murphy, H.M., 2019. Bacterial contamination on latrine surfaces in community and household latrines in Kathmandu, Nepal. Int. J. Environ. Res. Publ. Health 16 (2).
McLellan, S.L., Eren, A.M., 2014. Discovering new indicators of fecal pollution. Trends Microbiol. 22 (12), 697–706.
McMahon, S., Caruso, B.A., Obure, A., Okumu, F., Rheingans, R.D., 2011. Anal cleansing practices and faecal contamination: a preliminary investigation of behaviours and conditions in schools in rural Nyanza Province, Kenya. Trop. Med. Int. Health 16 (12), 1536–1540.
Mekonnen, G.K., Alemu, B.M., Mulat, W., Sahilu, G., Kloos, H., 2019. Risk factors for acute childhood diarrhea: a cross-sectional study comparing refugee camps and host communities in Gambella Region, Ethiopia. Trav. Med. Infect. Dis. 31.
Mengistu, B., Wirtu, F., Alemayehu, A., Alene, S., Asmare, A., Backers, S., Bakhtiari, A., Brady, M., Butcher, R.M.R., Dayessa, M., Frawley, H., Gebru, G., Jimenez, C., Kebede, F., Kejela, A., McPherson, S., Mihret, A., Negussu, N., Ngondi, J.M., Taddese, F., Willis, R., Wondimu, A., Dejene, M., Solomon, A.W., Harding-Esch, E. M., 2022. Prevalence of trachoma in Benishangul Gumuz region, Ethiopia, after implementation of the safe strategy: results of four population-based surveys. Ophthalmic Epidemiol. 1–9.
Mills, F., Willetts, J., Petterson, S., Mitchell, C., Norman, G., 2018. Faecal pathogen flows and their public health risks in urban environments: a proposed approach to inform sanitation planning. Int. J. Environ. Res. Publ. Health 15 (2), 181.
Momberg, D.J., Ngandu, B.C., Voth-Gaeddert, L.E., Cardoso Ribeiro, K., May, J., Norris, S.A., Said-Mohamed, R., 2021. Water, sanitation and hygiene (WASH) in sub-Saharan Africa and associations with undernutrition, and governance in children under five years of age: a systematic review. J. Dev. Orig. Health Dis. 12 (1), 6–33.
Montgomery, M.A., Desai, M.M., Elimelech, M., 2010a. Assessment of latrine use and quality and association with risk of trachoma in rural Tanzania. Trans. R. Soc. Trop. Med. Hyg. 104 (4), 283–289.
Montgomery, M.A., Desai, M.M., Elimelech, M., 2010b. Short report: comparing the effectiveness of shared versus private latrines in preventing trachoma in rural Tanzania. Am. J. Trop. Med. Hyg. 82 (4), 693–695.
Muhammad, N., Mohammed, A., Isiyaku, S., Adamu, M.D., Gwom, A., Rabiu, M.M., 2014. Mapping trachoma in 25 local government areas of Sokoto and Kebbi states, northwestern Nigeria. Br. J. Ophthalmol. 98 (4), 432–437.
Murenjekwa, W., Makasi, R., Ntozini, R., Chasekwa, B., Mutasa, K., Moulton, L.H., Tielsch, J.M., Humphrey, J.H., Smith, L.E., Prendergast, A.J., Bourke, C.D., Team, S. T., 2021. Determinants of urogenital schistosomiasis among pregnant women and its association with pregnancy outcomes, neonatal deaths, and child growth. J. Infect. Dis. 223 (8), 1433–1444.
Myaux, J., Ali, M., Felsenstein, A., Chakraborty, J., de Francisco, A., 1997. Spatial distribution of watery diarrhoea in children: identification of "risk areas" in a rural community in Bangladesh. Health Place 3 (3), 181–186.
Nasim, N., El-Zein, A., Thomas, J., 2022. A review of rural and peri-urban sanitation infrastructure in South-East Asia and the Western Pacific: highlighting regional inequalities and limited data. Int. J. Hyg Environ. Health 244, 113992-113992.
Nasir, W.A., Saw, Y.M., Jawidh, S., Kariya, T., Yamamoto, E., Hamajima, N., 2020. Determinants of diarrhea in children under the age of five in Afghanistan: a secondary analysis of the Afghanistan Demographic and Health Survey 2015. Nagoya J. Med. Sci. 82 (3), 545–556.
Nath, T.C., Eom, K.S., Choe, S., Mukutmoni, M., Khanum, H., Bhuiyan, J.U., Islam, K.M., Islam, S., Zohra, F., Park, H., Lee, D., 2022. An update of intestinal helminth infections among urban slum communities in Bangladesh. IJID Regions 5, 1–7.
Natnael, T., Lingerew, M., Adane, M., 2021. Prevalence of acute diarrhea and associated factors among children under five in semi-urban areas of northeastern Ethiopia. BMC Pediatr. 21 (1).
Newsom, S.W.B., 1972. Microbiology of hospital toilets. Lancet 300 (7779), 700–703.
Nigo, M.M., Odermatt, P., Salieb-Beugelaar, G.B., Morozov, O., Battegay, M., Hunziker, P.R., 2021. Epidemiology of schistosoma mansoni infection in ituri province, north-eastern democratic republic of the Congo. PLoS Neglected Trop. Dis. 15 (12).
Nigusie, A., Berhe, R., Gedefaw, M., 2015. Prevalence and associated factors of active trachoma among childeren aged 1-9 years in rural communities of Gonji Kolella district, West Gojjam zone, North West Ethiopia. BMC Res. Notes 8, 641-641.
Odagiri, M., Schriewer, A., Daniels, M.E., Wuertz, S., Smith, W.A., Clasen, T., Schmidt, W.-P., Jin, Y., Torondel, B., Misra, P.R., Panigrahi, P., Jenkins, M.W., 2016. Human fecal and pathogen exposure pathways in rural Indian villages and the effect of increased latrine coverage. Water Res. 100, 232–244.
Oduro-Kwarteng, S., Awuah, E., Nyarko, K.B., 2009. Shifting from Public Shared Toilets to Home Toilets in Urban Settlements: Implications of Household Demand in Kumasi, Ghana. 34th WEDC International Conference, Addis Ababa, Ethiopia.
Ojima, M., Toshima, Y., Koya, E., Ara, K., Kawai, S., Ueda, N., 2002a. Bacterial contamination of Japanese households and related concern about sanitation. Int. J. Environ. Health Res. 12 (1), 41–52.
Ojima, M., Toshima, Y., Koya, E., Ara, K., Tokuda, H., Kawai, S., Kasuga, F., Ueda, N., 2002b. Hygiene measures considering actual distributions of microorganisms in Japanese households. J. Appl. Microbiol. 93 (5), 800–809.
Olamiju, F., Nebe, O.J., Mogaji, H., Marcus, A., Amodu-Agbi, P., Urude, R.O., Apake, E., Olamiju, O., Okoronkwo, C., Achu, I., Mpama, O., 2022. Schistosomiasis outbreak during COVID-19 pandemic in Takum, Northeast Nigeria: analysis of infection status and associated risk factors. PLoS One 17 (1).
Omer, N., Bitew, B.D., Engdaw, G.T., Getachew, A., 2022. Utilization of latrine and associated factors among rural households in Takussa district, northwest Ethiopia: a community-based cross-sectional study. Environ. Health Insights 16.
Opara, K.N., Wilson, E.U., Yaro, C.A., Alkazmi, L., Udoidung, N.I., Chikezie, F.M., Bassey, B.E., Batiha, G.E.-S., 2021. Prevalence, risk factors, and coinfection of urogenital schistosomiasis and soil-transmitted helminthiasis among primary school children in Biase, southern Nigeria. J. Parasitol. Res. 2021, 6618394–12.
Palmeirim, M.S., Mrimi, E.C., Minja, E.G., Samson, A.J., Keiser, J., 2021. A cross-sectional survey on parasitic infections in schoolchildren in a rural Tanzanian community. Acta Trop. 213.
Phillips, A.E., Ower, A.K., Mekete, K., Liyew, E.F., Maddren, R., Belay, H., Chernet, M., Anjulo, U., Mengistu, B., Salasibew, M., Tasew, G., Anderson, R., 2022. Association between water, sanitation, and hygiene access and the prevalence of soil-transmitted helminth and schistosome infections in Wolayita, Ethiopia. Parasites Vectors 15 (1).
Pickering, A.J., Julian, T.R., Marks, S.J., Mattioli, M.C., Boehm, A.B., Schwab, K.J., Davis, J., 2012. Fecal contamination and diarrheal pathogens on surfaces and in soils among Tanzanian households with and without improved sanitation. Environ. Sci. Technol. 46 (11), 5736–5743.
Prüss-Ustün, A., Wolf, J., Bartram, J., Clasen, T., Cumming, O., Freeman, M.C., Gordon, B., Hunter, P.R., Medlicott, K., Johnston, R., 2019. Burden of disease from inadequate water, sanitation and hygiene for selected adverse health outcomes: an updated analysis with a focus on low- and middle-income countries. Int. J. Hyg Environ. Health 222 (5), 765–777.
Raj, S.J., Wang, Y., Yakubu, H., Robb, K., Siesel, C., Green, J., Kirby, A., Mairinger, W., Michiel, J., Null, C., 2020. The SaniPath exposure assessment tool: a quantitative approach for assessing exposure to fecal contamination through multiple pathways in low resource urban settlements. PLoS One 15 (6), e0234364.
Ramírez-Castillo, F.Y., Loera-Muro, A., Jacques, M., Garneau, P., Avelar-González, F.J., Harel, J., Guerrero-Barrera, A.L., 2015. Waterborne pathogens: detection methods and challenges. Pathogens 4 (2), 307–334.

Riahi, M., Mohammadi, A.A., Moghadam, V.K., Robati, Z.S., Bidkhori, M., 2018. Diarrhea deaths in children among countries with different levels of the human development index. Data Brief 17, 954–960.
Riess, H., Clowes, P., Kroidl, I., Kowuor, D.O., Nsojo, A., Mangu, C., Schuele, S.A., Mansmann, U., Geldmacher, C., Mhina, S., Maboko, L., Hoelscher, M., Saathoff, E., 2013. Hookworm infection and environmental factors in Mbeya region, Tanzania: a cross-sectional, population-based study. PLoS Neglected Trop. Dis. 7 (9).
Rusin, P., Maxwell, S., Gerba, C., 2002. Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. J. Appl. Microbiol. 93 (4), 585–592.
Rusin, P., Orosz-Coughlin, P., Gerba, C., 1998. Reduction of faecal coliform, coliform and heterotrophic plate count bacteria in the household kitchen and bathroom by disinfection with hypochlorite cleaners. J. Appl. Microbiol. 85 (5), 819–828.
Saha, J., Mondal, S., Chouhan, P., Hussain, M., Yang, J., Bibi, A., 2022. Occurrence of diarrheal disease among under-five children and associated sociodemographic and household environmental factors: an investigation based on national family health survey-4 in rural India. Children 9 (5).
Salam, A.S., Qayumi, R., Siddiqi, A.M., Naseem, M., Mansoor, M., Butcher, R., Bakhtiari, A., Renneker, K., Willis, R., Jimenez, C., Dejene, M., Safi, N., Heggen, A., Solomon, A.W., Harding-Esch, E.M., Alizoi, N., 2022. Prevalence of trachoma in 72 districts of Afghanistan in 2018-2019: results of 35 population-based prevalence surveys. Ophthalmic Epidemiol. 1–11.
Saxton, R.E., Yeasmin, F., Alam, M.U., Al-Masud, A., Dutta, N.C., Yeasmin, D., Luby, S.P., Unicomb, L., Winch, P.J., 2017. If I do not have enough water, then how could I bring additional water for toilet cleaning?! Addressing water scarcity to promote hygienic use of shared toilets in Dhaka, Bangladesh. Trop. Med. Int. Health 22 (9), 1099–1111.
Schmid-Hempel, P., Frank, S.A., 2007. Pathogenesis, virulence, and infective dose. PLoS Pathog. 3 (10), e147.
Schulz, S., Kroeger, A., 1992. Soil contamination with Ascaris lumbricoides eggs as an indicator of environmental hygiene in urban areas of north-east Brazil. J. Trop. Med. Hyg. 95 (2), 95–103.
Sclar, G.D., Penakalapati, G., Amato, H.K., Garn, J.V., Alexander, K., Freeman, M.C., Boisson, S., Medlicott, K.O., Clasen, T., 2016. Assessing the impact of sanitation on indicators of fecal exposure along principal transmission pathways: a systematic review. Int. J. Hyg Environ. Health 219 (8), 709–723.
Scott, E., Bloomfield, S.F., Barlow, C.G., 1982. An investigation of microbial contamination in the home. J. Hyg. 89 (2), 279–293.
Scoullos, I.M., 2020. Assessment of the Fate of Surrogates for Enteric Pathogens Resulting from the Surcharging of Combined Sewer Systems. PhD Thesis. IHE Delft.
Seyum, D., Fetene, N., Kifle, T., Negash, H., Kabeto, T., Gebre, M., Data, T., Tadele, T., Abayo, G., Wondimu, A., Butcher, R., Bakhtiari, A., Willis, R., Boyd, S., Jimenez, C., Negussu, N., Tadesse, F., Kebede, F., Dejene, M., Solomon, A.W., Harding-Esch, E.M., Sisay, A., 2022. Prevalence of trachoma from 66 impact surveys in 52 woredas of southern nations, nationalities and peoples' and Sidama regions of Ethiopia, 2017-2019. Ophthalmic Epidemiol. 1–10.
Shimelash, A., Alemayehu, M., Dagne, H., Mihiretie, G., Lamore, Y., Tegegne, E., Kumlachew, L., 2022. Prevalence of active trachoma and associated factors among school age children in Debre Tabor Town, northwest Ethiopia, 2019: a community based cross-sectional study. Ital. J. Pediatr. 48 (1).
Sinclair, R.G., Gerba, C.P., 2011. Microbial contamination in kitchens and bathrooms of rural Cambodian village households. Lett. Appl. Microbiol. 52 (2), 144–149.
Sinha, A., Nagel, C.L., Schmidt, W.P., Torondel, B., Boisson, S., Routray, P., Clasen, T.F., 2017. Assessing patterns and determinants of latrine use in rural settings: a longitudinal study in Odisha, India. Int. J. Hyg Environ. Health 220 (5), 906–915.
Sinharoy, S.S., Pittluck, R., Clasen, T., 2019. Review of drivers and barriers of water and sanitation policies for urban informal settlements in low-income and middle-income countries. Util. Pol. 60, 100957.
Soboksa, N.E., 2021. Associations between improved water supply and sanitation usage and childhood diarrhea in Ethiopia: an analysis of the 2016 demographic and health survey. Environ. Health Insights 15.
Soboksa, N.E., Hailu, A.B., Gari, S.R., Alemu, B.M., 2019. Water supply, sanitation and hygiene interventions and childhood diarrhea in Kersa and Omo Nada districts of Jimma Zone, Ethiopia: a comparative cross-sectional study. J. Health Popul. Nutr. 38 (1).
Solo-Gabriele, H.M., Wolfert, M.A., Desmarais, T.R., Palmer, C.J., 2000. Sources of Escherichia coli in a coastal subtropical environment. Appl. Environ. Microbiol. 66 (1), 230–237.
Sood, A., Amre, D., Midha, V., Sharma, S., Sood, N., Thara, A., Bansal, M., Juyal, G., Thelma, B.K., Seidman, E., 2014. Low hygiene and exposure to infections may be associated with increased risk for ulcerative colitis in a North Indian population. Ann. Gastroenterol. 27 (3), 219–223.
Steinbaum, L., Mboya, J., Mahoney, R., Njenga, S.M., Null, C., Pickering, A.J., 2019. Effect of a sanitation intervention on soil-transmitted helminth prevalence and concentration in household soil: a cluster-randomized controlled trial and risk factor analysis. PLoS Neglected Trop. Dis. 13 (2).
Stenström, T.A., Seidu, R., Ekane, N., Zurbrügg, C., 2011. Microbial Exposure and Health Assessments in Sanitation Technologies and Systems. Stockholm Environment Institute Stockholm.
Stephens, B., Azimi, P., Thoemmes, M.S., Heidarinejad, M., Allen, J.G., Gilbert, J.A., 2019. Microbial exchange via fomites and implications for human health. Current Pollution Reports 5, 198–213.
Ster, I.C., Niaz, H.F., Chico, M.E., Oviedo, Y., Vaca, M., Cooper, P.J., 2021. The epidemiology of soil-transmitted helminth infections in children up to 8 years of age: findings from an Ecuadorian birth cohort. PLoS Neglected Trop. Dis. 15 (11).
Stocks, M.E., Ogden, S., Haddad, D., Addiss, D.G., McGuire, C., Freeman, M.C., 2014. Effect of water, sanitation, and hygiene on the prevention of trachoma: a systematic review and metaanalysis. PLoS Med. 11 (2).
Strunz, E.C., Addiss, D.G., Stocks, M.E., Ogden, S., Utzinger, J., Freeman, M.C., 2014. Water, sanitation, hygiene, and soil-transmitted helminth infection: a systematic review and meta-analysis. PLoS Med. 11 (3).
Sun, S., Han, J., 2021a. Open defecation and squat toilets, an overlooked risk of fecal transmission of COVID-19 and other pathogens in developing communities. Environ. Chem. Lett. 19 (2), 787–795.
Sun, S., Han, J., 2021b. Unflushable or missing toilet paper, the dilemma for developing communities during the COVID-19 episode. Environ. Chem. Lett. 19 (1), 711–717.
Taleo, F., Macleod, C.K., Marks, M., Sokana, O., Last, A., Willis, R., Garae, M., Chu, B.K., Courtright, P., Kool, J., Taleo, G., Rory, J.J., Solomon, A.W., Global Trachoma Mapping, P., 2017. Integrated mapping of yaws and trachoma in the five northern-most provinces of Vanuatu. PLoS Neglected Trop. Dis. 11 (1).
Thomas, J., Gold, M., 2020. Resource Recovery from Human Excreta in Urban and Regional Settlements, the Palgrave Encyclopedia of Urban and Regional Futures. Springer International Publishing, Cham, pp. 1–13.
Tilley, E., Ulrich, L., Lüthi, C., Reymond, P., Schertenleib, R., Zurbrügg, C., 2014. Compendium of Sanitation Systems and Technologies, second revised edn. Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland.
Tsunoda, A., Otsuka, Y., Toguchi, A., Watanabe, K., Nishino, R., Takahashi, T., 2019. Survey on bacterial contamination of bidet toilets and relation to the interval of scrubbing these units. J. Water Health 17 (6), 863–869.
Tulu, B., Taye, S., Amsalu, E., 2014. Prevalence and its associated risk factors of intestinal parasitic infections among Yadot primary school children of south eastern Ethiopia: a cross-sectional study. BMC Res. Notes 7, 848-848.
UNDP, 2022. Human Development Index (HDI). https://hdr.undp.org/data-center/human-development-index#/indicies/HDI. (Accessed 22 June 2022).
United Nations, 2015a. The Millennium Development Goals Report 2015. https://www.un.org/millenniumgoals/2015_MDG_Report/pdf/MDG%202015%20rev%20(July%201).pdf. (Accessed 20 June 2022).
United Nations, 2015b. Transforming Our World: the 2030 Agenda for Sustainable Development. https://sustainabledevelopment.un.org/content/documents/21252030%20Agenda%20for%20Sustainable%20Development%20web.pdf. (Accessed 20 June 2022).
United Nations, 2021. The Sustainable Development Goal Reports 2021. https://unstats.un.org/sdgs/report/2021/The-Sustainable-Development-Goals-Report-2021.pdf. (Accessed 15 June 2022).
Verdon, T.J., Mitchell, R.J., van Oorschot, R.A., 2014. Swabs as DNA collection devices for sampling different biological materials from different substrates. J. Forensic Sci. 59 (4), 1080–1089.
Vilcins, D., Sly, P.D., Jagals, P., 2018. Environmental risk factors associated with child stunting: a systematic review of the literature. Ann. Glob. Health. 84 (4), 551.
Vujcic, J., Ram, P.K., Hussain, F., Unicomb, L., Gope, P.S., Abedin, J., Mahmud, Z.H., Islam, M.S., Luby, S.P., 2014. Toys and toilets: cross-sectional study using children's toys to evaluate environmental faecal contamination in rural Bangladeshi households with different sanitation facilities and practices. Trop. Med. Int. Health 19 (5), 528–536.
Wagner, E.G., Lanoix, J.N., 1958. Excreta Disposal for Rural Areas and Small Communities. World Health Organization, Geneva.
WHO, 2022. Sanitation. https://www.who.int/news-room/fact-sheets/detail/sanitation. (Accessed 16 August 2023).
WHO and UNICEF, 2015. Progress on Sanitation and Drinking Water: 2015 Update and MDG Assessment. World Health Organization, Geneva.
WHO and UNICEF, 2022. WHO/UNICEF JMP Monitoring. https://washdata.org/monitoring/sanitation (accessed. 2023.08.16).
Wogu, M., Okubotimibi, F., 2020. Enteric helminth parasites contamination of surfaces in selected public restrooms in Benin city, Nigeria. BIU. J. Basic Appl. Sci. 5 (1).
WoldeKidan, E., Daka, D., Legesse, D., Laelago, T., Betebo, B., 2019. Prevalence of active trachoma and associated factors among children aged 1 to 9 years in rural communities of Lemo district, southern Ethiopia: community based cross sectional study. BMC Infect. Dis. 19 (1).
Wolf, J., Prüss-Ustün, A., Cumming, O., Bartram, J., Bonjour, S., Cairncross, S., Clasen, T., Colford Jr., J.M., Curtis, V., De France, J., 2014. Assessing the impact of drinking water and sanitation on diarrhoeal disease in low-and middle-income settings: systematic review and meta-regression. Trop. Med. Int. Health 19 (8), 928–942.
Woyessa, E.T., Ashenafi, T., Ashuro, Z., Ejeso, A., 2022. Latrine utilization and associated factors among community-led total sanitation and hygiene (CLTHS) implemented kebeles in Gurage zone, southern Ethiopia: a cross-sectional study. Environ. Health Insights 16.
Wright, J., Liu, J., Bain, R., Perez, A., Crocker, J., Bartram, J., Gundry, S., 2014. Water quality laboratories in Colombia: a GIS-based study of urban and rural accessibility. Sci. Total Environ. 485–486, 643–652.
Ziegelbauer, K., Speich, B., Mäusezahl, D., Bos, R., Keiser, J., Utzinger, J., 2012. Effect of sanitation on soil-transmitted helminth infection: systematic review and meta-analysis. PLoS Med. 9 (1), e1001162.

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Association between exposure to wind turbines and sleep disorders: A systematic review and meta-analysis

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ABSTRACT

To date, there is scarce evidence on the association between sleep disorders and noise generated by wind turbines.

We searched six relevant electronic databases from the inception to May 2023 for relevant articles. The methodological quality of the included articles was evaluated using the US National Institutes of Health tool.

Fifteen articles met the inclusion criteria. The overall prevalence of sleep disorders among residents close to wind turbines was 34% (95% Confidence Interval, 0.22–0.47). Univariate meta-regressions for distance and sound power level showed that at higher distance the prevalence of sleep disorders decreases ($p = 0.010$) and with a higher sound power level the prevalence increases ($p = 0.037$). Furthermore, this systematic review and meta-analysis highlighted that the overall quality of current research on this topic is poor, and the methods to measure the results are often based on subjective assessments and not validated questionnaires.

In conclusion, our preliminary findings suggest that there may be a possible relation between exposure to wind turbines and sleep disorders, although no conclusions can be drawn in terms of causality due to the nature of the retrieved data and the poor quality of current evidence. Future studies should adopt a longitudinal design and focus on objective measurements, supported by validated subjective methods such as questionnaires.

1. Introduction

Over the past 15 years, European legislation has significantly promoted the development of renewable energy sources and set new targets and deadlines in order to answer the global increase in energy demand. The next commitment is to meet 32% of the energy needs through renewable energy by 2030, halve CO2 emissions compared to 1990, and move closer to near 100% independence from fossil fuels by the middle of this century. Despite the impact of the COVID-19 pandemic, renewable energy set a record for new power capacity in 2020 and was the only source of electricity generation to register a net increase in total capacity (REN21, 2021).

Wind energy is one of the fastest-growing renewable energy sources and the number of future plant installations is expected to substantially increase over the next few decades (Li et al., 2022). The wind energy industry recently enjoyed its second-best economic year, with almost 94 GW of installed capacity added, bringing the global wind power capacity to 837 GW, that is, up to 12% year-on-year growth. Europe played an important role in this growth, with new onshore installations increasing by 19% (GWEC, 2022).

The wind industry has confirmed its key role in the energy transition by presenting itself as one of the best technologies capable of ensuring compliance with international climate targets and reducing dependence on fossil fuels (Solarin and Bello, 2022). The wind is used to produce electricity by exploiting the kinetic energy of air moving through wind turbines (WTs) and other wind energy conversion systems. To ensure optimal efficiency, WTs are preferentially installed on hills, mountains, and in places that guarantee adequate convective conditions; however,

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these can also be placed in the open sea (the so-called offshore wind energy).

WTs are classified primarily based on their installation characteristics (onshore/offshore) and WT type (vertical/horizontal axis). The main difference between onshore and offshore wind farms is the foundation structure—while an onshore WT is based on concrete land, offshore turbines have foundations in water (floating) or on the seabed (fixed bottom). Compared to onshore WT, offshore wind power has the advantages of high wind speed, regional climate stability, and no significant visual impact; however, it has higher operating and maintenance costs and poor accessibility.

With the evidence of the various benefits of renewable energy sources over the last two decades, scientific research has focused on their potential environmental and health risks (Stefko et al., 2021).

Generally, wind energy has fewer environmental effects than other energy sources. WTs usually do not release air or water pollutants and do not require a water-cooling system. However, the reported impacts include changes in atmospheric conditions (Bodini et al., 2021) and accidental deaths of migratory birds colliding with the WTs (Katzner et al., 2017). In terms of possible human health effects, the noise produced by WTs is considered the main pollutant; however, other pollutants, such as vibrations and light contamination, could contribute to sleep disturbances. Furthermore, other issues such as noise sensitivity, visual impact, and landscape expectations can be considered as modifiers of the relationship between WTs exposure and health effects. The noise produced by the WT is both mechanical, owing to the friction of the rotor components and the generator transmission system, and aerodynamic, caused by the interaction of airflow with the blades (Lane et al., 2016). The WT blades moving through the air are capable of generating a broad spectrum of sounds, particularly low-frequency noise (LFN) in the range of 20–200 Hz, that can spread over long distances, potentially causing annoyance, sleep disturbances, and other adverse health effects (Smith et al., 2020). The latter include nausea, headaches, dizziness, fatigue, tinnitus, and cardiovascular symptoms (Turunen et al., 2021). The term “wind turbine syndrome” was coined by Pierpont (2009) to describe the association between these symptoms and exposure to WT noise.

Two previous systematic reviews and meta-analyses focused on sleep disorders in residents living near WTs plants (Liebich et al., 2020; Onakpoya et al., 2015). However, the results of these studies were inconclusive and partially contradictory. Specifically, Liebich et al. focused on studies reporting sleep outcomes in the presence of WTs using polysomnography and actigraphy and found that WT exposure did not affect key indicators of objective sleep. Onakpoya et al. analyzed self-reported sleep disturbance data and suggested that WT noise may be associated with increased odds of annoyance and sleep disorders. However, both studies concluded that there is a strong need for further evidence on this topic. Given the rapid technological development of this energy source and increasing worldwide spread, we considered it useful to update the results of previous studies focusing on subjective health assessment methodologies.

Therefore, given the relevance of this topic, the purpose of this systematic review and meta-analysis was to provide comprehensive data on the prevalence of sleep disorders among residents living near WTs and explore the possible associations between the distance from the WT and WT sound power levels.

2. Material and methods

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2015). We systematically searched for relevant articles in six electronic databases, including MEDLINE, PubMed, Embase, Cochrane Library, Web of Science, Scopus, and Reaxys from their inception to March 2022, and updated in May 2023.

2.1. Search strategy and study selection

The initial search strategy was exploratory and extensive and included studies on health outcomes, environmental issues, emissions, and experimental toxic effects of several renewable energy sources, such as biofuels, green hydrogen, solar power, carbon capture and storage, and nuclear fusion. Subsequently, a series of studies were conducted on the health outcomes of wind energy. Since health outcomes other than sleep disorders were sparse and highly heterogeneous, we decided to focus only on articles on sleep disorders. The search strategy was first executed on PubMed and then adapted for all databases. The following example search terms were used: “Wind" [Mesh], (“wind power" [Tiab, “wind turbine*" [Tiab], “wind farm*" [Tiab], “wind resource*" [Tiab], “wind energ*" [Tiab], “wind plant*" [Tiab]. An expert librarian was involved in database searches to ensure methodological rigor. Reference lists of the included articles were manually selected to identify other relevant articles.

Two researchers independently evaluated titles and abstracts. After the initial selection, two investigators independently evaluated the full text for potentially relevant articles. Any disagreements were resolved by consensus among the investigators or with the help of a third reviewer.

2.2. Inclusion and exclusion criteria

This systematic review and meta-analysis included data on sleep disorder rates among residents living in a wind farm area from cross-sectional investigations. The included articles investigated the presence of sleep disturbance using various questionnaires and tools. Despite methodological differences between studies, all examined the prevalence of sleep disorders related to noise exposure for subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and/or daytime dysfunction. No restrictions were applied for sound power level or distance from the dwellings. Similarly, no restrictions were applied to network connections or WT types.

Workers were excluded because of their different exposure patterns compared with residents and uncomparable results in terms of sleep disturbance. Offshore installations were excluded because they were a source of exposure mainly to workers and had less effect on onshore residents.

Only the articles published in peer-reviewed journals were included. Experimental studies, systematic reviews or meta-analyses, conference proceedings, theses, and letters to the editor were excluded. Articles for which the full text was not available online or upon request from the journal were excluded. Language exclusion criteria were not applied.

For multiple reports from the same sample, the most complete results (i.e., those based on the largest number of cases) were used.

2.3. Data extraction

In each study, the number of residents with sleep disorders was used as the primary outcome measure. ‘Sleep disorders’ is an umbrella term including sleep disturbance, poor sleep quality, insomnia, and restless leg syndrome (Karna et al., 2022). If more than one disorder was considered, the data with the highest prevalence for each disorder were used as the main outcome.

When prevalence rates were not directly reported, the results were extrapolated from the retrieved questionnaire scores. Residents with scores >5 on the Pittsburgh Sleep Quality Index (PSQI), or $\geq$ 6 on the Athens Insomnia Scale (AIS) were considered affected by a sleep disorder. Moreover, for studies that used a single question to assess perceived sleep quality, we used the same criteria adopted by the authors to identify residents reporting sleep disorders. Furthermore, if reported in the study, the data were stratified according to the distance from the WT (<500 m, 500–1000 m, 1000–1500 m, and >1500 m) and outdoor A-

weighted sound power level (SPL; <30 dB, 30–35 dB, 35–40 dB, 40–45 dB, and >45 dB).

The following study characteristics were extracted if reported in the article: publication year, country, study design, cohort size, socio-demographic characteristics of the respondents, number of wind farms, number of WTs, and WT power. Data were extracted by three independent reviewers, and any disagreements were resolved by a fourth reviewer.

2.4. Quality assessment

The methodological quality of articles was assessed using the National Institutes of Health Quality Assessment Tool (NIH, 2014). As the assessment tool did not provide cut-off values, the median score (median = 8) was calculated to define poor (score = 4–7), fair (score = 8–10), or good (score = 11–12) quality articles. Quality assessment was performed by three independent reviewers and the results were discussed with a fourth reviewer until a consensus was reached.

2.5. Statistical analysis

All analyses were performed using STATA SE/17 (StataCorp LLC, College Station, TX, USA) to estimate pooled mean effects and 95% confidence intervals (CI) using random-effects models. Before conducting the overall pooled prevalence meta-analysis, the heterogeneity of the prevalence estimates was evaluated by calculating the I^2 index and performing Cochran's Q test. An $I^2 > 50\%$ and Cochran's Q test p-values <0.05 represented a high degree of heterogeneity.

As high heterogeneity was expected due to the study design, random-effects meta-analyses with 95% CI were performed. Because the random-effects model resulted in a high mean square error in highly heterogeneous meta-analyses, a series of meta-analyses stratified by study quality was also performed. This assessment provided more robustness and led to the correct interpretation of the probability of coverage of the confidence interval regardless of heterogeneity. As the data were not normally distributed, a Freeman-Tukey double arcsine transformation was used to obtain the proportions collected from the included articles. This approach was used to stabilize variance in the data (Barendregt et al., 2013; Nyaga et al., 2014).

Subgroup meta-analyses were performed for distance and SPL. We performed a series of meta-regressions to examine the association between environmental factors and the prevalence of sleep disorders. We used a best-fit model to describe the relationship between sleep disturbances, the distance between dwellings and the WTs, and SPL.

We evaluated the presence of publication bias and small study effects by visual inspection of funnel plots and through a test proposed by Egger et al. (1997). Sensitivity analyses included repetitions of the main meta-analysis in which one article was removed to observe any effects.

3. Results

The exploratory database search yielded 12,242 articles. The initial screening of titles and abstracts, aimed at selecting articles related to health effects, and 618 articles were potentially relevant to our search. A manual reference search identified additional 171 potentially relevant studies. After duplicates were removed, 206 articles remained. These

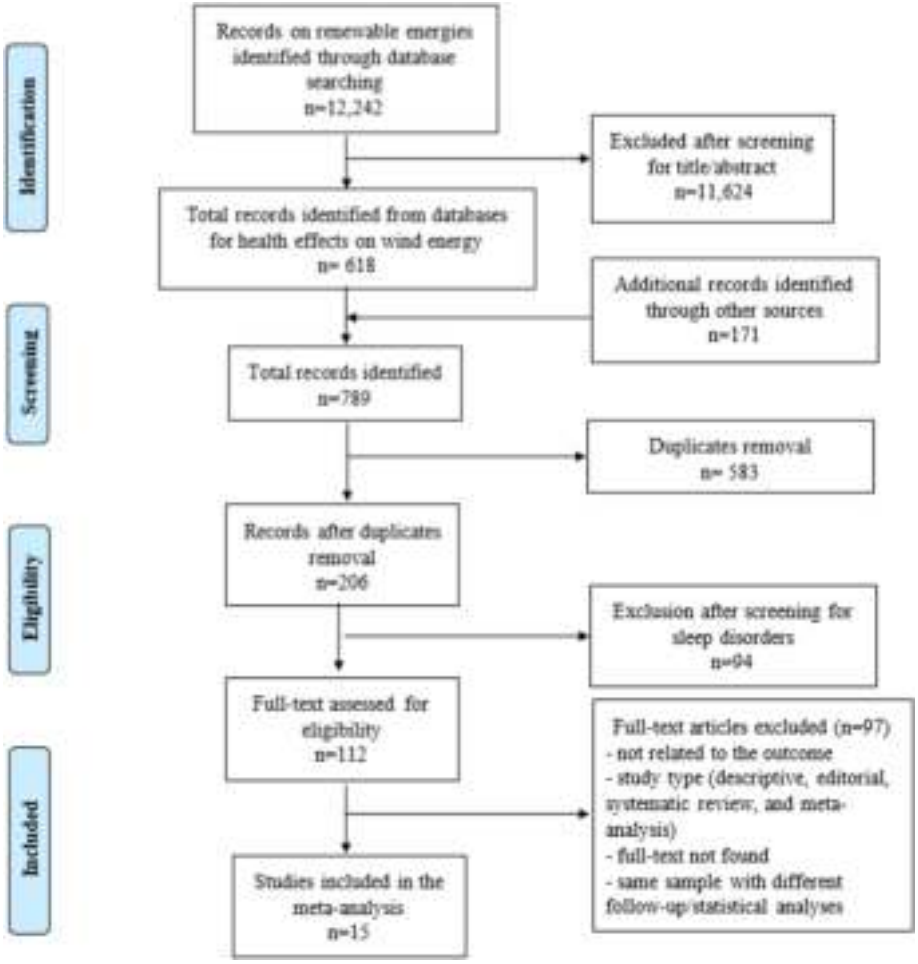

Fig. 1. Flow diagram for study selection.

articles were further screened to select those that were specifically relevant to sleep disorders. Thus, 110 studies were considered relevant for inclusion. The full texts of these articles were examined in detail and assessed according to the inclusion and exclusion criteria. Finally, 15 articles (Bakker et al., 2012; Ishitake et al., 2019; Jalali et al., 2016; Krogh et al., 2011; Magari et al., 2014; Michaud et al., 2016; Mroczek et al., 2015; Nissenbaum et al., 2012; Pawlaczyk-Łuszczyńska et al., 2018; Pedersen and Persson Waye, 2004; Pedersen and Waye, 2007; Qu and Tsuchiya, 2021; Song et al., 2016; Turunen et al., 2021a; 2021b) were included in the final analysis. The screening process is illustrated in Fig. 1.

Most studies were conducted in Europe (n = 8), followed by the United States and Canada (n = 5), Asia and Australia (n = 2). The selected articles were published between 2004 and 2021. In total, the data of 8,867 participants were analyzed.

All the studies evaluated subjective sleep quality using self-reported measures. Eleven studies used a single question to assess perceived sleep quality, whereas the remaining four studies adopted validated questionnaires. In particular, three studies used the PSQI, which measures seven components of self-perceived sleep quality (i.e., sleep quality, latency, duration, efficiency, disturbance, use of sleep medication, and daytime disturbance), and AIS, designed to measure the severity of insomnia symptoms.

The distance between the dwellings and WTs ranged from 495 to 3,093 m. The number of WTs ranged from 16 to 1,836 and their powers ranged from 0.5 to 3.5 MW. The mean SPL measured using A-weighting curves ranged from 33.4 dB to 42.6 dB. Four studies (Bakker et al., 2012; Ishitake et al., 2019; Michaud et al., 2016; Song et al., 2016) reported the prevalence rates of sleep disorders stratified by SPL, and three (Ishitake et al., 2019; Krogh et al., 2011; Nissenbaum et al., 2012) were stratified by distance from the WTs.

The mean age of the study participants, among the 13 studies, was

Table 1
Main findings of studies on sleep disorders.

Study	Country	Respondents	Age	Sleep disorders (Type of assessment)	Other outcomes investigated	Study Findings
Bakker et al. (2012)	Netherlands	725	52	Subjective sleep quality (Single question[b])	Annoyance	Sleep disturbance increased with SPL, especially above 45 dB (A), where 48% of the respondents reported sleep disturbance.
Ishitake et al. (2019)	Japan	2,192	58.1	Insomnia (AIS)	Annoyance	Audible noise (frequency of 20 Hz or over) produced by WTs can be a risk factor for sleep disorders
Jalali et al. (2016)	Canada	37	54	Subjective sleep quality (PSQI)	General attitude	Participants reported poorer sleep quality if they had a negative attitude toward WTs
Krogh et al. (2011)	Canada	97	52	Subjective sleep quality (Single question[c])	QoL, Signs and symptoms	Dose–response relationships between sleep disturbance, excessive tiredness, and headaches and distance from WTs
Magari et al. (2014)	US	62	51	Subjective sleep quality (Single question[b])	General attitude Annoyance Visual impact	No exposure-response relationship between annoyance levels and sound measurements. Positive correlation between general concerns about health effects and prevalence of sleep disturbances.
Michaud et al. (2016)	Canada	1,208	–	Subjective sleep quality (PSQI) Objective sleep quality (Actigraphy)	–	The results of the study do not support an association between exposure to outdoor WT noise up to 46 dB(A) and an increase in the prevalence of disturbed sleep.
Mroczek et al. (2015)	Poland	1,277	45.5	Subjective sleep quality (Single question[c])	QoL	The WTs in residential areas do not have a negative influence on quality of life. Sleep disorders were reported by 28.34% of the respondents.
Nissenbaum et al. (2012)	USA	38	–	Subjective sleep quality (PSQI)	QoL	Participants living within 1.4 km of a WT had worse sleep. Significant dose-response relationships between PSQI, ESS, SF36 mental component score, and log distance to the nearest WT were identified.
Pawlaczyk-kuszczyriska et al., (2018)	Poland	517	46.7	Subjective sleep quality (Single question[b])	Annoyance General attitude Mental health	There was no significant association between SPL/distance and various aspects of health.
Pedersen and Waye, (2007)	Sweden	754	51	Subjective sleep quality (Single question[b])	Sensitivity to noise Annoyance	Noise annoyance was associated with sleep quality and negative emotions. The odds of being annoyed by wind turbine noise also increased with increasing SPLs.
Pedersen and Persson Waye, (2004)	Sweden	351	48	Subjective sleep quality (Single question[b])	Sensitivity to noise Annoyance Visual impact	No correlations were found between sleep quality in general and outdoor noise, annoyance, indoor noise annoyance, attitude to visual impact and to WTs in general, or sensitivity to noise.
Qu and Tsuchiya, (2021)	UK	359	56	Subjective sleep quality (Single question[a])	QoL, Signs and symptoms	The WT noise was associated with some aspects of self-reported health, including raised health concerns, headaches, nausea, and ear discomfort, but was not directly related to sleep disturbances.
Song et al. (2016)	China	227	57	Subjective sleep quality (Single question[b])	Sensitivity to noise Annoyance	Noise sensitivity, noise annoyance, and noise intensity were significantly correlated with sleep disturbance.
Turunen et al. (2021a)	Finland	70	59	Subjective sleep quality (Single question[b])	Annoyance Signs and symptoms	Symptoms intuitively associated with wind turbine infrasound were relatively common (15%) within 2.5 km of the closest wind turbine and less common (5%) in the whole study area.
Turunen et al. (2021b)	Finland	1,180	60	Self reported[b]	Annoyance Signs and symptoms	Beyond annoyance and disturbance of sleep, there were no consistent associations between wind turbine exposure and self-reported health problems.

Abbreviations: AIS Athens Insomnia Scale, PSQI Pittsburgh Sleep Quality Index, WT Wind Turbine, QoL Quality of life; SPL Sound Power Level, dB Decibel, SF-36 Short Form Health Survey 36, GHQ-12 General Health Questionnaire 1

[a] Self-reported single question on presence of sleep disturbances (6-point Likert-type scale).

[b] Self-reported single question on presence of sleep disturbances (5-point Likert-type scale).

[c] Self-reported single question on presence of sleep disturbances (Yes vs No).

53.04 (SD ± 4.75). The number of respondents ranged from 37 to 1,965. Other sociodemographic characteristics such as marital status, smoking habits, and alcohol consumption were only occasionally reported.

The characteristics of the included studies, along with a summary of the main findings, are summarized in Table 1.

A complete quality assessment is reported in the Supplementary Material (Table S1). Two articles were of high quality, eight were of poor quality, and the remaining five were of fair quality.

3.1. Meta-analyses and meta-regression

The overall prevalence of sleep disorders among residents close to WTs was 34% (95% CI, 0.22–0.47) (Fig. 2).

The prevalence of sleep disorders among residents living <500 m from WTs was 79% (95% CI, 0.58–0.93), while the sleep disorders rates in the intervals 500–1000, 1000–1500, 1500–2000, 2000–3000, >3000 were, respectively, 65% (95% CI, 0.36–0.89), 41% (95% CI, 0.34–0.48), 29% (95% CI, 0.24–0.33), 22% (95% CI, 0.19–0.24), and 27% (95% CI, 0.22–0.33).

The lowest prevalence of sleep disorders was found at SPL <30 dB (31%; 95% CI, 0.17–0.46). Progressively higher rates were found at higher dB intervals as follows: 36% (95% CI, 0.25–0.48) at 30–35 dB, 49% (95% CI, 0.28–0.69) at 35–40 dB, 60% (95% CI, 0.22–0.92) at 40–45 dB, and 82% (95% CI, 0.75–0.88) at >45 dB.

Univariate meta-regression for distance (Ishitake et al., 2019; Krogh et al., 2011; Nissenbaum et al., 2012) and SPL (Bakker et al., 2012; Ishitake et al., 2019; Michaud et al., 2016; Song et al., 2016) showed that at a higher distance, the prevalence of sleep disorders decreased ($p = 0.010$) (Fig. 3a) and with a higher SPL, the prevalence increased ($p = 0.037$) (Fig. 3b).

3.2. Sensitivity analyses

The omission of any single study from the main meta-analysis did not significantly influence the pooled prevalence of sleep disorders, with a maximum variation of 3% in the outcome ($p < 0.01$). Furthermore, the meta-analysis performed after excluding low-quality articles did not show a significant difference in the prevalence rate, with an overall prevalence of sleep disorders of 31% (95% CI, 0.19–0.44). Univariate meta-regression of the quality scores revealed no significant association with the prevalence of sleep disorders.

The funnel plot for the overall meta-analysis was scattered and asymmetrical, indicating the presence of reporting bias (Supplementary Material, Fig. S1). Similarly, the results of Egger's test were statistically significant ($p < 0.05$) for the presence of a small study effect.

4. Discussion

This systematic review and meta-analysis investigated the prevalence of sleep disorders among residents living near WTs. Our results showed an overall prevalence of 34% in all the included studies. The actual impact of WTs noise exposure on the development of this disturbance is difficult to address given the possible exposure to other environmental sources of noise and the presence of many confounders and modifiers that can affect the prevalence of sleep disorders.

4.1. Sleep disorders in the general population

Several epidemiological studies have attempted to determine the prevalence of sleep disorders in the general population. An international survey by Leger et al. (Léger et al., 2008) conducted on a representative sample of the general population of the United States, France, Germany, Italy, Spain, the United Kingdom, and Japan, aged ≥15 years, showed that the prevalence of sleeping problems was 56% in the USA, 31% in Western Europe, and 23% in Japan. A recent article by Jahrami et al. (2021) published during the COVID-19 pandemic, highlighted that the global pooled prevalence rate of sleep problems among all included populations was 35.7%. As shown by the authors, there are consistent variations in the prevalence of sleep disorders in the general population (Jahrami et al., 2021). These variations could be explained by clinical and epidemiological difficulties in defining the diagnostic criteria for sleep disorders and, as a result, the heterogeneity of these definitions. Additionally, the inclusion criteria considered by different authors vary widely, and environmental noise exposure has been poorly reported. Other concerns regarding sleep disorders include a diagnosis not obtained by a specialist and the application of different scales to measure

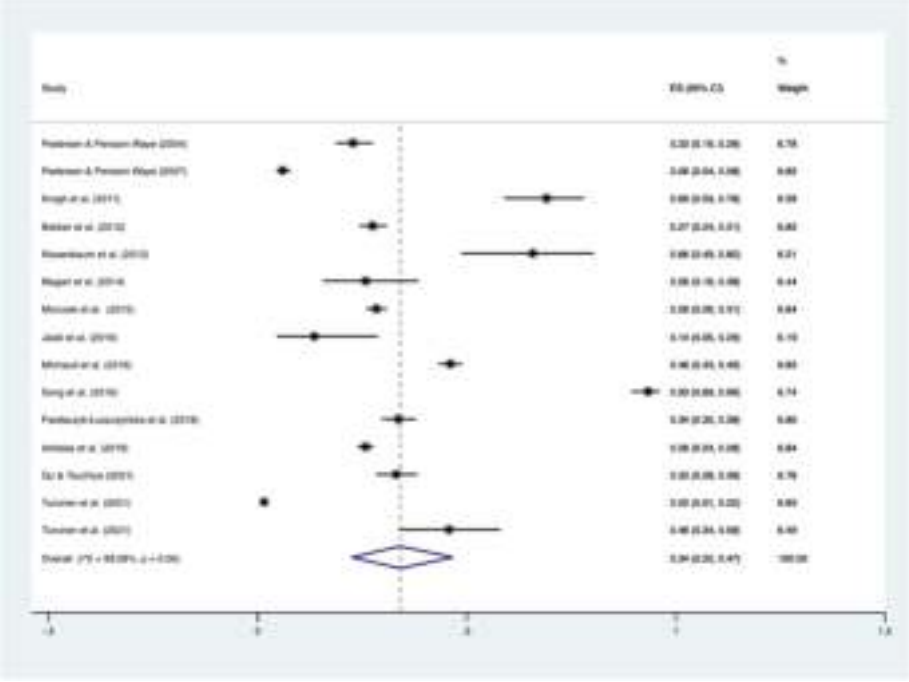

Fig. 2. Meta-analysis of the prevalence of sleep disorders in residents living near wind turbines. (ES: Effect Size, CI: Confidence Interval).

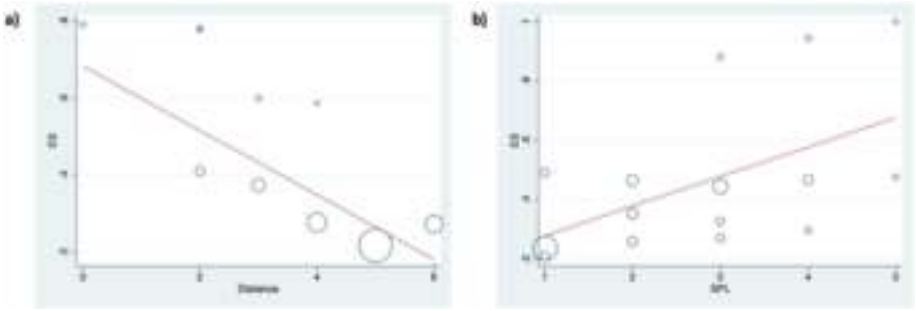

Fig. 3. Univariate meta-regression of the prevalence of sleep disorders by a) distance (p = 0.010) and b) sound power level (SPL) according to the following categories (1=<30 dB, 2 = 30–35 dB, 3 = 35–40 dB, 4 = 40–45 dB, 5= >45 dB (p = 0.037). Individual studies are represented by circles, with the size of the circle being inversely proportional to the variance of the estimated treatment effect (i.e. the larger the circle, the more precise the estimated treatment effect). The dotted line represents the regression line for the analysis.

sleep disorders that are difficult to compare. These confounders may have resulted in less reliable data from the original studies. Finally, sleep disorders are highly prevalent in the general population, with heterogeneous prevalence rates that vary between countries. For this reason, and due to the methodological limitations of the general population-based study mentioned above, it is difficult to understand the impact of WTs exposure on sleep disturbance. Consequently, we cannot state whether our value is higher or lower in absolute terms.

4.2. Sleep disorders due to transportation noise

WTs are responsible for producing a relatively large audible/subaudible noise in low-frequency and infrasound spectra. Another source of low-frequency emissions is background noise from many environmental sources such as road traffic, railways, and aircraft. Several studies are in agreement on the association of exposure with worse sleep outcomes (European Environmental Agency, 2022; Perron et al., 2012; Smith et al., 2022). The World Health Organization (WHO) recently published the Environmental Noise Guidelines for the European Region (Clark and Paunovic, 2018), highlighting knowledge gaps and research needs on this matter. They found limited evidence on the health impacts of transportation noise from large-scale cohort and case-control studies with objective measurements of both noise exposure and health outcomes. Moreover, it is difficult to establish whether the actual cause of sleep disturbances due to transportation noise is fully related to the low-frequency spectrum.

4.3. Relationship between sleep disorders and distance/SPL

As expected, in our study, the prevalence of sleep disorders decreased with increasing distance from WTs and decreasing SPL. Our results are similar to those reported by Onakpoya et al. (2015), where higher exposure to SPL revealed a significant increase in the chances of reporting sleep disturbances (OR 2.94; 95% Cl, 1.98–4.37). It should be highlighted that while the relationship between sleep disorders and SPL is based only on noise emissions, distance can be related to all WTs emissions, including visual impact. However, only four studies stratified sleep disorders by SPL and three by distance, increasing the risk of overfitting, thus reducing the generalizability of our conclusions.

4.4. Sleep disorders and objective measures of sleep quality

A systematic review and meta-analysis conducted by Liebich et al. (2020) focused on the impact of exposure to WTs using objective measures of sleep assessment, such as polysomnography and actigraphy. Their results showed no significant differences in objective sleep onset latency (0.03; 95% CI, -0.34–0.41), total sleep time (−0.05; 95% CI, -0.77–0.67), sleep efficiency (−0.25; 95% CI, -0.71–0.22), or wake-up after sleep onset (1.25; 95% CI, -2.00–4.50) in the presence versus absence of WTs (all p > 0.05). The authors did not stratify health outcomes and WTs exposure according to the SPL or distance.

4.5. Sleep disorders and experimental studies

Other studies have used experimental approaches to verify the hypothesis that WT noise is responsible for sleep-related health effects. However, it is difficult to simulate real-life conditions, such as exposure to WT noise, and consider the dose and duration of exposure. Moreover, experimental studies did not consider other sources of emissions. Finally, experimental studies cannot consider other factors related to WT-related annoyance, such as visual landscape impact, visual annoyance caused by stroke effects, moving shadows, safety issues, or social aspects (Simos et al., 2019). Experimental studies typically benefit from both controlled and replicable exposure conditions. For instance, Dunbar et al. (2021) examined the effect of WT noise compared with road traffic noise on sleep using quantitative electroencephalogram (EEG) power spectral analysis. Twenty-three participants were exposed to 3-min samples of WT noise and road traffic noise at three sound pressure levels (33, 38, and 43 dBA) in random order during established sleep. Their spectral analysis results showed subtle effects of noise on sleep, and that EEG changes after WT noise and road traffic noise onset differed depending on the SPLs. However, all the reported effects were mostly transient and had little impact on sleep scores. A study by Kasprak et al. (Kasprzak, 2014) investigated EEG variations in the delta, theta, alpha, SMR, Beta1, and Beta2 waves in humans exposed to infrasound noise. The experiment consisted of playing acoustic signals recorded from the WT at 750 m while testing EEG electric signals from 35 subjects. Their results showed changes in the EEG signal patterns registered under exposure to WT noise, and the specific frequency ranges of the EEG signals were altered.

4.6. Sleep disorders and factors not related to noise exposure

Another set of studies (Crichton et al., 2014a, 2014b; Crichton and Petrie, 2015) hypothesized that other factors, in addition to noise exposure, may contribute to the occurrence of health disorders and sleep disorders related to WT exposure. Some studies indicate that perceived symptoms can be explained by the nocebo response, whereby health concerns and negative expectations created from social discourse and media reports could trigger the reporting of symptoms. Other studies have suggested that negative expectations through WTs can create symptoms, or that positive expectations can produce the opposite effect, in terms of a reduction in symptoms and improvements in reported health. Moreover, several studies included in our review emphasized that sleep disturbance was highly correlated to subjective annoyance.

4.7. Limitations

This systematic review and meta-analysis has some limitations. The design of the included studies was cross-sectional and therefore could not be used to determine any specific causal relationships. The outcome was frequently measured using a single-question with various ranges of response scales and different reference timeframes for sleep disorders, making it difficult to compare the results of the included studies.

There was substantial heterogeneity in the definition of sleep disorders, and the response rates were often less than 50%, with an increased risk of selection bias. The quality of the included studies varied significantly. However, the overall level of evidence for the included studies was considered poor, with some of the elements considered in the quality assessment tool generally receiving low scores. Only a few studies have provided justifications for the sample size or discussed the statistical power of the study. Furthermore, less than half of the included studies stratified the prevalence results according to distance or SPL. Moreover, SPL measurements slightly differed between studies.

Experimental and laboratory studies were excluded because there were few reports with modalities of execution and experimental conditions that were very dissimilar or hardly comparable. Moreover, experimental and laboratory studies have been conducted on highly selected populations, making the results difficult to generalize. Observational studies are conducted in real-life contexts that are crucial for epidemiology as they allow researchers to test their assumptions and provide reliable evidence for making decisions in real-life population health interventions.

Additionally, self-reports can suffer from recall bias, particularly when the questions relate to the previous 12 months, as is typical for questions about sleep disturbance in most of the studies included in our meta-analysis. The results were not adjusted for all plausible confounders such as annoyance and other environmental stressors, including air pollution, light, temperature, and humidity. Furthermore, it is not possible to determine whether the association between exposure to WTs and sleep disorders is only caused by exposure to noise or whether other aspects, such as visual disturbances, economic problems, or attitudes toward noise, can affect the prevalence of sleep disorders.

5. Conclusions

These findings suggest that there may be a dose-response relationship between exposure to WTs and sleep disorders, although no conclusions can be drawn for causality. Future research should better define the pathologies that should be considered under the umbrella term ‘sleep disorders’ to compare the results of different studies. Future large-scale studies should adopt a longitudinal design and focus on objective measurements for the evaluation of sleep disorders, supported by validated subjective methods, such as questionnaires. Experimental studies in the same population could also provide information on the mechanisms linking exposure to WTs and sleep disorders.

Author contribution

Conceptualization, BD, NN, PB; Methodology, AG, MC, PB; Literature search and data abstraction: IM, GC, AF, ECa, VDP; Statistical analysis, AG, MC, CC; Writing – Original Draft Preparation, AG, MC, CC; Writing – Review & Editing, EP, BD, NN, PB; Supervision, AG, EP, PB; Project Administration, PB; Funding Acquisition, BD, NN, PB.

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Declaration of competing interest

Paolo Boffetta, Bruno Dallapiccola and Nicola Normanno received a honorarium as members of the scientific committee of Fondazione Eni Enrico Mattei (FEEM). Other Authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2023.114273.

References

Bakker, R.H., Pedersen, E., Berg, G.P. Van Den, Stewart, R.E., Lok, W., Bouma, J., 2012. Impact of wind turbine sound on annoyance , self-reported sleep disturbance and psychological distress. Sci. Total Environ. 425, 42–51. https://doi.org/10.1016/j.scitotenv.2012.03.005.

Barendregt, J.J., Doi, S.A., Lee, Y.Y., Norman, R.E., Vos, T., 2013. Meta-analysis of prevalence. J. Epidemiol. Community Health 67, 974–978. https://doi.org/10.1136/jech-2013-203104, 1978.

Bodini, N., Lundquist, J.K., Moriarty, P., 2021. Wind plants can impact long-term local atmospheric conditions. Sci. Rep. 11 https://doi.org/10.1038/s41598-021-02089-2.

Clark, C., Paunovic, K., 2018. WHO environmental noise guidelines for the European region: a systematic review on environmental noise and quality of life, wellbeing and mental health. Int. J. Environ. Res. Publ. Health 15. https://doi.org/10.3390/ijerph15112400.

Crichton, F., Dodd, G., Schmid, G., Gamble, G., Cundy, T., Petrie, K.J., 2014a. The power of positive and negative expectations to influence reported symptoms and mood during exposure to wind farm sound. Health Psychol. 33, 1588–1592. https://doi.org/10.1037/hea0000037.

Crichton, F., Dodd, G., Schmid, G., Gamble, G., Petrie, K.J., 2014b. Can expectations produce symptoms from infrasound associated with wind turbines? Health Psychol. 33, 360–364. https://doi.org/10.1037/a0031760.

Crichton, F., Petrie, K.J., 2015. Health complaints and wind turbines: the efficacy of explaining the nocebo response to reduce symptom reporting. Environ. Res. 140, 449–455. https://doi.org/10.1016/j.envres.2015.04.016.

Dunbar, C., Catcheside, P., Lechat, B., Hansen, K., Zajamsek, B., Liebich, T., Phuc, D., Hannah, N., Lack, L., Decup, F., Vakulin, A., Micic, G., 2021. RESEARCH ARTICLE EEG power spectral responses to wind farm compared with road traffic noise during sleep. A laboratory study 1–11. https://doi.org/10.1111/jsr.13517.

Egger, M., Smith, G.D., Schneider, M., Minder, C., 1997. Bias in meta-analysis detected by a simple, graphical test. BMJ 315, 629–634. https://doi.org/10.1136/bmj.315.7109.629.

European Environmental Agency, 2022. Health Impacts of Exposure to Noise from Transport [WWW Document].

GWEC, 2022. Global Wind Report-2022 n.d.

Ishitake, T., Morimatsu, Y., Hara, K., 2019. Epidemiological Study on Long-Term Health Effects of Low-Frequency Noise Produced by Wind Power Stations in Japan 1455–1462.

Jahrami, H., BaHammam, A.S., Bragazzi, N.L., Saif, Z., Faris, M., Vitiello, M. v, 2021. Sleep problems during the COVID-19 pandemic by population: a systematic review and meta-analysis. J. Clin. Sleep Med. 17, 299–313. https://doi.org/10.5664/jcsm.8930.

Jalali, L., Nezhad-Ahmadi, M.R., Gohari, M., Bigelow, P., McColl, S., 2016. The impact of psychological factors on self-reported sleep disturbance among people living in the vicinity of wind turbines. Environ. Res. 148, 401–410. https://doi.org/10.1016/j.envres.2016.04.020.

Karna, B., Sankari, A., Tatikonda, G., 2022. Sleep Disorder.

Kasprzak, C., 2014. The influence of infrasound noise from wind turbines on EEG signal patterns in humans. Acta Phys. Pol. 125, 20–23. https://doi.org/10.12693/APhysPolA.125.A-20.

Katzner, T.E., Nelson, D.M., Braham, M.A., Doyle, J.M., Fernandez, N.B., Duerr, A.E., Bloom, P.H., Fitzpatrick, M.C., Miller, T.A., Culver, R.C.E., Braswell, L., DeWoody, J. A., 2017. Golden Eagle fatalities and the continental-scale consequences of local wind-energy generation. Conserv. Biol. 31, 406–415. https://doi.org/10.1111/cobi.12836.

Krogh, C.M.E., Gillis, L., Kouwen, N., Aramini, J., 2011. WindVOiCe , a self-reporting survey : adverse health effects , industrial wind turbines , and the need for vigilance monitoring. https://doi.org/10.1177/0270467611412551.

Lane, J.D., Bigelow, P.L., Majowicz, S.E., McColl, R.S., 2016. Impacts of industrial wind turbine noise on sleep quality: results from a field study of rural residents in ontario, Canada. J. Environ. Health 79, 8–12.

Léger, D., Poursain, B., Neubauer, D., Uchiyama, M., 2008. An international survey of sleeping problems in the general population. Curr. Med. Res. Opin. 24, 307–317. https://doi.org/10.1185/030079907x253771.

Li, C., Mogollón, J.M., Tukker, A., Steubing, B., 2022. Environmental impacts of global offshore wind energy development until 2040. Environ. Sci. Technol. 56, 11567–11577. https://doi.org/10.1021/acs.est.2c02183.

Liebich, T., Lack, L., Hansen, K., Zajamšek, B., Lovato, N., Catcheside, P., Micic, G., 2020. A systematic review and meta-analysis of wind turbine noise effects on sleep using validated objective and subjective sleep assessments 1–23. https://doi.org/10.1111/jsr.13228.

Magari, S.R., Smith, C.E., Schiff, M., Rohr, A.C., 2014. Evaluation of community response to wind turbine-related noise in Western. New York State 16, 228–239. https://doi.org/10.4103/1463-1741.137060.

Michaud, D.S., Feder, K., Keith, S.E., Voicescu, S.A., Marro, L., Than, J., Guay, M., Denning, A., Murray, B.J., Weiss, S.K., Villeneuve, P.J., van den Berg, F., Bower, T., 2016. Effects of wind turbine noise on self-reported and objective measures of sleep. Sleep 39, 97–109. https://doi.org/10.5665/sleep.5326.

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L.A., 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst. Rev. 4, 1. https://doi.org/10.1186/2046-4053-4-1.

Mroczek, B., Banaś, J., Machowska-Szewczyk, M., Kurpas, D., 2015. Evaluation of quality of life of those living near a wind farm. Int. J. Environ. Res. Publ. Health 12, 6066–6083. https://doi.org/10.3390/ijerph120606066.

NIH, 2014. Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies [WWW Document]. URL. https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools. accessed 6.26.23.

Nissenbaum, M.A., Aramini, J.J., Hanning, C.D., 2012. Effects of industrial wind turbine noise on sleep and health. Noise Health 14, 237–243. https://doi.org/10.4103/1463-1741.102961.

Nyaga, V.N., Arbyn, M., Aerts, M., 2014. Metaprop: a Stata command to perform meta-analysis of binomial data. Arch. Publ. Health 72, 39. https://doi.org/10.1186/2049-3258-72-39.

Onakpoya, I.J., O'Sullivan, J., Thompson, M.J., Heneghan, C.J., 2015. The effect of wind turbine noise on sleep and quality of life: a systematic review and meta-analysis of observational studies. Environ. Int. 82, 1–9. https://doi.org/10.1016/j.envint.2015.04.014.

Pawlaczyk-Łuszczyńska, M., Zaborowski, K., Dudarewicz, A., Zamojska-Daniszewska, M., Waszkowska, M., 2018. Response to noise emitted by wind farms in people living in nearby areas. Int. J. Environ. Res. Publ. Health 15. https://doi.org/10.3390/ijerph15081575.

Pedersen, E., Persson Waye, K., 2004. Perception and annoyance due to wind turbine noise—a dose–response relationship. J. Acoust. Soc. Am. 116, 3460–3470. https://doi.org/10.1121/1.1815091.

Pedersen, E., Waye, K.P., 2007. Wind turbine noise, annoyance and self-reported health and well-being in different living environments. Occup. Environ. Med. 64, 480–486. https://doi.org/10.1136/oem.2006.031039.

Perron, S., Tétreault, L.-F., King, N., Plante, C., Smargiassi, A., 2012. Review of the effect of aircraft noise on sleep disturbance in adults. Noise Health 14, 58–67. https://doi.org/10.4103/1463-1741.95133.

Pierpont, N., 2009. Wind Turbine Syndrome: A Report on a Natural Experiment.

Qu, F., Tsuchiya, A., 2021. Perceptions of wind turbine noise and self-reported health in suburban residential areas. Front. Psychol. 12, 1–12. https://doi.org/10.3389/fpsyg.2021.736231.

REN21, 2021. Renewables 2021 Global Status Report.

Simos, J., Cantoreggi, N., Christie, D., Forbat, J., 2019. Wind turbines and health: a review with suggested recommendations. Environnement, Risques & Santé 18, 149–159. https://doi.org/10.1684/ers.2019.1281.

Smith, M.G., Cordoza, M., Basner, M., 2022. Environmental noise and effects on sleep: an update to the WHO systematic review and meta-analysis. Environ. Health Perspect. 130, 76001 https://doi.org/10.1289/EHP10197.

Smith, M.G., Ögren, M., Thorsson, P., Hussain-Alkhateeb, L., Pedersen, E., Forssén, J., Ageborg Morsing, J., Persson Waye, K., 2020. A laboratory study on the effects of wind turbine noise on sleep: results of the polysomnographic WiTNES study. Sleep 43. https://doi.org/10.1093/sleep/zsaa046.

Solarin, S.A., Bello, M.O., 2022. Wind energy and sustainable electricity generation: evidence from Germany. Environ. Dev. Sustain. 24, 9185–9198. https://doi.org/10.1007/s10668-021-01818-x.

Song, K., Di, G., Xu, Y., Chen, X., 2016. Community survey on noise impacts induced by 2MW wind turbines in China. J. Low Freq. Noise Vib. Act. Control 35, 279–290. https://doi.org/10.1177/0263092316676399.

Stefko, R., Gavurova, B., Kelemen, M., Rigelsky, M., Ivankova, V., 2021. Relationships between renewable energy and the prevalence of morbidity in the countries of the European union: a panel regression approach. Int. J. Environ. Res. Publ. Health 18. https://doi.org/10.3390/ijerph18126548.

Turunen, Anu W., Tiittanen, P., Yli-Tuomi, T., Taimisto, P., Lanki, T., 2021. Symptoms intuitively associated with wind turbine infrasound. Environ. Res. 192, 110360 https://doi.org/10.1016/j.envres.2020.110360.

Turunen, Anu W., Tiittanen, P., Yli-Tuomi, T., Taimisto, P., Lanki, T., 2021a. Self-reported health in the vicinity of five wind power production areas in Finland. Environ. Int. 151, 106419 https://doi.org/10.1016/j.envint.2021.106419.

Turunen, Anu W., Tiittanen, P., Yli-Tuomi, T., Taimisto, P., Lanki, T., 2021b. Symptoms intuitively associated with wind turbine infrasound. Environ. Res. 192, 110360 https://doi.org/10.1016/j.envres.2020.110360.

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Exposure of the general French population to herbicides, pyrethroids, organophosphates, organochlorines, and carbamate pesticides in 2014–2016: Results from the Esteban study

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ABSTRACT

Esteban is a nationwide cross-sectional study conducted in France in 2014–2016, including 2503 adults aged 18–74 years old and 1104 children aged 6–17 years old, as part of the French Human Biomonitoring programme. The present paper describes the biological levels of five families of pesticides analysed on random sub-samples of 900 adults and 500 children for urine concentrations, and 759 adults and 255 children for serum concentrations, and the determinants of exposure. Organophosphates, carbamates and herbicides were measured in urine by UPLC-MS/MS; chlorophenols and pyrethroids were measured in urine by GC-MS/MS; specific organochlorines were measured in serum by GC-HRMS. Multivariate analyses were performed to identify the determinants of exposure using a generalized linear model. Pyrethroid metabolites were quantified in 99% of adults and children, with the exeption of F-PBA, which was quantified in 31% of adults and 27% of children, respectively. Carbamates and some specific organophosphates were barely or not quantified. DMTP was quantified in 82% of adults and 93% of children, and γ-HCH (lindane) was quantified in almost 50% of adults and children. Concentration levels of pesticide biomarkers were consistent with comparable international studies, except for β-HCH, DMTP, and the deltamethrin metabolite Br2CA, whose levels were sometimes higher in France. Household insecticide use and smoking were also associated with higher levels of pyrethroids. All pyrethroids concentration levels were below existing health-based HBM guidance values, HBM-GV$_{GenPop}$, except for 3-PBA, for which approximately 1% and 10% of children were above the lower and upper urine threshold values of 22 μg/L and 6.4 μg/L, respectively. Esteban provides a French nationwide description of 70 pesticide biomarkers for the first time in children. It also describes some pesticide biomarkers for the first time in adults, including glyphosate and AMPA. For the latter, urine concentration levels were overall higher in children than in adults. Our results highlight a possible beneficial impact of existing regulations on adult exposure to organochlorine and organophosphate pesticides between 2006 and 2016, as concentration levels decreased over this period.

1. Introduction

A pesticide is any substance or combination of chemical or biological substances intended to repel, destroy or control harmful organisms, or to be used as a plant growth regulator (FAO, 2021). The term 'pesticides' also refers to a broad classification of plant protection products, which includes various categories of chemical substances according to their main targets (insecticides and acaricides, herbicides, fungicides, nematicides, rodenticides). France is one of the world's largest users of plant protection products, with approximately 83,983 tonnes of pesticides

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being sold there in 2018, the largest user over the period in the European Union (Eurostat data[1]). Approximately 90% of total pesticide consumption in France is used in agriculture (Aubertot et al., 2005); this facilitates the ubiquitous presence of pesticides in the human environment (in particular through pesticide spreading) and widespread contamination of the whole food chain. People come into contact with pesticides in several ways, including their use in food, in the community or household environment, in and around homes and gardens, on pets, and at work (NPIC, 2022).

Many pesticides have been associated with health and environmental issues (Nicolopoulou-Stamati et al., 2016). Exposure can be overt or subacute, and effects range from acute to chronic (Roberts et al., 2012). Health effects depend on the type of pesticide. Some, such as organophosphates and carbamates, affect the nervous system. Others can irritate the skin or eyes. Some are carcinogenic while others can affect the body's hormonal or endocrine system. The risk of pesticide exposure to human health depends on both the toxicity or hazard of the pesticide and the likelihood of exposure (EPA, 2022). Epidemiological studies have shown the strong likelihood that exposure to certain pesticides is associated with the occurrence of certain diseases, such as non--Hodgkin's lymphoma, multiple myeloma, prostate cancer, and Parkinson's disease in adults (INSERM, 2022). This is especially true for people most exposed to pesticides because of their regular use in their occupational activities. In addition, studies have also shown the strong likelihood that acute leukemia in children is associated with the domestic use of pesticides by mothers during pregnancy (INSERM, 2022). Repetitive low-level and combined non-acute exposure to pesticides is also a cause for concern. In children, the health effects associated with chronic exposure to pesticides are diverse, reflecting the diversity of the toxicological properties of this broad group of different chemicals (Roberts et al., 2012).

Human biomonitoring (HBM) is a valuable and pratical tool for assessing human exposure to chemicals, including toxicants (Angerer et al., 2007). It integrates all routes of uptake and all sources of exposure through different pathways, such as the living or working environment, diet, and the use of consumer products (Gilles et al., 2021). It allows scientists and public health actors to identify new chemical exposures, trends and changes in exposure, vulnerable groups and populations with higher exposure, and environmental risks at specific contaminated sites with relatively low expenditure. It also allows them to establish exposure distribution in the general population (Ougier et al., 2021). For several years, national HBM programmes in several countries have continuously assessed and monitored population exposure to various chemical substances (Seifert et al., 2000; Becker et al., 2006; Schoeters et al., 2017; Health Canada and Health, 2020; CDC, 2021a; b; Schoeters et al., 2022). The French national HBM programme was established in 2010 to provide a national representative estimation of the French population's exposure to various environmental chemicals, and to better investigate the determinants of exposure (Dereumeaux et al., 2017). The Esteban survey, conducted in 2014–2016, was one component of this programme. It targeted the general population of adults and children living in mainland France. In addition, Esteban will also make it possible, for certain chemical substances, to assess changes in exposure over time compared with the previous French national representative study ENNS (Fréry et al., 2013). Previous bans on certain pesticides does not automatically eliminate exposure, especially for the most persistent chemical substances in the environment. The pesticides measured in Esteban were all selected as part of the French national HBM programme, depending on their biomonitoring feasibility, exposure relevance, existing regulations, and priorities in terms of health effects (Fillol et al., 2014).

The aim of this article was to describe the biomonitoring concentration levels of 70 biomarkers of pesticides in the general French population, as well as the determinants of exposure levels. Five families of pesticides were studied, covering regulated and unregulated chemical substances which were in use at the time of Esteban, namely organochlorines, organophosphates, carbamates, pyrethroids, and herbicides.

2. Material and methods

2.1. Study design and individual data collection

Esteban is a cross-sectional study based on a representative sample of the general population living in mainland France during the period 2014–2016. The survey protocol was approved by an Ethical Committee and the French Data Protection Authority (authorization no 2012-A00456-34).The study design is described in detail elsewhere (Balicco et al., 2017). Briefly, 2503 adults and 1104 children were included between April 2014 and March 2016. Three-stage sampling was implemented as follows: a sample of municipalities was first selected; then a sample of households was selected from each selected municipality; finally, a sample of individuals was selected from each selected household (one eligible individual per household). Two independent samples were formed, one including children 6–17 years old, and the other including adults 18–74 years old. Data were collected using sociodemographic, behavioral and environmental questionnaires, food questionnaires, and a health examination comprising anthropometric measurements and biological samples. The latter were collected either at a National Health Insurance examination centre or at participants' homes. Participants collected a first morning urine sample at home in a polypropylene container. A nurse collected fasting blood samples by venous catheter. Once in the laboratory, after centrifugation, urine and serum samples were aliquoted in polypropylene cryotubes (10 mL, 5 mL, 2 mL and 1 mL), before being stored at −80 °C in the biobank for long-term conservation.

A sub-sample of participant health examinations was randomly selected to measure a range of pesticides (organophosphates, organochlorines included chlorophenols, pyrethroids, carbamates, and herbicides). Laboratory analyses were performed on random sub-samples of all the children and adults who had a sufficient quantity of serum or urine in the biobank, specifically 900 adults and 500 children for urine, and 759 adults and 255 children for serum.

2.2. Pesticide biomarker measurements

Seventy pesticide biomarkers were selected in Esteban after a prioritization process which took into account their importance in terms of public health (Fillol et al., 2014). The laboratories that performed the biomarker measurements were selected by *Santé publique France* (the French Public Health Agency) in a call for tenders based on price, quality, performance, delivery, suitability, and experience in HBM studies (Fillol et al., 2021). Each laboratory was provided either a volume of urine or serum to determine all the metabolites of a specific family of pesticide biomarkers. The limits of detection (LOD) of analytical methods in France are determined according to the European Directive 2002/657/EC, which specifies the number of identification points for each different method. Laboratories must define the limit of quantification (LOQ) by performing repeated measurements ($n = 10$) in the assay matrix (serum, urine). They must also evaluate the accuracy and intermediate precision of the analytical method at this concentration level. This corresponds to the limit of the method used in these conditions and not to the instrumental limit. For each pesticide biomarker measured, the LOD and LOQ were constant for all subsamples analysed. The process followed by the laboratories to validate certain analytical methods is covered in Supplementary Information.

Labocea (Plouzané, France) performed the analyses of organophosphates, pyrethroids, chlorophenols, and carbamates. Urinary concentrations of organophosphates and carbamates were quantified using

[1] https://ec.europa.eu/eurostat/databrowser/view/aei_fm_salpest09/default/table?lang=en.

Acquity ultra-high-performance liquid chromatography H-Class system with tandem mass spectrometry Xevo TQ-S (UPLC-MS/MS) (Waters, Milford, USA). Data analysis was performed using TargetLynx software from Waters. Analyses were performed using multiple reaction monitoring (MRM) in positive/negative electrospray ionization (ESI) mode measuring at least two specific transitions. Urinary concentrations of pyrethroids and chlorophenols were quantified using gas chromatography (Agilent 700B, USA) with tandem mass spectrometry (GC-MS/MS) (with the MRM mode measuring at least three specific transitions). Data analysis was performed using MassHunter software from Agilent. Labocea's characterisations are carried out in accordance with standards NF T90-210 and SH GTA 14. Labocea is also participating in EQUAS for pentachlorophenol (the only compound in the chlorophenol family proposed by G-EQUAS). A quality control was analysed every ten samples and a blank every twenty samples.

The analyses of specific organochlorines were performed by Laberca/Oniris (Nantes, France). Serum concentrations were quantified using gas chromatography (Agilent 7890A) coupled with high resolution mass spectrometry (GC-HRMS) on electromagnetic sector instruments (JEOL MS 700D or 800D), operating at 10,000 resolution and in the single ion monitoring (SIM) acquisition mode. Laberca ensures the performance of the method used and the maintenance of its skills by taking part in inter-laboratory tests at national and international level. This participation also makes it possible to check and the following points: data quality and validity, laboratory performance (robustness, accuracy), the competence of its staff, and possible sources of error and ways of for improvement. For specific organochlorines, Laberca participates every year in at least two proficiency tests on food and feed organized by the European Reference Laboratory POPs and in several tests on human serum samples organised by the QUEBEC Toxicology Centre.

Labéo (Saint-Contest, France) performed the analyses of herbicides. Urinary concentrations were quantified using ultra-high-performance liquid chromatography (Agilent 6495) with tandem mass spectrometry (UPLC-MS/MS) with at least two specific MRM transitions for each compound. The standard solutions used for internal calibration were prepared in synthetic urine with same protocol than samples. The control quality procedure was performed with analysis of negative, and 3 positive control urine samples (low-medium and high level) every ten samples in each batch of analysed samples. The control quality samples followed all the analytical process. The laboratory performance (LOD, LOQ, robustness, accuracy) were determined for each method according to NF AFNOR T90-210. LABEO participates every year in proficiency testing program OSEQAS (Organic Substances in urine Quality Assessment Scheme) for Glyphosate and AMPA in urine organized by the QUEBEC Toxicology Centre/INSPQ.

Analytical methods: i) The extraction of **organophosphates** (chlorpyrifos, chlorpyrifos-methyl, chlorpyrifos methyl-oxon, chlorpyrifos-oxon, TCPy, parathion, parathion-methyl and para-nitrophenol) was performed with Solid Phase Extraction-off line, Oasis HLB well Plate, 30 μm, 2 mg, Waters (Milford, Massachusetts, USA). Five internal standards were used for extraction and detection control: Chorpyrifos D_{10}, chlorpyrifos-methyl D_6, parathion-methyl D_6, parathion D_{10} and para-nitophenol D_4. The sample volume used was 300 μl, diluted by 300 μl of water 4% formic acid. The elution was done using a methanol–acetonitrile solution. A volume of 10 μl was injected in a column Acquity UPLC® BEH C18 1.7 μm 2.1 × 50 mm (Waters, Milford, USA). The eluents used were water/acetonitrile +0.01% formic acid with a gradient from 95 to 5% water 0.01% formic acid, in an oven at 40 °C temperature with a flow rate of 0.4 ml/min ii) The extraction method of **dialkyl phosphate metabolites** (DMP, DMTP, DMDTP, DEP, DETP, DEDTP) was 96-well μElution Solid Phase Extraction-off line, Oasis Wax well Plate, 30 μm, 2 mg, Waters (Milford, Massachusetts, USA) for purification and concentration purposes. Two internal standards were used for extraction and detection control: DETP D10 and DMTP D6. The sample volume used was 200 μl, diluted by 200 μl of water 4% formic acid. The compounds were eluted using a 5% ammonium hydroxide solution in acetonitrile. Volume of 15 μl was injected in a column Acquity UPLC® BEH amide 1.7 μm 2.1 × 100 mm (Waters, Milford, USA) in an oven at 40 °C. The eluents used were water 50 mM ammonium acetate/acetonitrile with a gradient from 90% to 20% acetonitrile, with a flow rate of 0.3 ml/min iii) **Pyrethroids** (3-PBA, F-PBA, cis-DBCA, cis-DCCA, trans-DCCA) were simultaneously extracted with dichloromethane and derivatized with PFBBr (PentaFluoroBenzyl Bromide) in the presence of TBHAS buffer (tetrabutylammonium hydrogenated sulphate). The internal standard (cis-DCCA – D6) was added directly to 5 mL of sample at the start of the analysis for extraction and detection controls. A purification phase on a Florisil column was carried out, then analytes were eluted and concentrated. iv) The extraction of **chlorophenols** (4-MCP; 2,4-DCP; 2,5-DCP; 2,6-DCP; 2,3,4-TCP; 2,4,5-TCP; 2,4,6-TCP; 2,3,4,6-TeCP; PCP) was performed with liquid-liquid extraction in acid conditions. The internal standards (pentachlorophenol 13C6 and 2,4 Dichlorophenol D3) were added directly to 5 mL of sample at the start of the analysis for extraction and detection controls. A purification phase on a Florisil column was carried out, then analytes were eluted, concentrated and a derivatization with acetic anhydride was realized. v) The extraction of **carbamates** (Propoxur, 2-IPP, Carbofuranphenol) was performed with Solid Phase Extraction-off line, Oasis HLB well Plate, 30 μm, 2 mg, Waters (Milford, Massachusetts, USA). Two internal standards were used for extraction and detection control: propoxur D7, carbofuran D3. The sample volume used was 300 μl, diluted by 300 μl of water 4% formic acid. The elution was done using a methanol–acetonitrile solution. A volume of 10 μl was injected in a column Acquity UPLC® BEH C18 1.7 μm 2.1 × 100 mm (Waters, Milford, USA). The eluents used were water/acetonitrile with a gradient from 90% to 10% water in an oven at 40 °C temperature with a flow rate of 0.3 ml/min vi) The methodology applied to isolate, detect, and quantify **specific organochlorines** is that described earlier by other authors (Ploteau et al., 2016). Briefly, 13C-labeled congeners were added to each sample for quantification according to the isotopic dilution method. Serum (4–6 mL) samples were first submitted to an acidic degradation with formic acid during 15 min. The reaction was stopped using water. The extraction of specific organochlorines was then performed on a C18 Solid Phase Extraction (SPE) column followed by a Florisil SPE column. All internal exposure data were generated blindly among the different collected samples whatever the status of individuals. QA/QC procedures included systematic analysis of negative blank following the entire process, and 3 positive control samples (low-medium and high level) in each batch of analysed samples. All the analyses were conducted in an ISO 17025:2017 accredited laboratory. Recoveries were in the 70–120% range, and the method's extended uncertainty was calculated at low, medium and high level in serum for each compound. vii) Two methods were used for the analysis of **herbicides**. Atrazine, atrazine-desethyl, atrazine-desethyl-2-hydroxy, atrazine-desisopropyl, atrazine-desethyl-desisopropyl, atrazine-2-hydro xy, atrazine mercapturate, 2,4-Dichlorophenoxyacetic acid, alachlor, alachlore mercapturate, 2,6-Diethylaniline, simazine, simazine-2-hydroxy, simazine mercapturate, Isoproturon-monodemethyl (IPPMU), diuron, chlortoluron, isoproturon, and dimetachlor were extracted with Solid Phase Extraction (SPE) online. The urine sample used was 100 μl. 7 internal standards were added and the sample was diluted with water until 1 ml. A volume of 450 μl was introduced in analytic system for SPE extraction using Oasis HLB (2,1 mm × 20 mm x 25 μm), then the chromatographic separation was performed on a BEH C18 1.7 μm 2.1 × 150mm column). The column temperature was kept at 40 °C during the analysis and a gradient elution with flow rate of 0.35 ml/min was used with ultrapure water and acetonitrile with both 0.005% of formic acid. The analysis of glyphosate and its metabolite AMPA required derivatization with fluorenylmethoxycarbonyl chloride (FMOCCl) in order to reduce the polarity and increase the retention of the molecules during separation on a reversed phase polarity chromatography column. This was followed by purification. 200 μl urine sample

were diluted with 700 µl of water and 100 µl of internal standard solution (glyphosate 13C215N and AMPA 13C15N). The derivatization was done during the night with 1 ml FMOC-cl and 1 ml Borate buffer (pH 9.5). This step was stopped by adding 2 ml of dichloromethane and the mixture was shaked. This extract was centrifugated and 500 µl of supernatant was purified and concentrated with online SPE using Oasis HLB (2,1 mm × 20 mm x 25 µm). The analysis was performed on Acquity UPLC BEH C18 1.7 µm 2.1 × 150mm column (Waters, Milford, USA) kept at 60 °C with an elution gradient using acetonitrile and water 2 mM ammonium bicarbonate with a 0.4 ml/min flow rate.

Table 1 describes the type and volume of biological matrix, as well as the analytical method used and associated limits of detection (LOD) and of quantification (LOQ) for each pesticide biomarker analysed in the Esteban study.

2.3. Urinary creatinine and serum lipids measurements

Analyses of creatinine were performed by Chemtox, France, using the kinetic Jaffe method (JAFFE, 1886). Cholesterol and triglycerides levels were determined by Laberca using the ISO-17025 accredited enzymatic-colorimetric method, on serum obtained at the same time as the serum used for the analyses of persistent organic pollutants. Total lipid (TL) concentration was calculated using the following formula: TL = 1.677 * (TC − FC) + FC + TG + PL (all expressed in grammes per litre), where TC is the total cholesterol, FC is free cholesterol, TG is triglycerides and PL is phospholipids (Akins et al., 1989). Urinary concentrations of pesticide biomarkers were expressed as volume-weighted concentrations - specifically microgrammes per litre - and adjusted for urine creatinine by including creatinine in the results (Barr et al., 2005). Serum concentrations of specific organochlorines were expressed on a wet-weight basis - specifically nanogrammes per litre -, and were adjusted for serum lipids as nanogrammes per gramme of total serum lipids, to take their lipophilic nature into account (Mussalo-Rauhamaa, 1991).

2.4. Statistical analysis

The statistical analysis method used was broadly similar to that previously described by Fillol et al. (2021) (i.e., first published results from the Esteban survey). All statistical analyses were performed separately for children and for adults (Balicco et al., 2017). All statistical analyses (descriptives and multivariates) were weighted except for the sample of children measured in serum due to the small sample size (255 children). The geometric mean, median, and 25th, 75th, 90th and 95th percentiles were estimated for each biomarker. When the proportion of values below the LOQ was greater than 40%, geometric means were not estimated.

Factors influencing pesticide biomarker levels were identified through a multivariate analysis using a generalized linear model (GLM). Pesticide level values were log-transformed to ensure that the residuals were normally distributed. Some factors were included in the model *a priori* after a literature search on the relationship between these factors and the specified biomarker. Other factors were selected during the modelling process based on statistical criteria, including the Akaike Information Criterion (AIC). To take into account the possible nonlinear relationship between a given biomarker and continuous factors, a natural cubic spline function was fitted. Models were not run when the subsample was small (i.e., included few individuals, for example as was the case with children).

Four different types of factors were considered as follows: i) individual and dietary factors: tobacco consumption; eating and drinking habits; consumption of various products from one's own garden or livestock; consumption of various products from organic farming; ii) domestic parameters and do-it-yourself activities: machining, manipulation of treated wood; working in old houses; wood renovation; domestic use of pesticides; time spent in house; home ventilation frequency; iii) housing and environmental parameters: type of habitat; characteristics of the dwelling; place of residence; housing nearby a railway line, a cultivation area, a public garden, an incinerator or a waste dump; iv) occupational factors: current or past professional activities in agriculture, arboriculture, cereal crops, working as a florist, gardening, nursery plants, market gardening; professional activities with possible exposure to pesticides; certain exposures in current workplace. Confounding variables were: age and sex of participants, body mass index (BMI), educational level, marital status of head of the child's household (in child models only), number of children in the household (in adult models only), and urinary creatinine (for urine biomarkers only). Results are presented either as percentage changes of pesticide biomarker concentrations, associated with an interquartile increase of quantitative variables, or compared to reference groups for the qualitative variables.

Missing data in a questionnaire and left-censored data (i.e., missing data due to chemical levels below the LOD or LOQ) were imputed using multiple imputations (ICE: STATA module) (Little and Rubin, 2002; Royston and White, 2011).

The concentration levels of urinary biomarkers expressed per gram of creatinine allowto take into account the effects of urinary dilution among participants, as well as certain physiological differences (renal function, lean body mass) (Barr et al., 2005; Pearson et al., 2009). Creatinine excretion may vary depending on the age, sex and race/-ethnicity. It is not advisable to compare creatinine-corrected levels of different demographic groups (e.g. adults – children, men – women) (Barr et al., 2005). In Esteban, the detection rate of urine creatinine was 100%. The proportion of adults with urine creatinine concentrations below 0.3 g/L was 12.9% and the proportion above 3 g/L was 0.3%. In children, these proportions were 3.1% and 0.5%, respectively. In this study, individuals with creatinine concentrations <0.3 g/L and >3 g/L were all included in the various analyses. In addition, creatinine (log--transformed) was systematically included in the models as a covariate.

The statistical analyses were conducted using Stata software (ICE module) for data imputation and R software (package 'survey') to take into account the sample design (StataCorp.; Team.).

3. Results

3.1. Concentrations of pesticide biomarkers

Table 2 summarises descriptive statistics for pesticide biomarkers measured in urine samples of adults (n = 900) and children (n = 500), only for the chemical substances quantified to at least 10% (cf. Table 1).

3.1.1. Pyrethroid biomarkers

Pyrethroid metabolites were quantified in 99% of urine samples from the Esteban population, except for F-PBA which was quantified in 31% and 27% of the samples from children and adults, respectively. Globally, distribution levels of pyrethroid metabolite concentrations found in children were higher than those measured in adults, except for trans-Cl2CA, which were similar for both groups.

3.1.2. Organophosphate biomarkers

All specific organophosphate biomarkers measured in children and adults were barely or not quantified in urine samples (0–1% for chlorpyrifos, chlorpyrifos methyl-oxon, chlorpyrifos-methyl, chlorpyrifos-oxon, parathion, and parathion-methyl; 2–4% for TCPy and para-nitrophenol). DMTP was the most quantified dialkylphosphate metabolite in urine samples both in children and in adults (93% and 82%, respectively). DMP, DEP and DETP were quantified in 22%–46% of the urine samples (children and adults), whereas DMDTP and DEDTP were barely or not quantified (0–2%). Distribution levels of DMP, DMTP, and DETP urinary concentrations in children were all higher than those found in adults; the opposite was true for DEP.

Table 1
Analytical performance of pesticide biomarkers analysed in the Esteban study, 2014–2016.

Chemical substance (abbreviation)	Biomarker measured	Biological matrix	Volume needed (mL)	Analytical method	LOD (μg. L^{-1})	LOQ (μg. L^{-1})	% ≥ LOQ (children)	% ≥ LOQ (adults)
Pyrethroid pesticides								
Pyrethroid metabolites								
3-phenoxybenzoic acid	3-PBA	urine	5[e]	GC-MS/MS	0.005	0.015	99.6	100
4-fluoro-3-phenoxybenzoic acid	4-F-3-PBA (or F-PBA)	urine		GC-MS/MS	0.005	0.015	31.0	27.0
cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid	cis-BR2CA (or cis-DBCA)	urine		GC-MS/MS	0.005	0.015	99.6	99.4
cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1- carboxylic acid	cis-Cl2CA (or cis DCCA)	urine		GC-MS/MS	0.005	0.015	99.4	99.8
trans-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid	trans-Cl2CA (or trans DCCA)	urine		GC-MS/MS	0.005	0.015	98.6	98.6
Organophosphate pesticides								
Specific organophosphate compounds and metabolites								
Chlorpyrifos	Chlorpyrifos	urine	10[a]	UPLC-MS/MS	0.02	0.05	0	0.66
Chlorpyrifos-methyl-oxon	Chlorpyrifos-methyl-oxon	urine		UPLC-MS/MS	0.02	0.05	0	0
Chlorpyrifos-methyl	Chlorpyrifos-methyl	urine		UPLC-MS/MS	0.02	0.05	0.4	1.59
Chlorpyrifos-oxon	Chlorpyrifos-oxon	urine		UPLC-MS/MS	0.002	0.005	0.4	1.72
3,5,6-trichloro-2-pyridinol (TCPy)	TCPy	urine		UPLC-MS/MS	0.02	0.05	2.04	3.05
Parathion	Parathion	urine		UPLC-MS/MS	0.2	0.5	0	0
Parathion-methyl	Parathion-methyl	urine		UPLC-MS/MS	0.2	0.5	0	0
Para-nitrophenol	Para-nitrophenol	urine		UPLC-MS/MS	0.02	0.05	2.04	4.38
Common dialkyl phosphate metabolites								
Dimethylphosphate (DMP)	DMP	urine	2[b]	UPLC-MS/MS	0.8	2.0	39.2	35.5
Dimethylthiophosphate (DMTP)	DMTP	urine		UPLC-MS/MS	0.3	0.6	92.6	82.5
Dimethyldithiophosphate (DMDTP)	DMDTP	urine		UPLC-MS/MS	0.3	0.6	0.2	2.2
Diethylphosphate (DEP)	DEP	urine		UPLC-MS/MS	0.3	0.6	21.6	46.0
Diethylthiophosphate (DETP)	DETP	urine		UPLC-MS/MS	0.3	0.6	36.4	36.4
Diethyldithiophosphate (DEDTP)	DEDTP	urine		UPLC-MS/MS	0.3	0.6	0	0.33
Organochlorine pesticides								
Dichlorodiphenyltrichloroethanes and metabolites								
Dichlorodiphenyltrichloroethane (DDT)	p,p'-DDT	serum	5[c]	GC-HRMS	0.002	0.01	14.5	50.86
	o,p'-DDT	serum		GC-HRMS	0.002	0.01	2.35	2.37
Dichlorodiphenyldichloroethylene (DDE)	p,p'-DDE	serum		GC-HRMS	0.002	0.01	100	100
	o,p'-DDE	serum		GC-HRMS	0.002	0.01	0	0
Hexachlorocyclohexanes								
α-Hexachlorocyclohexane (α-HCH)	α-HCH	serum		GC-HRMS	0.002	0.01	1.18	2.63
β-Hexachlorocyclohexane (β-HCH)	β-HCH	serum		GC-HRMS	0.002	0.01	82.0	99.74
γ-Hexachlorocyclohexane (γ-HCH)	γ-HCH (lindane)	serum		GC-HRMS	0.002	0.01	50.2	49.28
Cyclodienes								
cis-chlordane	cis-chlordane	serum		GC-HRMS	0.002	0.01	0	0
trans-chlordane	trans-chlordane	serum		GC-HRMS	0.002	0.01	0	0
Oxychlordane	Oxychlordane	serum		GC-HRMS	0.002	0.01	19.6	91.8
cis-nonachlor	cis-nonachlor	serum		GC-HRMS	0.002	0.01	0	13.3
trans-nonachlor	trans-nonachlor	serum		GC-HRMS	0.002	0.01	15.7	88.7
cis-heptachlor epoxide	cis-heptachlor epoxide	serum		GC-HRMS	0.002	0.01	31.0	89.5
trans-heptachlor epoxide	trans-heptachlor epoxide	serum		GC-HRMS	0.002	0.01	0	0
Aldrin	Aldrin	serum		GC-HRMS	0.002	0.01	0	0
Dieldrin	Dieldrin	serum		GC-HRMS	0.002	0.01	58.0	94.9
Endrin	Endrin	serum		GC-HRMS	0.002	0.01	0	0
Mirex	Mirex	serum		GC-HRMS	0.002	0.01	0.39	19.4
Chlorophenols								
Monochlorophenols (MCP)	4-MCP	urine	5[d]	GC-MS/MS	0.05	0.15	1.8	11.6
Dichlorophenols (DCP)	2,4-DCP	urine		GC-MS/MS	0.05	0.15	4	7.6
	2,5-DCP	urine		GC-MS/MS	0.05	0.15	4.8	9.7
	2,6-DCP	urine		GC-MS/MS	0.05	0.15	0	0.11
Trichlorophenols (TCP)	2,3,4-TCP	urine		GC-MS/MS	0.05	0.15	0	0.11
	2,4,5-TCP	urine		GC-MS/MS	0.05	0.15	0	0.56
	2,4,6-TCP	urine		GC-MS/MS	0.05	0.15	0	0.56
Tetrachlorophenols (TeCP)	2,3,4,6-TeCP	urine		GC-MS/MS	0.05	0.15	1.6	3.9

(*continued on next page*)

Table 1 (*continued*)

Chemical substance (abbreviation)	Biomarker measured	Biological matrix	Volume needed (mL)	Analytical method	LOD (μg. L^{-1})	LOQ (μg. L^{-1})	% ≥ LOQ (children)	% ≥ LOQ (adults)
Pentachlorophenols (PCP)	PCP	urine		GC-MS/MS	0.05	0.15	14.6	17.7
Carbamate pesticides								
Carbamate compounds and metabolites								
Carbofuranphenol	Carbofuranphenol	urine	10[f]	UPLC-MS/MS	1	5	2.2	1.7
Propoxur	Propoxur	urine		UPLC-MS/MS	0.02	0.05	6.0	6.0
2-Isopropoxyphenol	2-IPP	urine		UPLC-MS/MS	0.02	0.05	11.6	8.5
Herbicides								
Herbicide compounds and metabolites								
Atrazine	Atrazine	urine	10[g]	UPLC-MS/MS	0.01	0.02	0	0
Atrazine-desethyl	Deethylatrazin	urine		UPLC-MS/MS	0.002	0.005	0.2	3.0
Atrazine-desethyl-2-hydroxy	Deethylhydroxyatrazine	urine		UPLC-MS/MS	0.05	0.2	0.2	0.1
Atrazine-desisopropyl	Desisopropylatrazine	urine		UPLC-MS/MS	0.05	0.2	0.2	0.2
Atrazine-desethyl-desisopropyl	Desethyldesisopropylatrazine	urine		UPLC-MS/MS	0.05	0.2	0	0.3
Atrazine-2-hydroxy	2-hydroxyatrazine	urine		UPLC-MS/MS	0.02	0.05	0	0.7
Atrazine mercapturate	Atrazine mercapturate	urine		UPLC-MS/MS	0.01	0.02	0.2	1.5
2,4-Dichlorophenoxyacetic acid	2,4-D	urine		UPLC-MS/MS	0.03	0.1	55.8	40.4
Alachlor	Alachlor	urine		UPLC-MS/MS	0.01	0.02	0	0.1
Alachlore mercapturate	Alachlor mercapturate	urine		UPLC-MS/MS	0.01	0.02	0.4	0.1
2,6-Diethylaniline	2,6-Diethylaniline	urine		UPLC-MS/MS	0.02	0.05	0.4	0.6
Simazine	Simazine	urine		UPLC-MS/MS	0.01	0.02	0	0.6
Simazine-2-hydroxy	2-Hydroxysimazine	urine		UPLC-MS/MS	0.01	0.02	0	0.3
Simazine mercapturate	Simazine mercapturate	urine		UPLC-MS/MS	0.02	0.05	1.4	0.7
Isoproturon-monodemethyl (IPPMU)	IPPMU	urine		UPLC-MS/MS	0.01	0.02	0.2	0.8
Diuron	Diuron	urine		UPLC-MS/MS	0.01	0.02	0	0.8
Chlortoluron	Chlortoluron	urine		UPLC-MS/MS	0.03	0.1	0	0.1
Isoproturon	Isoproturon	urine		UPLC-MS/MS	0.01	0.02	0	0.7
Dimetachlor	Dimetachlor	urine		UPLC-MS/MS	0.02	0.05	0	0
Glyphosate	Glyphosate	urine		UPLC-MS/MS	0.02	0.05	14.3	16.6
Aminomethylphosphonic acid (AMPA)	AMPA	urine		UPLC-MS/MS	0.02	0.05	93.4	74.0

Volume needed for the analysis of a family of chemical subtances:
[a] Specific organophosphate compounds and metabolites;
[b] Common metabolites of organophosphate pesticides,
[c] Specific organochlorine compounds and metabolites;
[d] Chlorophenol compounds;
[e] Pyrethroid metabolites;
[f] Carbamate compounds and metabolites;
[g] Herbicide compounds and metabolites.

3.1.3. Carbamate biomarkers

The three biomarkers of carbamate pesticides selected were also quantified in very few urine samples in Esteban. Specifically, carbofuranphenol, propoxur and its metabolite 2-isopropoxyphenol (2-IPP) were quantified in 2%, 6%, and 12% of child samples, respectively, and in 2%, 6%, and 8% of adult samples, respectively.

3.1.4. Herbicide biomarkers

Most of the herbicide biomarkers studied were barely or not quantified (0–3%), except for 2,4-D, glyphosate and its metabolite AMPA, which were quantified in 56%, 14%, and 93% of child urine samples, respectively, and in 40%, 17%, and 74% of the urine samples in adults, respectively. The distributions of both glyphosate and AMPA urinary concentrations were overall higher in children than those in adults, with respectively, P95 values of 0.57 μg/L (0.53 μg/g creatinine) and 0.45

Table 2
Description of urinary concentrations (µg/L and µg/g creatinine) of organophosphates, chlorophenols, pyrethroids, carbamates, and herbicide biomarkers in adults and children living in mainland France, Esteban study, 2014–2016.

Biomarker	Population	N	% ≥ LOQ	P25	P50	P75	P90	P95	GM [95% CI][a]
volume-based (in µg/L)									
Pyrethroid compounds									
3-PBA	Children	499	99.6	0.59	1.03	1.97	4.28	7.30	1.10 [0.93; 1.29]
	Adults	900	100	0.37	0.72	1.28	2.42	3.23	0.72 [0.66; 0.79]
4-F-3-PBA (or F-PBA)	Children	499	31.0	< LOQ	< LOQ	0.023	0.048	0.085	nc
	Adults	900	27.0	< LOQ	< LOQ	0.018	0.037	0.068	nc
BR2CA (or DBCA)	Children	499	99.6	0.53	1.16	2.41	4.47	5.70	1.09 [0.92; 1.28]
	Adults	900	99.4	0.31	0.69	1.30	2.89	4.43	0.64 [0.58; 0.71]
cis-Cl2CA (or cis DCCA)	Children	499	99.4	0.18	0.32	0.57	1.18	1.89	0.32 [0.27; 0.38]
	Adults	900	99.8	0.12	0.25	0.48	1.05	1.53	0.24 [0.22; 0.27]
trans-Cl2CA (or trans DCCA)	Children	499	98.6	0.10	0.18	0.40	0.79	1.14	0.19 [0.16; 0.22]
	Adults	900	98.6	0.08	0.17	0.35	0.79	1.46	0.18 [0.16; 0.20]
Chlorophenol compounds									
4-MCP	Adults	900	11.6	< LOQ	< LOQ	< LOQ	< LOQ	0.21	nc
PCP	Children	500	14.6	< LOQ	< LOQ	< LOQ	0.18	0.34	nc
	Adults	900	17.7	< LOQ	< LOQ	< LOQ	0.16	0.23	nc
Common organophosphates metabolites									
DMP	Children	500	39.2	<LOQ	<LOQ	3.93	10.98	16.00	nc
	Adults	899	35.5	<LOQ	<LOQ	3.07	8.31	14.08	nc
DMTP	Children	500	92.6	1.96	4.63	9.24	16.33	26.48	4.01 [3.38; 4.75]
	Adults	899	82.5	0.90	2.16	4.69	10.32	14.77	2.00 [1.74; 2.30]
DEP	Children	500	21.6	<LOQ	<LOQ	<LOQ	3.24	6.48	nc
	Adults	899	46.0	<LOQ	<LOQ	2.64	9.18	16.34	nc
DETP	Children	500	36.4	<LOQ	<LOQ	1.09	3.38	5.64	nc
	Adults	899	36.4	<LOQ	<LOQ	0.98	2.83	4.44	nc
Carbamate compounds									
2-IPP	Children	500	11.6	< LOQ	< LOQ	< LOQ	0.12	0.30	nc
	Adults	899	8.5	< LOQ	< LOQ	< LOQ	< LOQ	0.11	nc
Herbicide compounds									
2,4-D	Children	498	55.8	<LOQ	0.11	0.18	0.28	0.41	nc
	Adults	891	40.4	<LOQ	<LOQ	0.15	0.28	0.40	nc
Glyphosate	Children	498	14.3	<LOQ	<LOQ	<LOQ	0.20	0.57	nc
	Adults	891	16.6	<LOQ	<LOQ	<LOQ	0.15	0.36	nc
AMPA	Children	498	93.4	0.10	0.17	0.25	0.36	0.45	0.16 [0.14; 0.18]
	Adults	891	74.0	<LOQ	0.09	0.16	0.26	0.34	0.08 [0.08; 0.09]
creatinine-adjusted (µg/g creatinine)									
Pyrethroid compounds									
3-PBA	Children	499	_	0.56	1.07	2.21	4.50	7.34	1.11 [0.95; 1.30]
	Adults	900	_	0.56	0.96	1.71	3.08	4.45	1.00 [0.93; 1.08]
4-F-3-PBA (or F-PBA)	Children	499	_	< LOQ	< LOQ	0.02	0.06	0.09	nc
	Adults	900	_	< LOQ	< LOQ	0.025	0.057	0.093	nc
BR2CA (or DBCA)	Children	499	_	0.55	1.21	2.49	4.75	6.86	1.10 [0.93; 1.30]
	Adults	900	_	0.42	0.90	2.01	4.01	5.46	0.89 [0.80; 0.99]
cis-Cl2CA (or cis DCCA)	Children	499	_	0.18	0.32	0.64	1.12	1.59	0.33 [0.28; 0.38]
	Adults	900	_	0.18	0.32	0.65	1.21	1.97	0.34 [0.31; 0.37]
trans-Cl2CA (or trans DCCA)	Children	499	_	0.10	0.21	0.41	0.78	1.15	0.19 [0.16; 0.22]
	Adults	900	_	0.12	0.24	0.46	0.96	1.90	0.25 [0.22; 0.27]
Chlorophenol compounds									
4-MCP	Adults	900	_	< LOQ	< LOQ	< LOQ	< LOQ	0.35	nc
PCP	Children	500	_	< LOQ	< LOQ	< LOQ	0.40	0.40	nc
	Adults	900	_	< LOQ	< LOQ	< LOQ	0.27	0.39	nc
Common organophosphates metabolites									
DMP	Children	500	_	<LOQ	<LOQ	4.48	13.02	19.72	nc
	Adults	899	_	<LOQ	<LOQ	4.62	13.05	25.21	nc
DMTP	Children	500	_	1.80	4.09	9.67	18.82	30.76	4.06 [3.37; 4.87]
	Adults	899	_	1.20	2.71	6.72	13.39	24.37	2.78 [2.43; 3.17]
DEP	Children	500	_	<LOQ	<LOQ	<LOQ	3.49	11.39	nc
	Adults	899	_	<LOQ	<LOQ	3.72	13.15	26.65	nc
DETP	Children	500	_	<LOQ	<LOQ	1.31	3.62	5.23	nc
	Adults	899	_	<LOQ	<LOQ	1.51	3.72	6.58	nc
Carbamate compounds									
2-IPP	Children	500	_	< LOQ	< LOQ	< LOQ	0.12	0.26	nc
	Adults	899	_	< LOQ	< LOQ	< LOQ	< LOQ	0.19	nc
Herbicide compounds									
2,4-D	Children	498	_	<LOQ	0.10	0.18	0.31	0.39	nc
	Adults	891	_	<LOQ	<LOQ	0.20	0.41	0.66	nc
Glyphosate	Children	498	_	<LOQ	<LOQ	<LOQ	0.23	0.53	nc
	Adults	891	_	<LOQ	<LOQ	<LOQ	0.18	0.45	nc
AMPA	Children	498	_	0.11	0.16	0.27	0.39	0.52	0.16 [0.14; 0.18]
	Adults	891	_	0.07	0.12	0.21	0.30	0.42	0.11 [0.10; 0.13]

[a] For each biomarker, if more than 40% of samples were below the LOQ, the percentile distribution is reported but the geometric mean was not calculated (nc).

μg/L (0.52 μg/g creatinine) in children, and 0.36 μg/L (0.45 μg/g creatinine) and 0.34 μg/L (0.42 μg/g creatinine) in adults.

3.1.5. Chlorophenol biomarkers

Among the nine chlorophenols measured in Esteban, PCP was the biomarker with the highest quantification rate both in children (14.6%) and in adults (17.7%), with P95 values of 0.34 μg/L (0.40 μg/g creatinine) and 0.23 μg/L (0.39 μg/g creatinine), respectively. Four chlorophenols, 4-MCP, 2,4-DCP, 2,5-DCP, and TeCP, were quantified in 1.6%–4.8% of child urine samples, and in 3.9%–11.6% of adult urine samples, respectively. Four other chlorophenols, 2,6-DCP, 2,3,4-TCP, 2,4,5-TCP, and 2,4,6-TCP, were not quantified in children and in less than 1% of adult samples.

3.1.6. Specific organochlorine biomarkers

Table 3 summarises descriptive statistics for pesticide biomarkers measured in serum in adults (n = 759) and children (n = 255), only for the chemical substances quantified to at least 10% (cf. Table 1).

The following specific organochlorine biomarkers were barely quantified (<3%) in children and adult serum samples: o,p'-DDT, o,p'-DDE, α-HCH, cis-chlordane (both 0%), trans-chlordane (both 0%), cis-nonachlor (0% in children), trans-heptachlor epoxide (both 0%), Aldrin (both 0%), endrin (both 0%), and mirex (0.4% in children). Other organochlorine biomarkers were quantified between 13% and 40% of serum samples in adults (mirex (19.4%)) or in children ((p,p'-DDT (14.5%), trans-nonachlor (15.7%), oxychlordane (19.6%), and cis-heptachlor epoxide (39%)).

The remaining organochlorine biomarkers studied were quantified in at least half of the populations of children and adults: p,p'-DDE (both 100%), β-HCH (82.0% and 99.7%, respectively), γ-HCH (50.2% and 49.3%, respectively); or only in adults: p,p'-DDT (50.9%), oxychlordane (91.8%), trans-nonachlor (88.7%), cis-heptachlor epoxide (89.5%), and dieldrin (94.9%).

Overall, the distributions of concentration levels (geometric means) of the studied specific organochlorine biomarkers (dichlorodiphenyltrichloroethanes, hexachlorocyclohexanes, and cyclodienes) measured in adult serum samples were all higher than the distributions observed in children.

3.2. Factors influencing exposure levels to pesticide biomarkers

3.2.1. Pyrethroid biomarkers

Results of the multivariate analyses for pyrethroid biomarkers in adults are detailed in Supplementary Tables A1 and A2, for quantitative

Table 3
Description of the concentrations in serum (in ng/L and in ng/g lipids) of specific organochlorine pesticide compounds and metabolites in the population of adults and children living in mainland France, Esteban study, 2014–2016.

Biomarker	Population[a]	N	% ≥ LOQ[b]	P25	P50	P75	P90	P95	GM [95% CI]
volume-based (in ng/L)									
Dichlorodiphenyltrichloroethanes and metabolites									
p,p'-DDT	Children	255	14.5	<LOQ	<LOQ	<LOQ	12.32	20.06	nc
	Adults	759	50.9	<LOQ	9.25	15.00	25.02	38.44	nc
p,p'-DDE	Children	255	100	67.25	102.04	166.3	279.8	444.7	113.0 [112.9; 113.1]
	Adults	759	100	179.0	355.7	760.1	1583.9	2540.8	393.1 [350.6; 440.8]
Hexachlorocyclohexanes									
β-HCH	Children	255	82.0	11.81	17.8	24.23	43.05	55.68	18.10 [18.02; 18.17]
	Adults	759	99.7	34.37	70.96	164.4	402.7	596.5	79.23 [69.97; 89.73]
γ-HCH (lindane)	Children	255	50.2	<LOQ	10.02	12.35	17.09	20.14	nc
	Adults	759	49.3	<LOQ	9.75	12.41	17.13	26.74	nc
Cyclodienes									
Oxychlordane	Children	255	19.6	<LOQ	<LOQ	<LOQ	13.08	16.43	nc
	Adults	759	91.8	12.2	22.1	37.47	55.81	73.60	21.55 [19.67; 23.60]
cis-nonachlor	Adults	759	13.3	<LOQ	<LOQ	<LOQ	9.99	13.36	nc
trans-nonachlor	Children	255	15.7	<LOQ	<LOQ	<LOQ	12.08	15.45	nc
cis-heptachlor epoxide	Adults	759	88.7	11.28	19.70	34.52	52.83	62.08	19.15 [17.38; 21.09]
	Children	255	31.0	<LOQ	<LOQ	10.90	14.59	16.33	nc
Dieldrin	Adults	759	89.5	11.68	17.4	27.33	43.64	58.47	18.10 [16.75; 19.56]
	Children	255	58.0	<LOQ	10.96	15.11	20.84	25.84	nc
	Adults	759	94.9	14.69	23.34	36.46	58.05	80.78	23.78 [21.87; 25.87]
Mirex	Adults	759	19.4	<LOQ	<LOQ	<LOQ	11.45	14.28	nc
lipid-adjusted (ng/g lipids)									
Dichlorodiphenyltrichloroethanes and metabolites									
p,p'-DDT	Children	255	_	<LOQ	<LOQ	<LOQ	2.36	3.73	nc
	Adults	759	_	<LOQ	1.57	2.36	4.00	5.93	nc
p,p'-DDE	Children	255	_	13.65	20.35	33.8	56.84	82.15	22.70 [22.62; 22.80]
	Adults	759	_	30.18	57.76	126.1	242.4	422.2	64.85 [57.97; 72.55]
Hexachlorocyclohexanes									
β-HCH	Children	255	_	2.41	3.40	5.07	8.14	11.88	3.64 [3.56; 3.71]
	Adults	759	_	5.94	11.14	24.99	62.74	94.2	13.07 [11.58; 14.76]
γ-HCH (lindane)	Children	255	_	<LOQ	2.04	2.56	3.21	4.10	nc
	Adults	759	_	<LOQ	1.65	2.15	2.92	4.01	nc
Cyclodienes									
Oxychlordane	Children	255	_	<LOQ	<LOQ	<LOQ	2.58	3.04	nc
	Adults	759	_	2.14	3.48	5.96	8.56	10.53	3.55 [3.26; 3.87]
cis-nonachlor	Adults	759	_	<LOQ	<LOQ	<LOQ	2.00	2.00	nc
trans-nonachlor	Children	255	_	<LOQ	<LOQ	<LOQ	2.41	2.93	nc
	Adults	759	_	1.92	3.22	5.36	7.86	9.56	3.16 [2.89; 3.44]
cis-heptachlor epoxide	Children	255	_	<LOQ	<LOQ	2.09	2.74	3.25	nc
	Adults	759	_	2.02	2.87	4.34	6.67	8.74	2.99 [1.38; 2.02]
Dieldrin	Children	255	_	<LOQ	2.19	3.04	4.07	5.30	nc
	Adults	759	_	2.52	3.89	5.78	8.86	12.37	3.92 [3.63; 4.24]
Mirex	Adults	759	_	<LOQ	<LOQ	<LOQ	2.00	2.62	nc

[a] Descriptive statistics not weighted for children due to the small sub-sample.
[b] For each biomarker, if more than 40% of samples were below the LOQ, the percentile distribution is reported but the geometric mean was not calculated (nc).

and qualitative variables, respectively. The following exposure factors were associated with the highest levels of the sum of urine pyrethroid metabolite concentrations (∑pyrethroids = 3-PBA + F-PBA + Br2CA + cisCl2CA + transCl2CA) in adults (Table A2): tobacco consumption, the use of pest control on pets at least three times a year, exposure to plant or animal substances and dust at the workplace, and consumption of at least one product of animal origin from the participant's own garden or livestock (poultry, meat, milk and eggs). In addition, having one's home in a green area (e.g., forest, meadow, and scrubland) was associated with higher urine concentration levels of 3-PBA (Table A2). Moreover, beef consumption was associated with higher urine concentration levels of Br2CA (Table A1). In contrast, moderate consumption of vegetables from organic farming (1–3 times a week) was associated with lower urine concentration levels of 3-PBA, cisCl2CA, transCl2CA, and ∑pyrethroids (Table A2).

Results of the multivariate analyses for pyrethroid biomarkers in children are detailed in Supplemetnary Tables A3 and A4, for quantitative and qualitative variables, respectively. In children, the concentration levels of F-PBA, cisCl2CA and transCl2CA decreased with age (Table A3). The same trends were observed with 3-PBA, Br2CA, and ∑pyrethroids, but they were not significant (Table A3). Exposure factors associated with highest levels of the ∑pyrethroids in children were (Table A4): the use of pest control on pets at least once a year, and the frequent use of pesticides against dust mites in the home. In addition, living within 200 m of an agricultural area (e.g., cultivated fields, orchards, and greenhouses) was associated with higher urine concentration levels of F-PBA, and living within 200m of a garden was associated with higher urine concentration levels of transCl2CA (Table A4).

3.2.2. Organochlorine biomarkers

Results of the multivariate analyses in adults for the specific organochlorine biomarkers studied are presented in Supplementary Tables A5 and A6, for quantitative and qualitative variables, respectively.

The concentration levels of all the specific organochlorine biomarkers studied (p,p'-DDE, β-HCH, cis-heptachlor epoxide, oxychlordane, trans-nonachlor, and dieldrin) increased with age (Table A5); older adults had higher serum organochlorine concentration levels than younger adults. Serum concentration levels of these biomarkers also increased with body mass index (BMI), except for trans-nonachlor (Table A5).

There were significant differences in concentration levels of specific organochlorine biomarkers according to sex (Table A6). Levels of serum β-HCH and p,p'-DDE in women were 41.2% and 26.6% higher than in men, respectively. In contrast, levels in men were 27.2%, 21.9% and 16.0% higher than in women for dieldrin, cis-heptachlor epoxide, and trans-nonachlor, respectively.

Several dietary factors were associated with serum concentration levels for the studied specific organochlorines. Higher levels were observed for the following types of foodstuffs (Table A5): eggs (8.4% and 4.6% for p,p'-DDE and oxychlordane, respectively); fats (9.2%, 8.7% and 6.5%, for β-HCH, oxychlordane and dieldrin, respectively); fish and seafood (17.0% for trans-nonachlor); crustaceans, molluscs and shellfish (3.5% for oxychlordane); all vegetables (13.2% for p,p'-DDE and 9.5% for dieldrin); all fruits (18.3% for p,p'-DDE and 16.7% trans-nonachlor). Higher levels were also observed for the consumption of tap water (8.2% for oxychlordane), and alcoholic beverages (14.4%, 11.5% and 10.9%, for trans-nonachlor, dieldrin and cis-heptachlor epoxide, respectively) (Table A5). In contrast, beef consumption (bovine meat) were associated with lower serum concentration levels of both oxychlordane (−6.7%) and trans-nonachlor (−6.6%) (Table A5).

Serum concentration levels of trans-nonachlor, cis-heptachlor epoxide, and oxychlordane, were 22.5%, 20.4%, and 18.4% higher, respectively, in participants who moderately (i.e., 1–3 times a month) consumed eggs from their own garden or farm (Table A6). In contrast, the consumption of the following foodstuffs was associated with lower serum concentration levels of certain specific organochlorines (Table A6): meat or poultry from one's own livestock with cis-heptachlor epoxide levels (−18.2%), organically farmed meat with p, p'-DDE levels (−22.3%), and of organic food products (dairy products, eggs, poultry and other meats, fruits and vegetables, cereals or wholemeal bread) with β-HCH levels (−10.2%), respectively.

With regard to domestic and environmental factors (Table A6), adults who reported using pesticides on their houseplants (indoor plants) had higher serum organochlorine biomarker concentration levels than those who reported not having indoor plants. Specifically, percentages were 25.8%, 23.4%, 21%, 15%, 10%, and 14% higher for p, p'-DDE, β-HCH, cis-heptachlor epoxide, oxychlordane, transnonachlor, and dieldrin in the former group. Moreover, the β-HCH serum concentration level was 17.8% higher in adults who reported using pesticides on the lawn at their house than in those with no lawn (Table A6). Furthermore, serum concentration levels of trans-nonachlor and dieldrin were 11.8% and 26.3% higher, respectively, in users of body repellents than in non-users (Table A6).

The serum concentration level of p,p'-DDE was 21.4% higher in adult participants living in suburban districts than in those living in city centres (Table A6). In addition, serum concentration levels of trans-nonachlor and cis-heptachlor epoxide were 18.8% and 13% higher, respectively, in adults who declared aerating their home (ventilation of housing during spring and summer) once or twice daily than in those who aerated their home more often (Table A6).

With regard to occupational exposure factors (Table A6), adult participants exposed to plant, animal, or topsoil dusts had serum concentration levels of β-HCH 30.8% higher than that in unexposed individuals. Similarly, serum concentration levels of dieldrin and cis-heptachlor epoxide were 43.1% and 16.6% higher, respectively, in adult participants who worked in occupational fields related to agriculture, plants, pesticides or wood than in those with other occuptations.

3.2.3. Organophosphate biomarkers

In terms of exposure levels to organophosphate pesticides, multivariate analyses did not identify potential sources of exposure to DMTP in children. However, younger children had higher urine DMTP concentration levels than older children (Table A7). In adults, the consumption of food from organic farming (dairy products, eggs, poultry and other meats, fruits and vegetables, cereals or wholemeal bread) was associated with lower urine concentration levels of DMTP (Table A8).

3.2.4. Herbicide biomarkers

With respect to the exposure levels to the glyphosate metabolite AMPA, none of the potential exposure variables analysed was significantly associated with differences in urine concentration in children. However, children sampled in spring had higher urine concentration levels of AMPA than those in autumn or winter (Table A9). In adults, AMPA concentration levels in urine were higher in those who had an individual boiler for the main mode of heating (Table A10).

4. Discussion

4.1. Pesticide levels in France and comparison with international studies

4.1.1. Pyrethroid concentrations

In 2006–2007, the ENNS study provided the first distributions of urine pyrethroid metabolite exposure levels in the adult population living in mainland France (Fréry et al., 2013). The present study showed that the average concentration levels of urine F-PBA and urine trans-Cl2CA slightly decreased between 2006-2007 and 2014–2016 (Fig. 1). The overall distribution of urine 3-PBA and urine cis-Cl2CA remained stable over the decade 2006–2016, while urine Br2CA concentration levels increased.

In Esteban, younger children had higher pyrethroid concentrations than older children and adults, reflecting other studies in Poland (Wielgomas and Piskunowicz, 2013), Spain (Fernandez et al., 2020),

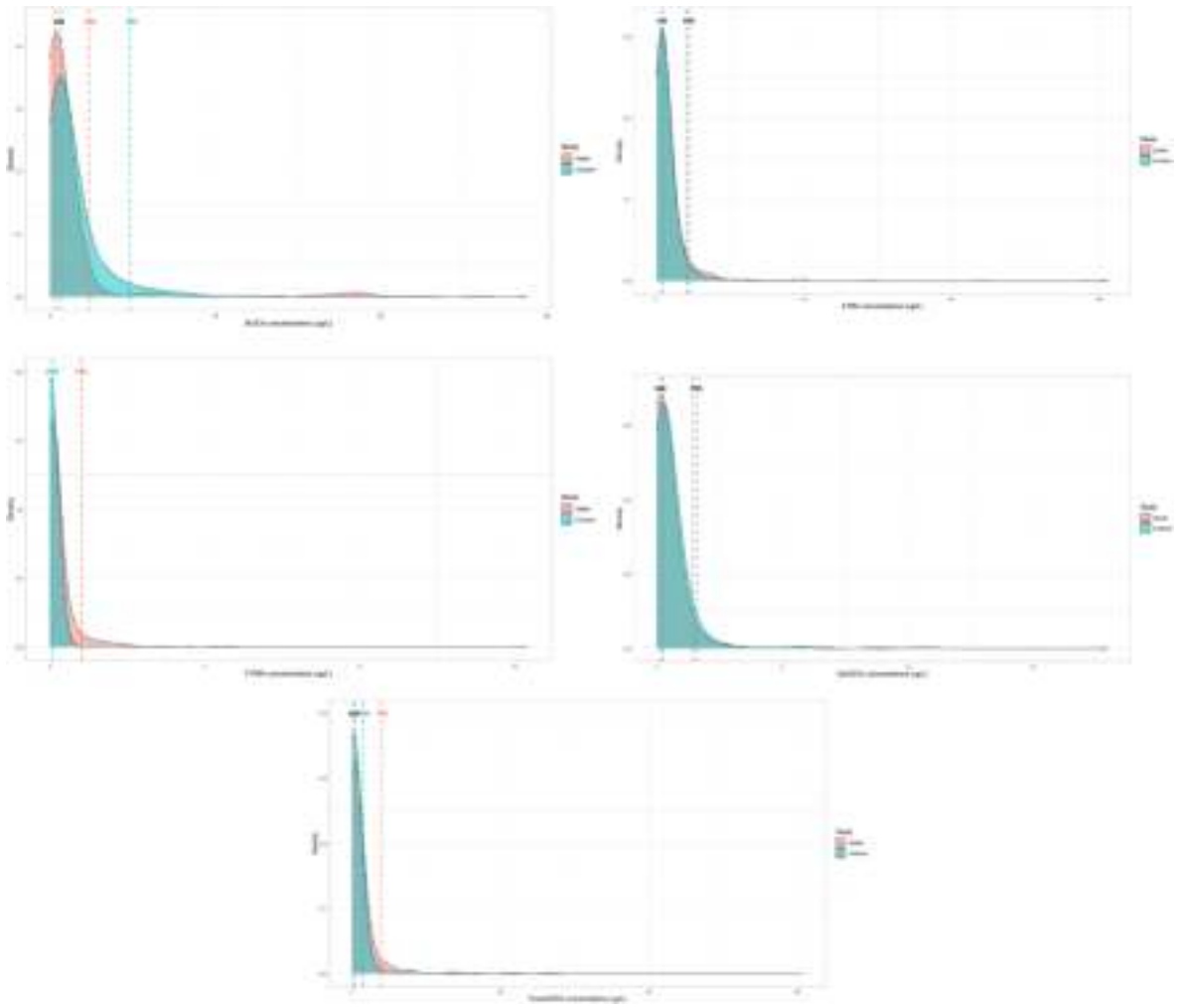

Fig. 1. Comparison of the distribution of urine concentration levels of pyrethroid metabolites in French adults aged 18–74 years between the ENNS survey (2006–2007) and the Esteban survey (2014–2016).

Slovenia (Bravo et al., 2020) and the United States (CDC, 2021a). This could be explained by the fact that younger children have more frequent hand-to-mouth activity, and are also more in contact with indoor dust containing pyrethroids (Berger-Preiss et al., 2002; Mandin et al., 2016). In addition, their food intake is higher than adults, in terms of body weight (Dewalque et al., 2014; Zentai et al., 2016; Katsikantami et al., 2019).

The only metabolite of pyrethroids to show an increase in concentration levels between the ENNS and Esteban surveys was Br2CA. This may reflect greater use of deltamethrin in France compared to other pyrethroid pesticides over 2006–2016, and also greater use of deltamethrin in France between the two surveys, probably mainly in houses. Deltamethrin is used very rarely in France for veterinary purposes. Currently, only three authorized veterinary drugs are based on deltamethrin, and sales are low (according to the French national databank for pesticide sales (BNVD)). In addition, deltamethrin is rarely used as an insecticide in vector control (adulticidal treatments) in mainland France, and was not used at all in 2014–2016. A possible increase in agricultural uses of deltamethrin in France could also be a source of the increase observed in concentration levels. The ban on several families of insecticides between the ENNS and Esteban studies, in particular carbamates in 2008, may have led to increased use of deltamethrin. This hypothesis however needs greater investigation, as French data did not show any substantial increase in the sale of deltamethrin-based plant protection products by authorized distributors nationally (12,500 kg and 12,582 kg for 2008 and 2016, respectively, for various commercial specialties). Finally, the lower concentration levels of transCl2CA in Esteban than in North American countries would suggest that related pesticides are used differently in France.

The results from the Esteban study would suggest that in 2014–2016, the French population did not have pyrethroid metabolite exposure levels different from those measured in international studies, except for Br2CA, a metabolite of deltamethrin. However, results from the ENNS survey in the general French population (2006–2007, see above) and from the French perinatal survey ELFE (2011) (Dereumeaux et al., 2018), showed that the adult population living in mainland France had higher exposure levels to pyrethroid metabolites than European and North American populations over the same period (i.e., 2006–2011). Furthermore, in France, the concentration levels of the different pyrethroid metabolites remained stable or declined between 2006-2007 and 2014–2016, while in Spain (Fernandez et al., 2020), Canada (Health Canada and Health, 2019) and in the United States (CDC, 2021a) they increased.

4.1.2. Organochlorine concentrations

Esteban is the first survey to measure exposure levels to organochlorines and their metabolites in French children aged 6–17 years.

Average organochlorine concentration levels in serum in the youngest children were slightly higher than those in older children (p,p'-DDE, β-HCH and γ-HCH (lindane)) (see Table A11). This indicates greater exposure of young children to organochlorines, perhaps due to possible greater exposure to household dust and/or food contaminated.

Few international studies have evaluated exposure levels to organochlorines in children. In Europe, the GerES V study conducted in 2014–2017 on German children aged 3–17 years (Bandow et al., 2020) is the closest temporal study to Esteban. The organochlorine p,p'-DDE was 100% quantified in both German and French children. However, the average serum concentration of p,p'-DDE was higher in German children than in French children, at 158 ng/L and 113 ng/L, respectively. The organochlorines p,p'-DDT, α-HCH, β-HCH and γ-HCH (lindane) were all quantified less in German children.

With regard to exposure to chlorophenolic compounds, 4-MCP, 2,4-MCP, 2,5-MCP, 2,4,5-TCP, 2,4,6-TCP and PCP were quantified in 59%–100% of adults in the French ENNS survey (Fréry et al., 2013), in contrast to only 0.6%–17.7% in Esteban; however, all LOQ were lower in Esteban.

The organochlorines p,p'-DDE and β-HCH were nearly 100% quantified in both ENNS (Fréry et al., 2013) and Esteban, with serum geometric means of 760 ng/L and 210 ng/L in the former, and 393 ng/L and 79 ng/L in the latter, respectively. The very marked difference in serum concentration levels these two biomarkers between both surveys (Fig. 2), reflects the definitive ban on organochlorine pesticides in France in 2009.

The concentration levels of all the organochlorines studied, with the exception of hexachlorocyclohexanes (β-HCH and γ-HCH), were all lower in French adults in 2014–2016 (i.e., Esteban) than in Canadian adults in 2007–2009 for p,p'-DDT, p,p'-DDE and cyclodiene compounds (oxychlordane, cis-nonachlor, trans-nonachlor, mirex) (Health Canada and Health, 2010). Although organochlorine-based pesticides were hardly used anymore in France at the time of Esteban, highest serum concentration levels were found for p,p'-DDE and β-HCH. This can be at least partly explained by the fact that these two chemical compounds are very persistent in the environment. For example, DDT, the parent substance of p,p'-DDE, has a reported half-life of 2–15 years in the environment (Blaylock, 2005) and 3–6 years in humans (Burr, 2014). In addition, DDT breaks down in the environment to more stable chemical forms, including p,p'-DDE and higher serum concentrations of p,p'DDE than p,p'DDT are usually found in human populations (Blaylock, 2005; Kang et al., 2008; Li et al., 2022). While French legislation has helped limit direct exposure to organochlorines, it cannot limit their long-term presence in the body.

4.1.3. Organophosphate concentrations

The very high quantification of the dialkylphosphate metabolite DMTP in Esteban is an indicator of permanent exposure of the French population to organophosphate compounds in 2014–2016. The second most quantified dialkylphosphate metabolite in the study was DMP. Although measuring DMTP and DMP does not allow the specific identification of their parent organophosphate compounds, they are nevertheless the only dialkylphosphate metabolites of certain pesticides, such

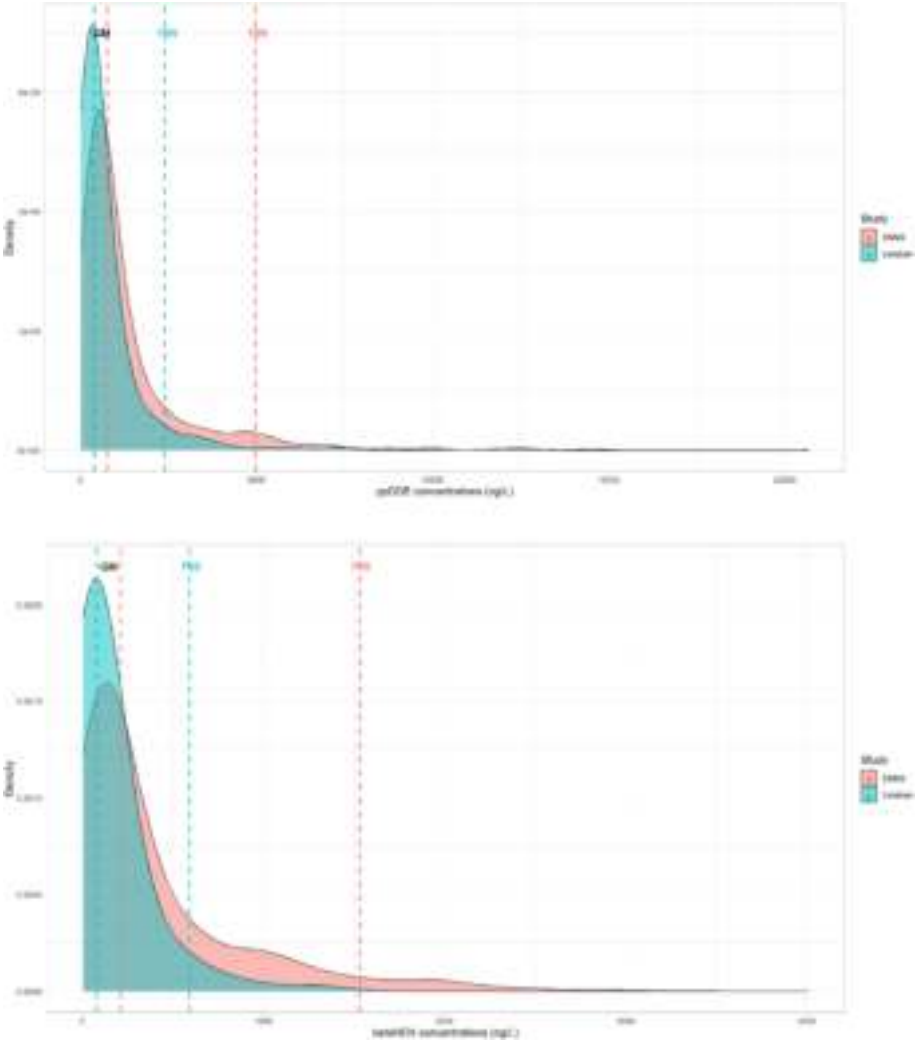

Fig. 2. Comparison of the distribution of serum concentration levels of specific organochlorine metabolites in French adults 18–74 years old between the ENNS survey (2006–2007) and the Esteban survey (2014–2016).

as chlorpyrifos methyl, which were still widely used in France at the time of Esteban.

All of the common dialkylphosphate metabolites in urines were less quantified in French adults in Esteban than in adults from the ENNS survey (Fréry et al., 2013). The average DMTP concentration in Esteban adults was one third that in ENNS adults. These results reflect a clear decrease in the exposure to organophosphorus compounds in France between 2006-2007 and 2014–2016. At the time of the ENNS survey, several organophosphorus pesticides such as trichlorfon, dichlorvos, malathion and diazinon were still authorized and widely used in agriculture in France, before a gradual ban on their use was introduced, starting in 2008.

The mean level of urine DMTP concentration levels in Esteban were higher in France than those observed in both US (CDC, 2021a) and Canada (Health Canada and Health, 2019). Note that the Canadian sample was more recent (2016–2017), while the United States sample was older (2007–2008). Similarly in Europe, the average DMTP concentration level in young French children in Esteban was double that observed in 2011 in young Danish children in the Democophes study (Morck et al., 2016). Despite older regulations in Europe on the agricultural uses of organophosphorus pesticides, and their low persistence in the environment, the higher concentration levels of DMTP in the French population than in Western countries may be explained by non-agricultural factors, such as non-agricultural uses of organophosphorus compounds and exposure to non-pesticidal parent compounds of DMTP. In addition, the overexposure to DMTP in French children compared to Western children could also be explained by local differences in environmental exposure to organophosphorus compounds, coupled with local singularities in children's behaviors and habits (e.g., hand-to-mouth contact, presence of pets in housing, outdoor hobbies).

4.1.4. Herbicide concentrations

Esteban is the first study to measure the concentration levels of several herbicides in both children and adults of the general population living in mainland France. In Esteban, only three herbicide biomarkers of all those measured (i.e., 2,4-D, and glyphosate and its metabolite AMPA) were quantified in urine samples in more than 2% of children and more than 5% of adults.

Glyphosate and AMPA were quantified, respectively, in 14% and 93% of child urine samples, and 17% and 74% of adult urine samples. AMPA exposure was therefore slightly higher in children than in adults. Urinary concentrations of 2,4-D measured in children and adults were generally lower than those in studies in North America (Nhanes in 2009–2010 (CDC, 2021a) and CHMS in 2009–2011 (Health Canada and Health, 2013)). In addition, urinary ghyphosate concentration levels in the Nhanes survey 2013–2014 were also higher than those in Esteban (2014–2016) for both children and adults (Ospina et al., 2022). One of the most likely explanations for this is the greater use of herbicides in the United States and Canada (FAO, 2021), as these two countries rank second and fifth, respectively, in terms of herbicide-intensive countries in the world, while France ranks ninth (FAO, 2022).

In Europe, urine concentration levels to glyphosate and AMPA differ greatly between countries, e.g., when comparing upper percentiles of their distributions (Conrad et al., 2017; Connolly et al., 2018; Nova et al., 2020; Soukup et al., 2020; Stajnko et al., 2020; Lemke et al., 2021; Ruiz et al., 2021; Buekers et al., 2022a, 2022b; Schoeters et al., 2022). Comparison is difficult however because of particularities specific to each of the studies concerned (e.g., target population, study period, analytical method).

4.1.5. Carbamate concentrations

Esteban is the first study to measure exposure levels of the French population to carbamate pesticides (carbofuranphenol and propoxur). Carbamates were barely quantified in Esteban, reflecting studies in the United States (CDC, 2021a), Canada (Haines et al., 2017), and the French ELFE cohort (see above) (Dereumeaux et al., 2016).

4.2. Factors influencing exposure levels to pesticides in the French population

4.2.1. Pyrethroid factors

Our analyses on pyrethroids exposure factors show that the domestic use of insecticides to control crawling insects, flying insects, fleas or lice was one of the main determinants of high urine pyrethroid concentrations in children and adults. This confirms results on the exposure to pyrethroids linked to the domestic use of insecticides in previous studies in France (Fréry et al., 2013; Dereumeaux et al., 2018).

No dietary factor was associated with pyrethroid concentration levels in children in Esteban. In Spain, higher urinary 3-PBA concentrations were observed in children with recent consumption of vegetables (Fernandez et al., 2020), but this was not the case for children from Belgium (Pirard et al., 2020).

In Esteban, we observed that adults who consumed organically grown vegetables had lower concentration levels of urine pyrethroid metabolites. On the contrary, higher levels were observed in adults who consumed their own livestock, and those who consumed beef (irrespective of its provenance). Using pyrethroids as antiparasitics for livestock could therefore be a source of contamination of related foods. In fact, several pyrethroid substances (in particular cyfluthrin, cypermethrin, deltamethrin and permethrin, for which a number of metabolites were measured in this study) are used in specific products designed for livestock breeding, in particular antiparasitic collars and ear tags. In other studies, the consumption of cereals and fruits or vegetables was associated with higher concentrations (Becker et al., 2006; Fortes et al., 2013; Ye et al., 2015; Glorennec et al., 2017).

Smoking was associated with higher pyrethroid concentration levels in adults in Esteban. This may be explained by direct exposure of smokers to pesticide residues used during tobacco growing, or indirect exposure through the ingestion of dust contaminated by pyrethroid residues. Expsoure through the latter pathway would be higher in smokers than in non-smokers, due to more frequent hand-to-mouth contact (Cai et al., 2002).

Finally, residential proximity (i.e., within 200 m) to a garden or cropland or living in a green area also seemed to have an influence on urine concentration levels of pyrethroid metabolites in both adults and children. These observations deserve further investigation; in particular using geographical indicators would make it possible to explore this link in greater detail, especially proximity to specific agricultural crops.

4.2.2. Organochlorine factors

The present study shows an overall increase with age of serum concentration levels of the specific organochlorines studied. For example, β-HCH concentration levels in adults increased by almost two hundred percent between the first and third age quartiles. This positive relationship is consistent with findings observed in the French ENNS study (Fréry et al., 2013; Saoudi et al., 2014) and in international studies (Ibarluzea et al., 2011; Brauner et al., 2012). This age effect can be explained by the long half-lives of the specific organochlorines in the human body (6–9 years for DDE and 7 years for β-HCH), as well as their bioaccumulation in body tissue (Genuis et al., 2016). Furthermore, older partcipants were more likely to have had greater exposure to the specific organochlorines before regulatory measures were introduced.

With regard to dietary exposure factors, in adults in Esteban, the biggest consumers of fish and seafood (excluding crustaceans, molluscs and shellfish), and the biggest consummers of crustaceans, molluscs and shellfish had higher levels of exposure to, respectively trans-nonachlor and oxychlordane. This result is consistent with findings in the French ENNS study (Saoudi et al., 2014) and other European and international studies focusing on general populations (Brauner et al., 2012; Arrebola et al., 2018; Gonzalez-Alzaga et al., 2018; Harmouche-Karaki et al., 2019). There is also a positive association in Esteban between egg consumption and higher concentration levels of p,p'-DDE, cis-heptachlor epoxide, oxychlordane and trans-nonachlor. These results

reflect those in international studies on different populations (students and employees at a university (Harmouche-Karaki et al., 2019), pregnant women (Cao et al., 2011), persons living in the vicinity of a chemical plant (Narduzzi et al., 2020)).

In Esteban, the consumption of vegetables (all kinds) and of fruits (all kinds) was associated with higher serum concentration levels of p,p'-DDE, trans-nonachlor and dieldrin. In the French ENNS study, fruit consumption (all types) was also associated with higher concentration levels of serum p,p'-DDE (Saoudi et al., 2014). However, it is important to point out that in Esteban, the consumption of fruits from one's own garden was associated with lower concentration levels of serum oxychlordane. Similarly, the consumption of meat or poultry from one's own livestock was associated with lower cis-heptachlor epoxide; the consumption of organically-farmed meat was associated with lower p, p'-DDE; and the consumption of organic food products was associated with lower β-HCH. Few studies to date have investigated the agricultural origin of the food consumed in terms of exposure factors to specific organochlorines (i.e., food from all origins vs. homegrown food/livestock vs. food from organic products). However, in contrast to the Esteban results, a few studies focusing on specific populations have reported higher concentration levels of specific organochlorines associated with the consumption of products from one's own crops or livestock; these associations were probably due to local soil contamination and uncontrolled homegrown food/livestock production (Stehr-Green et al., 1988; Aerts et al., 2019; Narduzzi et al., 2020).

Finally, certain geographical and household environment factors, such as living in a suburban area or having a lawn at home (despite not using pesticides on lawn), were also associated with higher concentration levels of some of the organochlorines studied in Esteban. This could be also explained by higher contamination of organochlorine compounds in soil due to past agricultural uses and the proximity of housing to agricultural sites. In France, almost one third of the national agricultural area lies within 150 m of residential buildings (Guilpart et al., 2022).

4.2.3. Organophosphate factors

In adults in Esteban, the consumption of organic food products were associated with lower urine concentration levels of DMTP. The potential protective effect of organic food consumption on exposure to organophosphorus pesticides is consistent with the fact that organic farming is, by definition, an agricultural production system that does not use synthetic pesticides. Accordingly, one can logically assume that people who report they regularly consume organic food products are less likely to be exposed to pesticide residues. However, it is important to note that when taken individually, the different organic foods we studied (dairy products, eggs, poultry and other meats, fruits and vegetables, cereals or wholemeal bread), did not show significant associations with urine DMTP concentration levels. This result needs future investigation.

4.2.4. Herbicide factors

The investigation of exposure factors to herbicides in Esteban focused only on factors associated with the urinary concentrations of AMPA, which was the only metabolite quantified at levels high enough to permit researching exposure factors. No exposure factors were identified for children aged 6–17 years old. However, it would appear that children who had their health examinations in winter or autumn were less exposed than those who had them in the spring. This could be related to the greater amount of time spent outdoors in spring and summer, which would lead to greater exposure to outdoor sources of AMPA metabolites (and to their parent compounds).

A positive association was observed between urinary AMPA concentration levels in adults and using an independent boiler for home heating. AMPA exposure may have been linked to the use of phosphonates (exposure not related here to pesticide use), which are commonly used for descaling boilers, and which degrade into AMPA (Studnik et al., 2015; Grandcoin et al., 2017).

4.3. Comparison with health guidance values

A new approach for the derivation of health-based HBM guidance values (HBM-GV) to assess health risks was recently derived as part of the European initiative HBM4EU (Nakayama et al., 2023). The HBM-GV derived for the general population (HBM-GV_{GenPop}) represents the concentration of a specific chemical substance or its specific metabolite (s) in human biological matrices (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., 2020). HBM-GV_{GenPop} are equivalent to the HBM-I values from the German Human Biomonitoring Commission (Angerer et al., 2011). They can also be derived for particularly vulnerable population groups and/or for certain phases of life by considering differences in physiology (e.g., women of child-bearing age, children, elderly persons) (Apel et al., 2020). The proposed HBM-GVs for biomarkers of pyrethroid exposure are still provisional and must obtain final approval (Tarazona et al., 2022). HBM-GVs are constructed according to specific critical effects. Therefore, for the same metabolite common to distinct active substances, different values can be proposed depending on the toxicological reference values used in the calculations. In France, the values for all the pyrethroid metabolites measured in Esteban in the general population were overall below existing HBM-GVs_{GenPop}, guideline threshold values, except for 3-PBA, for which approximately 1% and 10% of children were above the urine lower and upper threshold values of 22 μg/L and 6.4 μg/L, respectively (according to tau-fluvalinate exposure assumptions) (Table 4). Tau-fluvalinate is a synthetic pyrethroid characterized by high lipid solubility and a more specific combat spectrum, insects and mites being its main targets. It is used as a spray on a wide variety of crops, but also in beekeeping through medicated strips. Based on the French BNVD (see above), the use of tau-fluvalinate slightly increased in France between 2008 and 2018, with 35,375 kg and 42,000 kg of various analogous chemical substances sold, respectively.

4.4. Strengths and limitations

The results of the Esteban study described here are based on data collected according to rigorous standardized protocols and from a large sample of participants, representative of the population of mainland France. Certain limitations inherent in Esteban's design need to be considered in our present analyses.

First, there may have been selection bias arising from the process used to select households and non-response by households and individuals. More specifically, participation trends in Esteban were similar to those in previous related studies conducted in France (Falq et al., 2011; Saoudi et al., 2014) with less participation by young adults (18–35 years old), single adults, men and unemployed persons, and more participation by individuals with a high-school diploma and above, persons in a relationship, employed people, individuals close nearing retirement age, and those already retired. However, considering the weighting process used, it is reasonable to suppose that the potential impact of any selection bias was limited. This is why we consider the study population to be representative of the French population living in mainland France during 2014–2016.

Second, collecting a first voided urine specimen raises the question of the measurement variability of the chemical substances (Morgan et al., 2016). However, considering that the majority of pesticides included in the present study have short half-lives, ranging from a few hours to a few days (exept for organoclorine compounds, with half-lives in years), the biomarker concentration levels measured reflected more recent and/or occasional exposure to parent compounds.

Third, the associations we highlighted should be interpreted with caution as cross-sectional studies alone do not allow causal links between the sources of exposure identified and the pesticide exposure levels, especially for exposure biomarkers with relatively short half-lives. In addition, due to the short half-lives of most pesticide

Table 4
Existing health-based HBM guidance values (HBM-GVs) in children and adults for the pyrethroid biomarkers measured in the Esteban study, 2014–2016.

			Percentile and maximum values in Esteban (µg Metabolite/L Urine)						
Pyretroid metabolite	Active Substance	Population	P50	P75	P90	P95	P99	Max	Proposed HBM-GV_{GenPop}[a] (µg Metabolite/L Urine)
3-PBA	Tau-fluvalinate	Children	1.03	1.97	4.28	7.30	26.1	61.2	6.4–22[b]
		Adults	0.72	1.28	2.42	3.23	8.34	44.5	9.6–33[b]
4-F-3-PBA (or F-PBA)	Cyfluthrin	Children	< LOQ	0.023	0.048	0.085	0.35	1.30	80
		Adults	< LOQ	0.018	0.037	0.068	0.18	1.83	130
BR2CA (or DBCA)	Deltamethrin	Children	1.16	2.41	4.47	5.70	12.5	22.8	90
		Adults	0.69	1.30	2.89	4.43	8.08	25.8	130
Σ cis - trans Cl2CA (or DCCA)	Cypermethrin	Children	0.50	0.97	1.97	3.03	11.2	17.9	30
		Adults	0.42	0.83	1.84	2.99	8.82	14.9	45
Σ cis - trans Cl2CA (or DCCA)	Permethrin	Children	0.50	0.97	1.97	3.03	11.2	17.9	320
		Adults	0.42	0.83	1.84	2.99	8.82	14.9	480

[a] Reference: Tarazona et al. (2022).
[b] Values based on the acceptable daily intake (ADI) of 0.005 mg/kg bw grounded on generic toxicity for the most conservative scenario and the most realistic scenario, respectively.

biomarkers of interest studied, the absence of any association between a potential source of exposure and the measured biomarker concentrations does not mean that this potential source of exposure must be totally excluded. Similarly, other potential exposure factors, in particular environmental factors, could not be analysed very accurately in the present study. For this, more adapted and targeted ad-hoc studies would be necessary. Futhermore, some differences in the comparisons of pesticide concentration levels observed between Esteban and international studies could also be explained by some methodological and temporal differences between the studies. Moreover, our study did not allow us to examine exposure to multiple chemicals among Esteban's participants; this would have provided a more accurate characterization of multi-chemical burden in the general population, as demonstrated elsewhere (Pecheux et al., 2022). Having said that, one of the advantages of the substance-by-substance approach used in our analyses is that it helped to identify exposure factors to a single chemical regardless of simultaneous exposure to other substances. It was not possible to identify occupational exposure to pesticides from agricultural factors based on the present study, because the number of agricultural workers in Esteban was too small. For this, future studies with a more appropriate design would be required.

Finally, given the absence of an internal toxicological reference value for many of the pesticide biomarkers we studied, the biological levels measured in urine and serum samples in Esteban do not necessarily reflect a health risk.

5. Conclusions

Esteban is the first HBM study in France to describe exposure to five families of pesticides in the general French population (adults and children) in 2014–2016. This article describes the levels of exposure to certain pesticides already studied only in adults in the French national HBM survey ENNS conducted a decade before Esteban. It also presents for the first time data on a new selection of pesticide families, including carbamates and herbicides. We found that pesticide exposure levels in France varied by chemical substance and chemical family, and by age category. French adults and children were still widely exposed to pyrethroid compounds because organochlorine pesticides had been replaced first by organophosphorus pesticides, and then by pyrethroids (broad-spectrum insecticides, which are still used today against a wide variety of pests). Concentration levels for pesticide compounds were globally lower than those from the French ENNS survey, with the exeption of the deltamethrin metabolite Br2CA. However, a significant proportion of the population was still exposed to prohibited chemicals like lindane, with quantifiable concentrations in almost half of the adults and children. In addition, depending on the calculation assumptions, one to ten out of one hundred children in France could have urinary 3-BPA concentrations above the recent health-based HBM guidance values derived for the general population (HBM-GV_{GenPop}). We found that concentration levels of pesticide compounds in France were similar to those from studies elsewhere in Europe and in North America, with the exception of β-HCH, DMTP metabolite, and Br2CA, whose levels were higher in France. Esteban enabled us to identify some factors of pesticide exposure, which can be acted on to improve public health. This is the case for the use of antiparasitics on domestic animals, which was associated with higher exposure to pyrethroids in adults and children. Our results also showed that regular consumption of products from organic farming was associated with lower concentration levels of pesticide exposure, in particular for pyrethroid metabolites and specific organochlorine compounds. The further of the national HBM programme in France should confirm the current downward trends in exposure levels to organophosphate, organochlorine and pyrethroid pesticides observed in Esteban, and provide initial data on exposure trends for carbamates, cyclodienes, and herbicides, including glyphosate and its metabolite AMPA. Future surveys will also enable us to broaden the list of pesticide molecules to be measured, as those measured in Esteban represent only a tiny fraction of the pesticides which the French population is exposed to.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that may have influenced the work reported in this paper.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijheh.2023.114265.

References

Aerts, R., Van Overmeire, I., Colles, A., Andjelkovic, M., Malarvannan, G., Poma, G., Den Hond, E., Van de Mieroop, E., Dewolf, M.C., Charlet, F., Van Nieuwenhuyse, A., Van Loco, J., Covaci, A., 2019. Determinants of persistent organic pollutant (POP) concentrations in human breast milk of a cross-sectional sample of primiparous mothers in Belgium. Environ. Int. 131, 104979 https://doi.org/10.1016/j.envint.2019.104979.

Akins, J.R., Waldrep, K., Bernert Jr., J.T., 1989. The estimation of total serum lipids by a completely enzymatic 'summation' method. Clin. Chim. Acta 184, 219–226.

Angerer, J., Aylward, L.L., Hays, S.M., Heinzow, B., Wilhelm, M., 2011. Human biomonitoring assessment values: approaches and data requirements. Int. J. Hyg Environ. Health 214, 348–360. https://doi.org/10.1016/j.ijheh.2011.06.002.

Angerer, J., Ewers, U., Wilhelm, M., 2007. Human biomonitoring: state of the art. Int. J. Hyg Environ. Health 210, 201–228. https://doi.org/10.1016/j.ijheh.2007.01.024.

Apel, P., Rousselle, C., Lange, R., Sissoko, F., Kolossa-Gehring, M., Ougier, E., 2020. Human biomonitoring initiative (HBM4EU) - strategy to derive human biomonitoring guidance values (HBM-GVs) for health risk assessment. Int. J. Hyg Environ. Health 230, 113622. https://doi.org/10.1016/j.ijheh.2020.113622.

Arrebola, J.P., Castano, A., Esteban, M., Bartolome, M., Perez-Gomez, B., Ramos, J.J., Bioambient, Es, 2018. Differential contribution of animal and vegetable food items on persistent organic pollutant serum concentrations in Spanish adults. Data from BIOAMBIENT.ES project. Sci. Total Environ. 634, 235–242. https://doi.org/10.1016/j.scitotenv.2018.03.283.

Aubertot, J.N., Barbier, J.M., Carpentier, A., Gril, J.J., Guichard, L., Lucas, P., Savary, S., Savini, I., Voltz, M., 2005. Pesticides, agriculture et environnement. Réduire l'utilisation des pesticides et limiter leurs impacts environnementaux. Expertise scientifique collective, synthèse du rapport. INRA et Cemagref (France).

Balicco, A., Oleko, A., Szego, E., Boschat, L., Deschamps, V., Saoudi, A., Zeghnoun, A., Fillol, C., 2017. Esteban design: a cross-sectional health survey about environment, biomonitoring, physical activity and nutrition (2014–2016). Toxicol. Anal. Clin. 29, 517–537. https://doi.org/10.1016/j.toxac.2017.06.003.

Bandow, N., Conrad, A., Kolossa-Gehring, M., Murawski, A., Sawal, G., 2020. Polychlorinated biphenyls (PCB) and organochlorine pesticides (OCP) in blood plasma - results of the German environmental survey for children and adolescents 2014-2017 (GerES V). Int. J. Hyg Environ. Health 224, 113426. https://doi.org/10.1016/j.ijheh.2019.113426.

Barr, D.B., Wilder, L.C., Caudill, S.P., Gonzalez, A.J., Needham, L.L., Pirkle, J.L., 2005. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ. Health Perspect. 113, 192–200. https://doi.org/10.1289/ehp.7337.

Becker, K., Seiwert, M., Angerer, J., Kolossa-Gehring, M., Hoppe, H.W., Ball, M., Schulz, C., Thumulla, J., Seifert, B., 2006. GerES IV pilot study: assessment of the exposure of German children to organophosphorus and pyrethroid pesticides. Int. J. Hyg Environ. Health 209, 221–233. https://doi.org/10.1016/j.ijheh.2005.12.002.

Berger-Preiss, E., Levsen, K., Leng, G., Idel, H., Sugiri, D., Ranft, U., 2002. Indoor pyrethroid exposure in homes with woollen textile floor coverings. Int. J. Hyg Environ. Health 205, 459–472. https://doi.org/10.1078/1438-4639-00181.

Blaylock, B.L., 2005. DDT (dichlorodiphenyltrichloroethane). In: Wexler, P. (Ed.), Encyclopedia of Toxicology (Second Edition). Elsevier, New York.

Brauner, E.V., Raaschou-Nielsen, O., Gaudreau, E., Leblanc, A., Tjonneland, A., Overvad, K., Sorensen, M., 2012. Predictors of adipose tissue concentrations of organochlorine pesticides in a general Danish population. J. Expo. Sci. Environ. Epidemiol. 22, 52–59. https://doi.org/10.1038/jes.2011.39.

Bravo, N., Grimalt, J.O., Mazej, D., Tratnik, J.S., Sarigiannis, D.A., Horvat, M., 2020. Mother/child organophosphate and pyrethroid distributions. Environ. Int. 134, 105264 https://doi.org/10.1016/j.envint.2019.105264.

Buekers, J., Remy, S., Bessems, J., Govarts, E., Rambaud, L., Riou, M., Halldorsson, T.I., Olafsdottir, K., Probst-Hensch, N., Ammann, P., Weber, T., Kolossa-Gehring, M., Esteban-Lopez, M., Castano, A., Andersen, H.R., Schoeters, G., 2022a. Glyphosate and AMPA in human urine of hbm4eu-aligned studies: Part B adults. Toxics 10. https://doi.org/10.3390/toxics10100552.

Buekers, J., Remy, S., Bessems, J., Govarts, E., Rambaud, L., Riou, M., Tratnik, J.S., Stajnko, A., Katsonouri, A., Makris, K.C., De Decker, A., Morrens, B., Vogel, N., Kolossa-Gehring, M., Esteban-Lopez, M., Castano, A., Andersen, H.R., Schoeters, G., 2022b. Glyphosate and AMPA in human urine of HBM4EU aligned studies: Part A children. Toxics 10. https://doi.org/10.3390/toxics10080470.

Burr, S.A., 2014. DDT (dichlorodiphenyltrichloroethane). In: Wexler, P. (Ed.), Encyclopedia of Toxicology (Third Edition). Academic Press, Oxford.

Cai, J., Liu, B., Zhu, X., Su, Q., 2002. Determination of pyrethroid residues in tobacco and cigarette smoke by capillary gas chromatography. J. Chromatogr. A 964, 205–211. https://doi.org/10.1016/s0021-9673(02)00586-1.

Cao, L.L., Yan, C.H., Yu, X.D., Tian, Y., Zhao, L., Liu, J.X., Shen, X.M., 2011. Relationship between serum concentrations of polychlorinated biphenyls and organochlorine pesticides and dietary habits of pregnant women in Shanghai. Sci. Total Environ. 409, 2997–3002. https://doi.org/10.1016/j.scitotenv.2011.04.040.

CDC, 2021a. Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, March 2021. Volume One: NHANES 1999-2010. Center for Disease Control and Prevention.

CDC, 2021b. Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, March 2021. Volume Two: NHANES 2011-2016. Center for Disease Control and Prevention.

Connolly, A., Leahy, M., Jones, K., Kenny, L., Coggins, M.A., 2018. Glyphosate in Irish adults - a pilot study in 2017. Environ. Res. 165, 235–236. https://doi.org/10.1016/j.envres.2018.04.025.

Conrad, A., Schroter-Kermani, C., Hoppe, H.W., Ruther, M., Pieper, S., Kolossa-Gehring, M., 2017. Glyphosate in German adults - time trend (2001 to 2015) of human exposure to a widely used herbicide. Int. J. Hyg Environ. Health 220, 8–16. https://doi.org/10.1016/j.ijheh.2016.09.016.

Dereumeaux, C., Fillol, C., Charles, M.A., Denys, S., 2017. The French human biomonitoring program: first lessons from the perinatal component and future needs. Int. J. Hyg Environ. Health 220, 64–70. https://doi.org/10.1016/j.ijheh.2016.11.005.

Dereumeaux, C., Saoudi, A., Goria, S., Wagner, V., De Crouy-Chanel, P., Pecheux, M., Berat, B., Zaros, C., Guldner, L., 2018. Urinary levels of pyrethroid pesticides and determinants in pregnant French women from the Elfe cohort. Environ. Int. 119, 89–99. https://doi.org/10.1016/j.envint.2018.04.042.

Dereumeaux, C., Saoudi, A., Pecheux, M., Berat, B., de Crouy-Chanel, P., Zaros, C., Brunel, S., Delamaire, C., le Tertre, A., Lefranc, A., Vandentorren, S., Guldner, L., 2016. Biomarkers of exposure to environmental contaminants in French pregnant women from the Elfe cohort in 2011. Environ. Int. 97, 56–67. https://doi.org/10.1016/j.envint.2016.10.013.

Dewalque, L., Charlier, C., Pirard, C., 2014. Estimated daily intake and cumulative risk assessment of phthalate diesters in a Belgian general population. Toxicol. Lett. 231, 161–168. https://doi.org/10.1016/j.toxlet.2014.06.028.

EPA, 2022. Human Health Issues Related to Pesticides.

Falq, G., Zeghnoun, A., Pascal, M., Vernay, M., Le Strat, Y., Garnier, R., Olichon, D., Bretin, P., Castetbon, K., Frery, N., 2011. Blood lead levels in the adult population living in France the French Nutrition and Health Survey (ENNS 2006-2007). Environ. Int. 37, 565–571. https://doi.org/10.1016/j.envint.2010.11.012.

FAO, 2021. Pesticides use. Global, regional and country trends. In: 1990–2018. FAOSTAT Analytical Brief Series No. 16. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO, 2022. FAOSTAT. https://www.fao.org/faostat/en/#data.

Fernandez, S.F., Pardo, O., Corpas-Burgos, F., Yusa, V., force, B.t., 2020. Exposure and cumulative risk assessment to non-persistent pesticides in Spanish children using biomonitoring. Sci. Total Environ. 746, 140983 https://doi.org/10.1016/j.scitotenv.2020.140983.

Fillol, C., Garnier, R., Mullot, J.-U., Boudet, C., Momas, I., Salmi, L.R., Vandentorren, S., 2014. Prioritization of the biomarkers to be analyzed in the French biomonitoring program. Biomonitoring 1. https://doi.org/10.2478/bimo-2014-0010.

Fillol, C., Oleko, A., Saoudi, A., Zeghnoun, A., Balicco, A., Gane, J., Rambaud, L., Leblanc, A., Gaudreau, E., Marchand, P., Le Bizec, B., Bouchart, V., Le Gleau, F., Durand, G., Denys, S., 2021. Exposure of the French population to bisphenols, phthalates, parabens, glycol ethers, brominated flame retardants, and perfluorinated compounds in 2014-2016: results from the Esteban study. Environ. Int. 147, 106340 https://doi.org/10.1016/j.envint.2020.106340.

Fortes, C., Mastroeni, S., Pilla, M.A., Antonelli, G., Lunghini, L., Aprea, C., 2013. The relation between dietary habits and urinary levels of 3-phenoxybenzoic acid, a pyrethroid metabolite. Food Chem. Toxicol. 52, 91–96. https://doi.org/10.1016/j.fct.2012.10.035.

Fréry, N., Saoudi, A., Garnier, R., Zeghnoun, A., Bidondo, M.L., 2013. Exposition de la population française aux substances chimiques de l'environnement Tome2 - Polychlorobiphényles (PCB-NDL) et pesticides.

Genuis, S.J., Lane, K., Birkholz, D., 2016. Human elimination of organochlorine pesticides: blood, urine, and sweat study. BioMed Res. Int., 1624643 https://doi.org/10.1155/2016/1624643, 2016.

Gilles, L., Govarts, E., Rambaud, L., Vogel, N., Castano, A., Esteban Lopez, M., Rodriguez Martin, L., Koppen, G., Remy, S., Vrijheid, M., Montazeri, P., Birks, L., Sepai, O., Stewart, L., Fiddicke, U., Loots, I., Knudsen, L.E., Kolossa-Gehring, M., Schoeters, G., 2021. HBM4EU combines and harmonises human biomonitoring data across the EU, building on existing capacity - the HBM4EU survey. Int. J. Hyg Environ. Health 237, 113809. https://doi.org/10.1016/j.ijheh.2021.113809.

Glorennec, P., Serrano, T., Fravallo, M., Warembourg, C., Monfort, C., Cordier, S., Viel, J. F., Le Gléau, F., Le Bot, B., Chevrier, C., 2017. Determinants of children's exposure to pyrethroid insecticides in western France. Environ. Int. 104, 76–82. https://doi.org/10.1016/j.envint.2017.04.007.

Gonzalez-Alzaga, B., Lacasana, M., Hernandez, A.F., Arrebola, J.P., Lopez-Flores, I., Artacho-Cordon, F., Bonde, J.P., Olea, N., Aguilar-Garduno, C., 2018. Serum concentrations of organochlorine compounds and predictors of exposure in children living in agricultural communities from South-Eastern Spain. Environ. Pollut. 237, 685–694. https://doi.org/10.1016/j.envpol.2017.10.109.

Grandcoin, A., Piel, S., Baures, E., 2017. AminoMethylPhosphonic acid (AMPA) in natural waters: its sources, behavior and environmental fate. Water Res. 117, 187–197. https://doi.org/10.1016/j.watres.2017.03.055.

Guilpart, N., Bertin, I., Valantin-Morison, M., Barbu, C.M., 2022. How much agricultural land is there close to residential areas? An assessment at the national scale in France. Build. Environ. 226 https://doi.org/10.1016/j.buildenv.2022.109662.

Haines, D.A., Saravanabhavan, G., Werry, K., Khoury, C., 2017. An overview of human biomonitoring of environmental chemicals in the Canadian Health Measures Survey: 2007-2019. Int. J. Hyg Environ. Health 220, 13–28. https://doi.org/10.1016/j.ijheh.2016.08.002.

Harmouche-Karaki, M., Mahfouz, Y., Salameh, P., Matta, J., Helou, K., Narbonne, J.F., 2019. Patterns of PCBs and OCPs exposure in a sample of Lebanese adults: the role of diet and physical activity. Environ. Res. 179, 108789 https://doi.org/10.1016/j.envres.2019.108789.

Health Canada, 2010. In: Health, M.o. (Ed.), Report on Human Biomonitoring of Environmental Chemicals in Canada: Results of the Canadian Health Measures Survey Cycle 1 (2007-2009). Ottawa, ON.

Health Canada, 2013. In: Health, M.o. (Ed.), Second Report on Human Biomonitoring of Environmental Chemicals in Canada: Results of the Canadian Health Measures Survey Cycle 2 (2009-2011). Ottawa, ON.
Health Canada, 2019. In: Health, M.o. (Ed.), Fifth Report on Human Biomonitoring of Environmental Chemicals in Canada: Results of the Canadian Health Measures Survey Cycle 4 (2016–2017). Ottawa, ON.
Health Canada, 2020. In: Health, M.o. (Ed.), Report on Human Biomonitoring of Environmental Chemicals in Pooled Samples: Results of the Canadian Health Measures Survey Cycle 1 (2007–2009), Cycle 2 (2012–2013), Cycle 4 (2014–2015), and Cycle 5 (2016–2017. Ottawa, ON.
Ibarluzea, J., Alvarez-Pedrerol, M., Guxens, M., Marina, L.S., Basterrechea, M., Lertxundi, A., Etxeandia, A., Goni, F., Vioque, J., Ballester, F., Sunyer, J., Project, I., 2011. Sociodemographic, reproductive and dietary predictors of organochlorine compounds levels in pregnant women in Spain. Chemosphere 82, 114–120. https://doi.org/10.1016/j.chemosphere.2010.09.051.
JAFFE, M., 1886. Ueber den Niederschlang, welchen Pikrinsaoure in nomalen Harn erzeugt und uber eiue Reaktion des Kreatinins. Z. Physiol. Chem. 10, 391–400.
Kang, J.H., Park, H., Chang, Y.S., Choi, J.W., 2008. Distribution of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in human serum from urban areas in Korea. Chemosphere 73, 1625–1631. https://doi.org/10.1016/j.chemosphere.2008.07.087.
Katsikantami, I., Colosio, C., Alegakis, A., Tzatzarakis, M.N., Vakonaki, E., Rizos, A.K., Sarigiannis, D.A., Tsatsakis, A.M., 2019. Estimation of daily intake and risk assessment of organophosphorus pesticides based on biomonitoring data - the internal exposure approach. Food Chem. Toxicol. 123, 57–71. https://doi.org/10.1016/j.fct.2018.10.047.
Lemke, N., Murawski, A., Schmied-Tobies, M.I.H., Rucic, E., Hoppe, H.W., Conrad, A., Kolossa-Gehring, M., 2021. Glyphosate and aminomethylphosphonic acid (AMPA) in urine of children and adolescents in Germany - human biomonitoring results of the German Environmental Survey 2014-2017 (GerES V). Environ. Int. 156, 106769 https://doi.org/10.1016/j.envint.2021.106769.
Li, M., Wang, R., Su, C., Li, J., Wu, Z., 2022. Temporal trends of exposure to organochlorine pesticides in the United States: a population study from 2005 to 2016. Int. J. Environ. Res. Publ. Health 19. https://doi.org/10.3390/ijerph19073862.
Little, R.J., Rubin, D.B., 2002. Statistical Analysis with Missing Data, second ed. ed. John Wiley & Sons, New Jersey.
Mandin, C., Mercier, F., Ramalho, O., Lucas, J.-P., Gilles, E., Blanchard, O., Bonvallot, N., Glorennec, P., Le Bot, B., 2016. Semi-volatile organic compounds in the particulate phase in dwellings: a nationwide survey in France. Atmos. Environ. 136, 82–94. https://doi.org/10.1016/j.atmosenv.2016.04.016.
Morck, T.A., Andersen, H.R., Knudsen, L.E., 2016. Organophosphate Metabolites in Urine Samples from Danish Children and Women: Measured in the Danish DEMOCOPHES Population. The Danish Environmental Protection Agency.
Morgan, M.K., Sobus, J.R., Barr, D.B., Croghan, C.W., Chen, F.L., Walker, R., Alston, L., Andersen, E., Clifton, M.S., 2016. Temporal variability of pyrethroid metabolite levels in bedtime, morning, and 24-h urine samples for 50 adults in North Carolina. Environ. Res. 144, 81–91. https://doi.org/10.1016/j.envres.2015.11.003.
Mussalo-Rauhamaa, H., 1991. Partitioning and levels of neutral organochlorine compounds in human serum, blood cells, and adipose and liver tissue. Sci. Total Environ. 103, 159–175. https://doi.org/10.1016/0048-9697(91)90142-2.
Nakayama, S.F., St-Amand, A., Pollock, T., Apel, P., Bamai, Y.A., Barr, D.B., Bessems, J., Calafat, A.M., Castano, A., Covaci, A., Duca, R.C., Faure, S., Galea, K.S., Hays, S., Hopf, N.B., Ito, Y., Jeddi, M.Z., Kolossa-Gehring, M., Kumar, E., LaKind, J.S., Lopez, M.E., Louro, H., Macey, K., Makris, K.C., Melnyk, L., Murawski, A., Naiman, J., Nassif, J., Noisel, N., Poddalgoda, D., Quiros-Alcala, L., Rafiee, A., Rambaud, L., Silva, M.J., Ueyama, J., Verner, M.A., Waras, M.N., Werry, K., 2023. Interpreting biomonitoring data: introducing the international human biomonitoring (i-HBM) working group's health-based guidance value (HB2GV) dashboard. Int. J. Hyg Environ. Health 247, 114046. https://doi.org/10.1016/j.ijheh.2022.114046.
Narduzzi, S., Fantini, F., Blasetti, F., Rantakokko, P., Kiviranta, H., Forastiere, F., Michelozzi, P., Porta, D., 2020. Predictors of Beta-Hexachlorocyclohexane blood levels among people living close to a chemical plant and an illegal dumping site. Environ. Health 19, 9. https://doi.org/10.1186/s12940-020-0562-7.
Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., Hens, L., 2016. Chemical pesticides and human health: the urgent need for a new concept in agriculture. Front. Public Health 4, 148. https://doi.org/10.3389/fpubh.2016.00148.
Nova, P., Calheiros, C.S.C., Silva, M., 2020. Glyphosate in Portuguese adults - a pilot study. Environ. Toxicol. Pharmacol. 80, 103462 https://doi.org/10.1016/j.etap.2020.103462.
NPIC, 2022. Pesticides and Human Health.
Ospina, M., Schutze, A., Morales-Agudelo, P., Vidal, M., Wong, L.Y., Calafat, A.M., 2022. Exposure to glyphosate in the United States: data from the 2013-2014 national health and nutrition examination survey. Environ. Int. 170, 107620 https://doi.org/10.1016/j.envint.2022.107620.
Ougier, E., Ganzleben, C., Lecoq, P., Bessems, J., David, M., Schoeters, G., Lange, R., Meslin, M., Uhl, M., Kolossa-Gehring, M., Rousselle, C., Vicente, J.L., 2021. Chemical prioritisation strategy in the European human biomonitoring initiative (HBM4EU) - development and results. Int. J. Hyg Environ. Health 236, 113778. https://doi.org/10.1016/j.ijheh.2021.113778.
Pearson, M.A., Lu, C., Schmotzer, B.J., Waller, L.A., Riederer, A.M., 2009. Evaluation of physiological measures for correcting variation in urinary output: implications for assessing environmental chemical exposure in children. J. Expo. Sci. Environ. Epidemiol. 19, 336–342. https://doi.org/10.1038/jes.2008.48.
Pecheux, M., Saoudi, A., Zeghnoun, A., Oleko, A., Fillol, C., 2022. Assessment of multi-chemical exposure using human biomonitoring data from the French Esteban study using exposure load method. Int. J. Hyg Environ. Health 246, 114054. https://doi.org/10.1016/j.ijheh.2022.114054.
Pirard, C., Remy, S., Giusti, A., Champon, L., Charlier, C., 2020. Assessment of children's exposure to currently used pesticides in wallonia, Belgium. Toxicol. Lett. 329, 1–11. https://doi.org/10.1016/j.toxlet.2020.04.020.
Ploteau, S., Antignac, J.P., Volteau, C., Marchand, P., Venisseau, A., Vacher, V., Le Bizec, B., 2016. Distribution of persistent organic pollutants in serum, omental, and parietal adipose tissue of French women with deep infiltrating endometriosis and circulating versus stored ratio as new marker of exposure. Environ. Int. 97, 125–136. https://doi.org/10.1016/j.envint.2016.08.011.
Roberts, J.R., Karr, C.J., Council On Environmental, H., 2012. Pesticide exposure in children. Pediatrics 130, e1765–1788. https://doi.org/10.1542/peds.2012-2758.
Royston, P., White, I., 2011. Multiple imputation by chained equations (MICE): implementation in Stata. J. Stat. Software 45, 1–20.
Ruiz, P., Dualde, P., Coscolla, C., Fernandez, S.F., Carbonell, E., Yusa, V., 2021. Biomonitoring of glyphosate and AMPA in the urine of Spanish lactating mothers. Sci. Total Environ. 801, 149688 https://doi.org/10.1016/j.scitotenv.2021.149688.
Saoudi, A., Frery, N., Zeghnoun, A., Bidondo, M.L., Deschamps, V., Goen, T., Garnier, R., Guldner, L., 2014. Serum levels of organochlorine pesticides in the French adult population: the French National Nutrition and Health Study (ENNS), 2006-2007. Sci. Total Environ. 472, 1089–1099. https://doi.org/10.1016/j.scitotenv.2013.11.044.
Schoeters, G., Govarts, E., Bruckers, L., Den Hond, E., Nelen, V., De Henauw, S., Sioen, I., Nawrot, T.S., Plusquin, M., Vriens, A., Covaci, A., Loots, I., Morrens, B., Coertjens, D., Van Larebeke, N., De Craemer, S., Croes, K., Lambrechts, N., Colles, A., Baeyens, W., 2017. Three cycles of human biomonitoring in flanders - time trends observed in the flemish environment and health study. Int. J. Hyg Environ. Health 220, 36–45. https://doi.org/10.1016/j.ijheh.2016.11.006.
Schoeters, G., Verheyen, V.J., Colles, A., Remy, S., Martin, L.R., Govarts, E., Nelen, V., Den Hond, E., De Decker, A., Franken, C., Loots, I., Coertjens, D., Morrens, B., Bastiaensen, M., Gys, C., Malarvannan, G., Covaci, A., Nawrot, T., De Henauw, S., Bellemans, M., Leermakers, M., Van Larebeke, N., Baeyens, W., Jacobs, G., Voorspoels, S., Nielsen, F., Bruckers, L., 2022. Internal exposure of flemish teenagers to environmental pollutants: results of the flemish environment and health study 2016-2020 (FLEHS IV). Int. J. Hyg Environ. Health 242, 113972. https://doi.org/10.1016/j.ijheh.2022.113972.
Seifert, B., Becker, K., Hoffmann, K., Krause, C., Schulz, C., 2000. The German Environmental Survey 1990/1992 (GerES II): a representative population study. J. Expo. Sci. Environ. Epidemiol. 10, 103–114. https://doi.org/10.1038/sj.jea.7500075.
Soukup, S.T., Merz, B., Bub, A., Hoffmann, I., Watzl, B., Steinberg, P., Kulling, S.E., 2020. Glyphosate and AMPA levels in human urine samples and their correlation with food consumption: results of the cross-sectional KarMeN study in Germany. Arch. Toxicol. 94, 1575–1584. https://doi.org/10.1007/s00204-020-02704-7.
Stajnko, A., Snoj Tratnik, J., Kosjek, T., Mazej, D., Jagodic, M., Erzen, I., Horvat, M., 2020. Seasonal glyphosate and AMPA levels in urine of children and adolescents living in rural regions of Northeastern Slovenia. Environ. Int. 143, 105985 https://doi.org/10.1016/j.envint.2020.105985.
Stehr-Green, P.A., Farrar, J.A., Burse, V.W., Royce, W.G., Wohlleb, J.C., 1988. A survey of measured levels and dietary sources of selected organochlorine pesticide residues and metabolites in human sera from a rural population. Am. J. Publ. Health 78, 828–830. https://doi.org/10.2105/ajph.78.7.828.
Studnik, H., Liebsch, S., Forlani, G., Wieczorek, D., Kafarski, P., Lipok, J., 2015. Amino polyphosphonates - chemical features and practical uses, environmental durability and biodegradation. Nat. Biotechnol. 32, 1–6. https://doi.org/10.1016/j.nbt.2014.06.007.
Tarazona, J.V., Cattaneo, I., Niemann, L., Pedraza-Diaz, S., Gonzalez-Caballero, M.C., de Alba-Gonzalez, M., Canas, A., Dominguez-Morueco, N., Esteban-Lopez, M., Castano, A., Borges, T., Katsonouri, A., Makris, K.C., Ottenbros, I., Mol, H., De Decker, A., Morrens, B., Berman, T., Barnett-Itzhaki, Z., Probst-Hensch, N., Fuhrimann, S., Tratnik, J.S., Horvat, M., Rambaud, L., Riou, M., Schoeters, G., Govarts, E., Kolossa-Gehring, M., Weber, T., Apel, P., Namorado, S., Santonen, T., 2022. A tiered approach for assessing individual and combined risk of pyrethroids using human biomonitoring data. Toxics 10. https://doi.org/10.3390/toxics10080451.
Team, R.C.R., 2017. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Australia. Disponible: https://www.R-project.org/.
Wielgomas, B., Piskunowicz, M., 2013. Biomonitoring of pyrethroid exposure among rural and urban populations in northern Poland. Chemosphere 93, 2547–2553. https://doi.org/10.1016/j.chemosphere.2013.09.070.
Ye, M., Beach, J., Martin, J.W., Senthilselvan, A., 2015. Associations between dietary factors and urinary concentrations of organophosphate and pyrethroid metabolites in a Canadian general population. Int. J. Hyg Environ. Health 218, 616–626. https://doi.org/10.1016/j.ijheh.2015.06.006.
Zentai, A., Szabo, I.J., Kerekes, K., Ambrus, A., 2016. Risk assessment of the cumulative acute exposure of Hungarian population to organophosphorus pesticide residues with regard to consumers of plant based foods. Food Chem. Toxicol. 89, 67–72. https://doi.org/10.1016/j.fct.2016.01.016.

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Perceived difficulties in maintaining menstrual hygiene practices among indigenous adolescents during seasonal water scarcity periods in Bandarban hill district of Bangladesh: A cross-sectional study☆

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ABSTRACT

Background: Access to clean water is important for menstrual hygiene practices, an important aspect of health for adolescent girls. In Bangladesh, adolescent girls represent poor menstrual hygiene practices, whereas the practice is worse among vulnerable population groups living in areas experiencing seasonal water scarcity. This study portrays perceived difficulties in menstrual hygiene practices among indigenous adolescent girls during the period of seasonal water scarcity in Bandarban Hill District, Bangladesh.

Method: Data was collected from 242 indigenous adolescent girls through interviews during the period of water scarcity. Backward stepwise regression model was used to identify factors associated with perceived difficulty in maintaining menstrual hygiene (PD) practices.

Result: The study participants, mainly living in hard-to-reach areas, reported difficulty in getting adequate water during the water scarcity period, and the quality of water was reported to be poor. PD due to water scarcity was found to be significantly associated with water source degradation ($\beta = 0.247$, < 0.001), the need for boiling/purifying water before use for menstrual hygiene ($\beta = 0.203$, $p = 0.005$), and experience of water availability when it was necessary to maintain their optimal menstrual hygiene practice ($\beta = 0.449$, $p < 0.001$), time required to collect water ($\beta = 0.209$, $p < 0.001$), taking a bath every day ($\beta = -0.228$, $p < 0.001$), and frequency of washing genitals per day ($\beta = -0.094$, $p = 0.040$).

Conclusion: Indigenous adolescents perceive difficulty in menstrual hygiene practices during the period of water scarcity. Further research could be carried out to observe to what extent the seasonal water scarcity could be attributable to worsen the menstrual hygiene practices and to identify the need for addressing the problems.

1. Introduction

Water availability is essential for hygiene practices, including menstrual hygiene (Hussein et al., 2022). Water scarcity increases women's vulnerability regarding their menstrual health (Rossouw and Ross, 2021). Particularly in lower-middle-income countries (LMICs), studies showed that water scarcity significantly affects women's menstrual hygiene practices (Dr Shamima Yasmin, 2013; Van Eijk et al., 2016; Wali et al., 2020). Poor menstrual hygiene practices were found to be associated with urogenital infections such as bacterial vaginosis among reproductive-age women (Das et al., 2015; Torondel et al., 2018). School absenteeism among adolescent girls was also attributed to poor

Abbreviations: **LMIC**, Low- and middle-income countries; **CHT**, Chittagong Hill Tract; **MHP**, Menstrual hygiene practices; **PD**, Perceived difficulty in maintaining menstrual hygiene; **WASH**, Water, Sanitation and Hygiene.

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menstrual hygiene due to inadequate access to water, sanitation and hygiene (WASH) facilities (Shumie and Mengie, 2022; Tegegne and Sisay, 2014). In consequence, it increases the overall burden of disease, especially in the domain of women's reproductive health (Mills and Cumming, 2016). Studies conducted in LMICs demonstrated that water scarcity highly affected women's menstrual hygiene practices (Dr Shamima Yasmin, 2013; Van Eijk et al., 2016; Wali et al., 2020). For instance, a study in West Bengal, India, found that adequate water supply was significantly associated with quality menstrual hygiene practices among adolescents (Dr Shamima Yasmin, 2013).

In Bangladesh, many women face challenges maintaining menstrual hygiene regarding water quantity and quality. Although 98% of people have access to water, the overall water quality is poor in Bangladesh (World Bank, 2018). For instance, it was found that *E. coli* bacteria was present in 80% of private piped water taps and most of the pond water (World Bank, 2018). National hygiene survey 2018 reported that 8% of adolescents and 12% of women of reproductive age used unprotected water sources for drying and storing menstrual clothes (Government of the People's Republic of Bangladesh, 2018), incurring 41% of school absenteeism among adolescent girls (Alam et al., 2017).

Chittagong Hill Tracts (CHT) of Bangladesh are considered one of the most vulnerable areas that suffer from water scarcity all over the year, and the situation becomes worse during the dry seasons (Alam, 2022; Hossen et al., 2016). As the area is steep with high latitude compared to the mainland and the infrastructures are poor, people experience hardship in getting and reserving water for their daily use during dry season. A study found that 50% of dwellers of CHT districts, namely Bandarban, Rangamati and Khagrachari, suffer from severe water crises during the winter and summer when they collect water from the nearby springs, streams, and other natural sources (Hossen et al., 2016).However, this rate was 41% in Bandarban district (Hossen et al., 2016). It was reported that only 15.4% of adolescent girls in CHTs clean vagina during menstruation with clean water (Borua et al., 2019). A recent study shows 59.4% of indigenous people in Bandarban use spring water, 36% use surface water, and 10.8% use tube-well water for drinking (Mahmud et al., 2020). However, this may vary due to seasonal water availability changes in these sources. More than half of the indigenous populations in Bandarban district were found to have poor WASH practices which could be attributed to water scarcity (Mahmud et al., 2020). Evidence represents that this severe water scarcity heavily obstructs the menstrual hygiene management of the women of reproductive age in this region (Ahmed et al., 2017; Muhit and Chowdhury, 2013). The study by Muhit and Chowdhury (2013) showed that adolescent schoolgirls of the CHT are more vulnerable to water supply and sanitation conditions compared to those in other parts of Bangladesh, contributing to 39% school absenteeism.

Although studies were conducted on the water scarcity situation and its impact on the population health in CHT districts, there is no study exploring if the seasonal water scarcity has any association in maintaining adolescent girls' menstrual hygiene. To fill out this research gap, the present study aims at exploring the impact of water scarcity and its associated factors on the perception of difficulty faced by the adolescent ethnic minorities inhabiting the Bandarban district of CHT in maintaining their menstrual hygiene during the dry seasons.

2. Methods

2.1. Settings

Bandarban is in the south-eastern region under the Chattogram administrative division and is the remotest and least populated district in Bangladesh. Bandarban has some of the highest peaks in the country, and 11 different indigenous communities living along with mainstream Bengali people (Debnath et al., 2022). The indigenous communities have distinct languages, cultures, traditions, and religions. However, they experience discrimination, land extortion, ethnic prejudice, and poor health and nutritional status (Roy, 2012).

The total population of Bandarban is 436,950, and the total under-eighteen population is 173,351 (BBS, 2011a). Among them, 142,401 (44.39%) are from indigenous communities (BBS, 2011b). The population density in Bandarban is 87 per square km (BBS, 2011a), which is lower than the national average of 976 per square km (BBS, 2011a). It has seven sub-districts, called upazilas (the second smallest unit of local government/administration), and 29 unions (the smallest unit of local government). Most of the indigenous people live in rural small villages, called para (regarded as the smallest social institution administered by a local representative called Headman), near to hills, springs, rivers, and plains/valleys. For this study, the district of Bandarban was chosen for having higher water scarcity than the other two districts in the Chattrogram Hill Tracts (Chakma et al., 2021).

2.2. Study design, sampling, and sample size

A cross-sectional design was employed to collect information from 242 conveniently selected indigenous adolescent girls living in the water scarcity areas in Bandarban Hill District of Bangladesh. Water scarcity areas were identified with the help of local indigenous people during the planning phase of the study. There was no available data regarding what proportion of indigenous adolescent girls had access to quality water and sun drying the menstrual absorbent properly for menstrual hygiene practices. Therefore, population proportion was considered based on National Hygiene Survey 2018, indicating that 12% of the adolescents in Bangladesh had improved water source and adequate sundry of menstrual absorbent for menstrual hygiene practices (Government of the People's Republic of Bangladesh, 2018). The estimated population size was calculated as 10,295, considering a total of 76,036 adolescents, 44.39% indigenous population, and 61% people living in hilly areas (BBS, 2011b). Finally, sample size was determined considering a population proportion (p) of 0.12, 95% confidence level, and 5% margin of error, and 20% non-response (Kasiulevičius et al., 2006). The calculation provided at least 195 adolescents to be interviewed. In this study, 242 indigenous adolescents were interviewed, providing a representative sample of 10,295 indigenous adolescent girls.

2.3. Data collection and management

Data collection was conducted in 38 paras from 11 unions under the six upazilas from Bandarban district (Fig. 1). Data was collected between the period of February to May 2022. A total of 242 adolescent girls were interviewed using a pre-tested questionnaire. The questionnaire was translated from English to both Bengali and Marma languages (the most common indigenous language in the study area). Female interviewers were recruited from indigenous communities, including Marma, Tripura, and Tanchangya and were trained in data collection. The data collectors were able to speak both Bengali and indigenous. The purpose of the study, data privacy, potential impact was described, and verbal consents were taken before the interviews.

After interviewing, the questionnaire was transcribed into an SPSS datasheet. Each respondent was designated with an identity number (ID number) for anonymization during analysis and to maintain privacy of the respondent. Both versions of the dataset such as completed questionnaire and soft copy were stored securely.

2.4. Variables and covariates

Literature search was performed to understand how menstrual hygiene practices are associated with socioeconomic factors and water availability. Bangladesh National Hygiene survey 2018 and researches conducted in water scarcity settings were reviewed to select variables for formulating the questionnaire. Resource scarcity affects menstrual hygiene practice, knowledge, and awareness of different reproductive-aged women in crisis and resource-poor settings (Kuhlmann et al.,

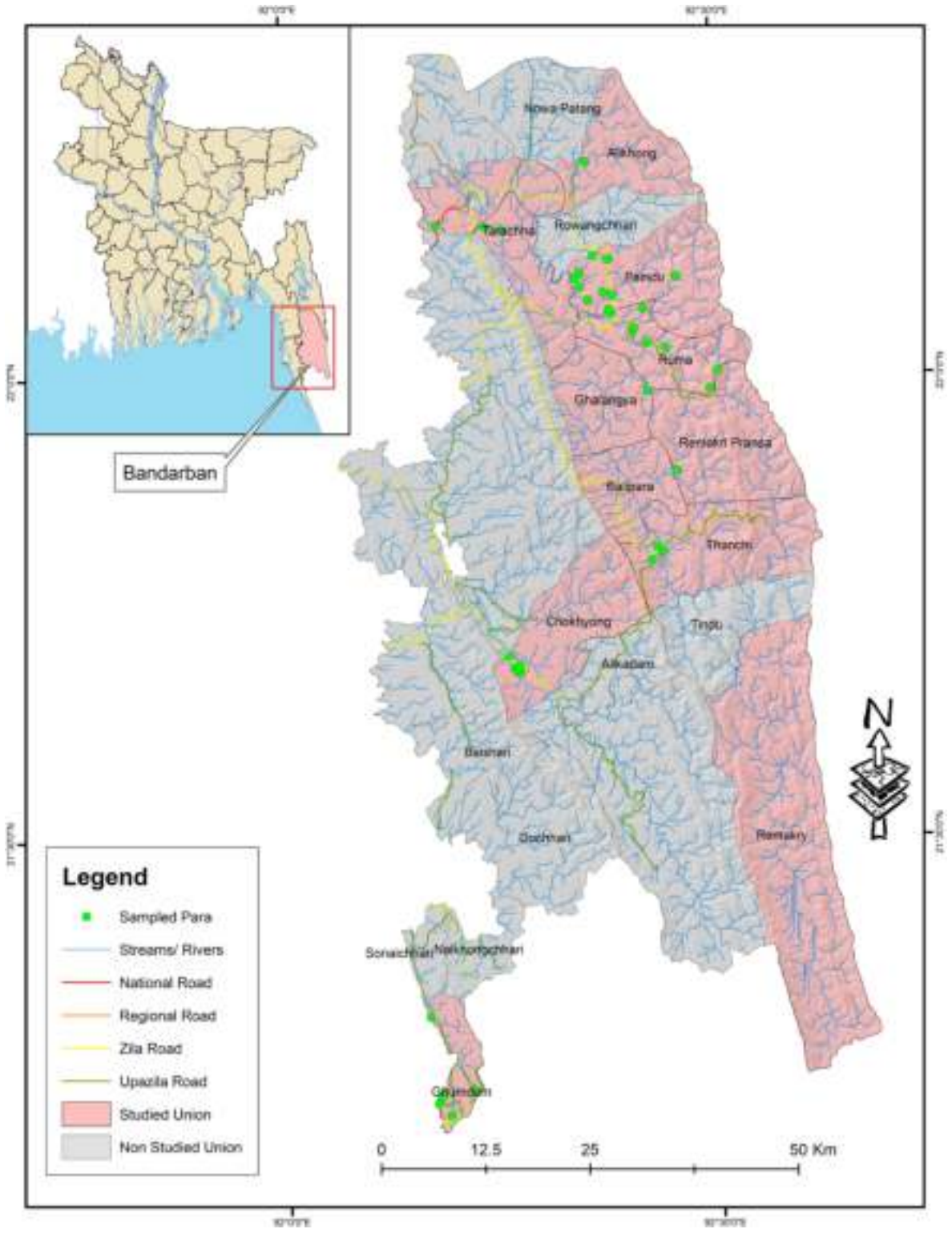

Fig. 1. Study area and data collection sites.

2017; Michael et al., 2020; Raina and Balodi, 2014; Thakre et al., 2011; VanLeeuwen and Torondel, 2018). Then, we narrowed our search to the associated factors of water scarcity and menstrual hygiene in similar socioeconomic and geographic contexts (Das et al., 2015; Garg et al., 2022; Ha and Alam, 2022).

Socio-demographic information included age, marital status, educational status, number of family members, place of residence, and ethnicity. Variables related to hardship in getting water included sources of water for family use and menstrual hygiene practices, the time required to collect water from these sources, degradation of water sources during the dry seasons, quality of water used in the last menstrual period, the hardship of getting sufficient water during the last menstrual period, if they need to boil or purify water before use, and event of family relocation due to water scarcity in last three years. Menstrual hygiene practices were assessed considering the frequency of taking baths and washing genitals in a day during the last menstrual period.

2.5. Psychometric toll development: outcome measurement

To address our research question, we needed a validated and reliable instrument to measure the perception of the indigenous adolescent girls. However, no pre-tested or validated scale or questionnaire was available in previous studies that could measure the perception of the adolescents who experiences the hardship of water scarcity in maintaining their menstrual hygiene. Thus, we endeavoured to construct a new validated instrument to measure adolescents' perceptions named as perceived difficulty in maintaining menstrual hygiene due to water scarcity (PD). In the initial stage of scale development, we performed a literature search and created an item pool which was used for measuring the different perceptions of the women who face difficulties in maintaining menstrual hygiene due to water scarcity (Dr Shamima Yasmin, 2013; Ellis et al., 2016; Michael et al., 2020; Patel et al., 2022; Van Eijk et al., 2016). Followed by that, the items were discussed with three women of reproductive age and three indigenous adolescents to get their feedback on it. Finally, a self-reported five-point Likert scale with five items was finalized considering that these were well structured, clear, comprehensive, and could sufficiently measure the adolescents' PD by the authors- IHT, MAR, and PS. To ensure content validity, all the disagreements were discussed and resolved by the other authors. The items of the scale are provided in Table 1.

Both classical and modern psychometric tests were performed to examine the reliability and validity of the newly constructed scale.

In classical item analysis, Cronbach's Alpha value was found 0.91 which is higher than widely accepted cut-off value 0.70 (Bolarinwa, 2015) that denotes high inter-item consistency. Exploratory factor analysis found one component determined by Eigenvalue greater than 1 which denotes that the five items combinedly create a common factor what we named PD. Moreover, Kaiser-Meyer-Olkin (KMO) Measure of Sampling Adequacy (0.88) denotes that the sample size of our study was enough to perform the factor analysis, whereas Bartlett's test of Sphericity (815.17) found significant ($p < 0.01$) which denotes that the items were correlated to each other to make a common factor or construct. The component matrix of each item was found to be more than the accepted cutoff value of 0.7 (Table 2), which means there were enough variabilities (not repetitive) among the items.

Rasch analysis was performed to evaluate the psychometric properties of the 5-item questionnaire that was aimed to measure a single construct named PD. Data of respondents in that questionnaire was tested for their fit to the Rasch model following the Joint Maximum Likelihood Estimation (JMLE) (Linacre, 2017) method in WINSTEP, version 5.4.1. The result of Rasch analysis shows that the questionnaire has accepted values of separation index (1.62) and reliability (0.72) and a high item separation index (4.51) and reliability (0.95) (Souza et al., 2017). Explanation of getting lower person separation index and reliability values might be homogeneity of the participants from all demographic perspectives. On the other hand, the item reliability index and value are higher than the accepted cutoff point of 3 and 0.90, which denotes that the items have a reasonably different range of variability.

Mean square values of infit and outfit for the person and item were within the cutoff range of 0.5–1.5, as recommended by Linacre (2017) (Table 2). Only one value of the MNSQ outfit for the person in item D1 was found to be 1.70, slightly higher than the recommended cutoff value. It indicates that the questionnaire is a reliable construct and statistically fit.

The structural validity of the questionnaire was examined by unidimensionality and local independence assumptions of the Rasch model. The assumption of unidimensionality was held according to three criteria. Firstly, 44.5% of the variance was explained by the Rasch measurement, which is more than the 40% recommended cutoff point (Linacre, 2017; McCreary et al., 2013). Secondly, Eigenvalue was found to be 1.49 for the first construct, which is less than 2, and denotes that the questionnaire expresses a single construct combinedly. Thirdly, all disattenuated correlation values were found to be 1 except one value of 0.89 (PCS contrast 3 item clusters 2–3: 0.89), which denotes that the items are highly concentrated to a common construct. Local independence assumption was also held considering standardized residual item correlation values were found to be all negative as negative or zero standardized residual item correlation coefficient values suggest that the items have local independence (Marais and Andrich, 2008).

Considering these psychometric properties of the constructed scale, the questionnaire's composite values were considered continuous numeric in the statistical analysis of the paper (Leung, 2011; Wu and Leung, 2017).

2.6. Statistical analysis

The study chose a backward elimination linear regression method to figure out the most relevant factors and their joint predictive ability from a wide range of factors presupposed in the study (Chowdhury and Turin, 2020). To avoid the possibility of including irrationally too many numbers of variables, *the 'one in ten rule'* (Harrell et al., 1984; Peduzzi et al., 1996), the role of including each variable for every 10 observations, for determining the total number of variables in the prediction model was strictly followed. Before performing stepwise multiple regression, a univariate analysis was performed to examine the association of each factor with the predicted variable PD. To avoid any possibility of exclusion of any important factor, we considered all the candidate variables in our backward stepwise multiple regression.

Statistical analyses were performed with Statistical Packages for Social Science (SPSS) version 28. Descriptive statistics were used to observe the distribution of demographic characteristics of the participants, their menstrual hygiene practice, and perceived difficulties. Regression analysis was performed to observe the variables associated with PD of menstrual hygiene practices, which is a major objective of the study. Backward stepwise multiple linear regression was performed to observe the variables associated with PD of the adolescents adjusted for age, number of family members, education level, place of residence, and ethnic identity. Quality of the regression model was assessed using R^2 value, variance inflation factors (VIF) of the independent variables and observing the heteroscedasticity of the model.

Table 1
Perceived difficulty in maintaining menstrual hygiene due to water scarcity questionnaire.

Item Number	Item Description
D1	The distance of the water source made it difficult to collect enough water for cleanliness during my last menstrual period.
D2	I did not get sufficient water to wash my genitals during my last menstruation period.
D3	I did not get sufficient water to bathe during my last menstruation period.
D4	Washing my absorbents and other menstruation-related materials was difficult due to sufficient water.
D 5	Felt difficulty in washing hand before and after changing absorber and clothes due to scarcity of safe water in the last menstrual period

Response options: 0 = completely disagree, 1 = disagree, 2 = neither agree nor disagree, 3 = agree, 4 = strongly agree.

Table 2
Item statistics of the perceived difficulty questionnaire.

Item	Exploratory Factor Analysis			Rasch Analysis			
	Component Matrix	% of variance explained	Cumulative % of variance explained	Item Endorsability (difficulty) *	Standard Error	MNSQ Infit	MNSQ Outfit
D1	0.86	72.95	72.95	−0.08	0.13	1.23	1.70
D2	0.87	11.50	84.44	−0.04	0.13	1.11	1.09
D3	0.91	6.212	90.65	−0.51	0.14	1.00	0.96
D4	0.90	5.99	96.64	−0.53	0.14	0.84	0.80
D5	0.72	3.37	100	1.17	0.12	0.74	0.69

2.7. Ethical issues

Ethical clearance was obtained from the ethical review committee of the Faculty of Biological Sciences of the University of Dhaka, Bangladesh with reference number: 176/Biol.Scs (Supplementary file 01). Before conducting data collection, informed consent was received from all the participants and from their guardians/legal guardians.

3. Result

3.1. Characteristics of the participants

Characteristics of the participants are presented in Table 3. The participants (n = 242) of the study belonged to Tripura (33.1%), Marma (32.2%), and other (34.7%) ethnic groups in the Bandarban hill district, and their mean age of the participants was 15.67 (SD = 2.07) years. 15.3% of adolescents were illiterate or had no formal education, 15.7% were educated until primary level, and 68.2% had more than primary level education. The mean number of family members was 5.87 (SD = 1.74). The Sources of water of their families were spring/fall (47.9%) and surface water (36.8%), while a minimal number of the families used underground water (12.8%) and multiple sources (2.5%). Similarly, sources of water for maintaining their menstrual hygiene were spring/waterfall (45.9%), surface water (36.4%), underground water (16.1%), and multiple sources (1.7%). Most adolescents reported using disposable sanitary pads (50%), while a significant proportion (27.3%) reported using cloth or towels for their menstrual secretion. On the other hand, 27.3% used multiple types of materials, while 2.9% reported that they did not use any material for menstrual secretion. Common coping strategies for maintaining menstrual hygiene were reported to be reducing their cleaning frequency (6.6%), using tissues or clothes rather than washing with water (1.2%), reducing the amount of water used for cleaning genitals (19%), and suboptimal use of water while bathing (26.9%). 45.9% reported that they did not need to cope with this situation at all. Average reported time to collect water was 19.24 (±14.85) minutes and 75.2% respondents reported that the quality of these sources of water degrades during the dry seasons and 69.8% reported that the quality of these water was not good as 75.2% reported that their sources of water degrade during the dry seasons. 67.2% reported that their place of residence was in hard-to-reach areas. 44.2% reported that they needed to boil or purify that water before use. 4.5% adolescents reported that their family has been relocated from their previous place of residence to another place due to water scarcity.

Table 3
Characteristics of participants.

Variable	Frequency (%)
Age (mean ± SD)	15.67 ± 2.07
Number of family members (mean ± SD)	5.87 ± 1.74
Marital status	
Unmarried	230 (95.5)
Married	11 (4.5)
Ethnicity	
Tripura	80 (33.1)
Marma	78 (32.2)
Others	84 (34.7)
Education level	
No formal education	37 (15.3)
Less than primary	38 (15.7)
More than primary	165 (68.2)
Source of water of the family	
Underground	31 (12.8)
Surface water	89 (36.8)
Spring/waterfall	116 (47.9)
Multiple sources	
Source of water for menstrual hygiene	
Underground	39 (16.1)
Surface water	88 (36.4)
Spring/waterfall	111 (45.9)
Multiple sources	4 (1.7)
Material used for menstrual secretion	
Disposable sanitary pad	121 (50)
Cloth/towel	41 (16.9)
More than one	66 (27.3)
Nothing	7 (2.9)
Methods of disposing absorbents	
Burning	99 (40.9)
Throwing away	33 (13.6)
Under soil	49 (20.2)
Toilet	10 (14)
Did not dispose	34 (14)
More than one method	3 (1.2)
Other ways than the abovementioned	7 (2.9)
Not applicable	5 (2.1)
Coping strategy during water scarcity	
Reduced the cleaning frequency	16 (6.6)
Used tissue or cloths for cleaning genitals	3 (1.2)
Reduced amount of water used for cleaning genitals	46 (19)
Suboptimal use of water while bathing	65 (26.9)
Not applicable	111 (45.9)

3.2. Factors associated with PD

3.2.1. Testing of assumptions in linear regression analysis

Before conducting multiple linear regression, the assumptions were tested. Normal PP plot showed normality of the outcome variable while residual plot showed no heteroscedasticity. No multicollinearity was found among the explanatory variables as the values of tolerance (>0.1) and variable inflation factor (VIF <10) were found within generally accepted values (Alin, 2010). No heteroscedasticity was found diagnosed with residual plot and standardized residuals in the scatterplot where the range of residual values were found between 1.01 and 2.15 (Supplementary file 02).

3.2.2. Univariate analysis

Univariate linear regression (Table 4) shows that number of family member, place of residence, time required to collect water, water source degradation, perceived water quality, took a bath every day, and experience of water scarcity in last menstruation period were positively and significantly associated with perceived difficulty while needed to boil or purify water and having of Marma ethnicity were negatively and significantly associated with perceived difficulty. On the other hand, other covariates were not found to have any significant association with the outcome variable.

Table 4
Association between predictor variable and perceived difficulty.

Factor	Coefficient (95% CI)	*p* value
Age	−0.042 (−0.094 to 0.010)	0.112
Number of family member	0.093 (0.032–0.154)	0.003
Education level (no formal Education as reference)		
Primary education	0.177 (.367 to −.209)	0.904
Above primary	0.086 (0.579 to −0.218)	0.555
Ethnic identity (other ethnic group as reference)		
Marma	−0.744 (−0.979 to −0.509)	<0.001
Tripura	0.106 (−0.128 to 0.339)	0.374
Living hard to reach area	0.318 (0.091–0.544)	0.006
Time required to collect water	0.032 (0.026–0.038)	<.001
Water source degradation	1.156 (0.903–1.409)	<.001
Family relocation due to water scarcity	−0.148 (−0.662 to 0.366)	0.571
Bad water quality during last menstruation	0.384 (0.155–0.612)	0.001
Need boil or purify water (no need to boil or purify as reference)	−0.522 (−0.727 to −0.316)	<.001
Frequency of washing genitals	0.011 (−0.056 to 0.078)	0.748
Took a bath everyday (not taking a batch as reference)	1.108 (0.636–1.581)	<.001
Experience of water scarcity in last menstrual period	1.123 (0.963–1.284)	<.001

3.2.3. Multivariable analysis

In the backward multiple linear regression model, cut-off p-value 0.01 was used to exclude the variables in each stage.

In the full model including all the candidate variables, multiple linear regression was statistically significant (R square: 0.632), F (13, 198) = 26.152, $p < 0.01$) (Table 5).

In the full model, all the variables related to hardship of getting water were significantly associated with PD (time required to collect everyday water: β = 0.013, $p = 0.003$; family relocation due to water scarcity: β = −0.485, $p = 0.006$; water source degradation (β = 0.541, $p < 0.01$), quality of used water: β = −0.335, $p = 0.004$), water source degradation (β = 0.541, $p < 0.001$), need to boil or purify water (β = 0.287, $p = 0.044$), and experience of facing hardship in getting water in last menstruation: β = 0.772, $p < 0.001$) except living hard to reach area (β = 0.048, $p = 0.623$) (Table 5).

Similarly, both variables related to menstrual hygiene practice were significantly and negatively associated with PD (frequency of washing genitals: β = −0.051, $p = 0.038$) whether took a bath every day during menstrual period: β = −0.975, $p < 0.001$) (Table 5, Full Model).

In the final model (Table 5), Marma (β = −0.116, $p = 0.028$) and Tripura (β = −0.192, $p = 0.009$) ethnic groups of peoples were found to be negatively associated with PD compared to the other ethnic group of peoples. The factors related to hardship of getting water-water source degradation (β = 0.247, p <0.001), whether needed to boil or purify water (β = 0.203, $p = 0.005$), and experience of difficult in getting water during the last menstrual period (β = 0.449, $p < 0.001$) were found negatively and significantly associated with PD, while time required to collect water (β = 0.209, $p < 0.001$) was found to be negatively associated with PD. Both variables related to menstrual hygiene practice-frequency of washing genitals (β = - 0.094, $p = 0.040$) and whether took a bath every day (β = −0.228, $p < 0.001$) were found to be negatively and significantly associated with PD, while the factor living hard to reach area was not found to be significant association with PD (β = 0.048, $p = 0.623$.

4. Discussion

4.1. Findings

To our best knowledge, this is the first study that investigated the status of PD by the indigenous adolescents in the Bandarban Hill District of Bangladesh. The major finding shows that indigenous adolescent girls

Table 5
Adjusted models of the association of predictor variable with perceived difficulty in maintaining menstrual hygiene due to water scarcity (N = 242).

	Full Adjusted Multiple Regression Model		Final Reduced Multiple Regression Model with Backward Stepwise Elimination	
Variable	Coefficient (95% CI)[a]	p-value	Coefficient (95% CI)[b]	p-value
Age	0.026 (−0.014 to 0.066)	0.206	–	–
Number of family member	0.010 (−0.032 to 0.052)	0.635	–	–
Education level (no formal education as reference)				
Primary education	0.079 (−.227 to .385)	.610	–	–
Above primary	−0.091-(.335 .153)	.463	–	–
Ethnic identity (other ethnic group as reference)				
Marma	−.206 (−.429 to .016)	.069	−0.116 (−0.403 to −0.023)	0.028
Tripura	−.311 (−.609 to −.012)	.041	−0.192 (−0.623 to −0.090)	0.009
Living hard to reach area	0.048 (−.145 to .241)	0.623		
Time required to collect water	0.013 (.004–.021)	0.003	0.209 (0.005–0.019)	<.001
Water source degradation	0.541 (.317–.766)	<.001	0.247 (0.323–0.756)	<.001
Family relocation due to water scarcity	−0.485 (−0.827 to −0.142)	0.006	−0.125 (−0.819 to −0.142)	0.006
Perceived water quality during last menstruation	−0.335 (−0.559 to −0.112)	0.004	−0.187 (−0.569 to −0.146)	0.001
Need boil or purify water	0.287 (0.008–0.565)	0.044	0.203 (0.108–0.596)	0.005
Frequency of washing genitals	−0.051 (−0.099 to −0.003)	.038	−0.094 (−0.097 to −0.002)	0.040
Whether took a bath everyday	−0.975 (−1.374 to −0.576)	<.001	−0.228 (−1.358 to −0.578)	<.001
Experience of water scarcity in last menstrual period	0.772 (0.567–0.977)	<.001	0.449 (0.571–0.970)	<.001
Model Fit Statistics	R square: 0.632, Adjusted R square: 0.608, F (13, 198) = 26.152, $p < 0.01$.		R square: 0.628, Adjusted R square: 0.608, F (13, 198) = 47.690, $p < 0.01$.	

[a] Unstandardised Beta.
[b] Standardised Beta.

of the study area faced difficulty in menstrual hygiene practices during water scarcity period, causing more than half of the respondents to undergo various coping strategies to maintain menstrual hygiene practices. Therefore, the finding complies with the fact that water inadequacy leads to poor menstrual hygiene practices (Blair et al., 2022; Chatterjee, 2020; Downing et al., 2021; Ellis et al., 2016; Michael et al., 2020; Van Eijk et al., 2016). However, on the other hand, 45.9% of respondents were found to maintain their usual menstrual hygiene practices, regardless of seasonal water scarcity in the study area. This could be due to the fact that seasonal water scarcity is geographical as a whole, whereas some families might still have access to water throughout the year. Furthermore, convenient sampling caused the exclusion of potential participants from some remotest and hard-to-reach areas where water scarcity is perceived as extreme.

Though studies have been conducted on the perception of menstruation (Parle and Khatoon, 2019) and menstrual hygiene (Hennegan and Sol, 2020, p. 20192019; Parle and Khatoon, 2019), no study was found on the perception of the difficulty of maintaining menstrual hygiene due to water scarcity, specifically, faced by menstruating women. However, Caruso et al., 2020 found that less access to water sources significantly increased menstrual insecurity among menstruating women in Odisha, India, where they included access to water as a component of menstrual security (Caruso et al., 2020).

In our study, PD was associated with a few factors of the hardship of getting water and menstrual hygiene practices. Among the factors related to the hardship of getting water, we found that water scarcity caused displacement, and the association of PD with that displacement was inversely related. This inverse association might be explained by the fact that families who changed their residences due to water scarcity already had their water situations resolved. Studies have reported forced displacement due to the adverse effects of climate change and water scarcity over the decades globally (Palattiyil et al., 2022). Another hardship factor, the need for purification or boiling the water, was also found to be positively and significantly associated with PD in our study. According to the National Hygiene Survey 2018 (Government of the People's Republic of Bangladesh, 2018), 14% of households in Bangladesh were found to purify water at household level (boiling, chemical, or filtered treatment) (Debnath et al., 2022), whereas, the frequency of boiling or purifying water was found to be comparatively high (44.2%) in our study. In previous studies, boiling or purifying water implied poor water quality (Debnath et al., 2022) posing a serious obstacle to maintaining menstrual hygiene (Elledge et al., 2018; Krishnan and Twigg, 2016; VanLeeuwen and Torondel, 2018). Time required to collect water, water source degradation, and water quality were also found to be associated with PD in our study, which is similar to the findings of previous studies in different water scarcity settings. For instance, a systematic review (Patel et al., 2022) found that access to water adversely affects menstrual hygiene management in humanitarian crisis settings. Our study also found poor menstrual hygiene practices to be associated with higher PD. Previous studies found poor menstrual hygiene practices to be associated with water access and availability. For instance, a study in the Sub-Saharan region found that inadequate water and sanitation facilities were associated with poor menstrual hygiene practices (Kuhlmann et al., 2017).

4.2. Methodological discussion

The study tried to objectively define the PD based on in-depth literature review, expert opinions, and developing a new measurement tool of PD in this context by piloting and testing the reliability and validity of that instrument. Both classical and modern methods of reliability and validity determination were used to confirm that the newly developed tool is competent enough to objectively measure the PD as a single construct. As its explorative nature, the study considered a considerable number of factors as independent variables and applied backward stepwise linear regression analysis to ensure all candidate variables to be included into the mode. That is likely to avoid the possibility of occurring omission bias. For having robust measurement of association, both univariate and multiple regression models were used. All possible demographic variables, assumed as potential confounders, were adjusted for each regression model.

No funding was received to conduct this study that provided the researchers scope of being objective and unbiased in the entire process of the study. Additionally, an ethical clearance has been taken from an authorized ethical committee and informed consent form was used prior to data collection. In every stage of the study, including data collection, data handling, and analyzing, highest personal secrecy and privacy of the participants was maintained.

Due to lack of previous theoretical ground and empirical evidence, it was difficult to scope out the possible phenomena of the PD and its associated factors. Thus, this study is not a conclusive one that could claim that the domain of interest has been scooped in the best way. Thus, generalizability of the study should be drawn carefully. Advanced statistical tools and methods could have been used, for instance, Structural Equation Modeling (SEM), Path Analysis, to draw more confirmed causal relationships.

4.3. Strengths and limitations of the study

Data was collected by volunteers from local indigenous communities which might help to reduce information bias arising from language barrier and cultural reservedness of the participants to talk about sensitive menstrual health issues.

Several limitations of the study could also be worth mentioning. Cross-sectional nature of the data doesn't imply any causal association between the variables of interest. Convenient sampling might lead to either underestimation or overestimation of both the PD score and the water scarcity situation because some of the remotest areas were not considered for data collection. Use of more advanced statistical methods, for instance, structural equation model, path analysis, or analysis of commonality, could have been applied to get a more robust and clearer picture of the associations of the considered variables. But due to lack of time and resources, further analysis was not possible to perform. Thus, the findings cannot be generalized with the same population groups in other areas of Bangladesh.

Exposing the frequency of washing genitals is sensitive personal information. Similarly, daily bathing represents a standard hygienic behavior in Bangladesh (Sultana, 2011), and the menstruation period is considered a social, cultural, and religious taboo (Mohammed and Larsen-Reindorf, 2020). Thus, daily bathing (94.6%) has been a regular cultural norm of the menstruating women of Bangladesh. These findings reflect the cognitive dissonance theory that claims that individuals tend to protect their positive self-image and feel uncomfortable while facing image-threatening events (Alicke and Sedikides, 2009). It is the self-protective behavior of the adolescent girls, as the indigenous communities of Bandarban district highly value hygiene and sanitation (Hussain et al., 2015). Thus, a great chance of over and under estimation considering self-image protecting tendency as a potential confounder can be attributable to the association of taking baths and washing genitals with PD of menstrual hygiene due to water scarcity of the participating adolescent girls.

5. Conclusion

This study shed light on how the PD was associated with the demographic factors, practical hardship of getting water, and menstrual hygiene practices of the indigenous adolescent girls in Bandarban Hill District, providing evidence that PD increases with poor menstrual hygiene practices and practical hardship of getting water. The further analytical study could be carried out to investigate, with more methodological rigor, if the seasonal water scarcity is attributable to the PD.

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Availability of data

Complete data set and analyzed files are available in "sav" format and accessible to all. Any further use of these data is subjected to have prior permission of the corresponding author.

Authorship contribution statement

IHT: Study design, analysis, writing and editing; MAR: Study design, writing and editing; PS: Conceptualization, study design, data collection, and writing and editing; NP: Data collection, manuscript editing; MKT: Data analysis; IM: Project administration, writing and editing. All authors carefully read and provided consensus to the submitted version of the manuscript.

Declaration of competing interest

No conflict of interest is declared. The contents of this manuscript are free of copyright and submitted nowhere for publication.

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Appendix A. Supplementary data

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References

Ahmed, S., Tarique, K.M., Arif, I., 2017. Service quality, patient satisfaction and loyalty in the Bangladesh healthcare sector. Int. J. Health Care Qual. Assur. 30 (5), 477–488. https://doi.org/10.1108/IJHCQA-01-2017-0004.

Alam, M.Z., 2022. Ethnic inequalities in access to WASH in Bangladesh. Lancet Global Health 10 (8), e1086–e1087. https://doi.org/10.1016/S2214-109X(22)00232-7.

Alam, M.-U., Luby, S.P., Halder, A.K., Islam, K., Opel, A., Shoab, A.K., Ghosh, P.K., Rahman, M., Mahon, T., Unicomb, L., 2017. Menstrual hygiene management among Bangladeshi adolescent schoolgirls and risk factors affecting school absence: results from a cross-sectional survey. BMJ Open 7 (7), e015508. https://doi.org/10.1136/bmjopen-2016-015508.

Alicke, M.D., Sedikides, C., 2009. Self-enhancement and self-protection: what they are and what they do. Eur. Rev. Soc. Psychol. 20 (1), 1–48. https://doi.org/10.1080/10463280802613866.

Alin, A., 2010. Multicollinearity: multicollinearity. Wiley Interdisciplinary Reviews: Comput. Stat. 2 (3), 370–374. https://doi.org/10.1002/wics.84.

BBS, 2011b. Bangladesh Population and Housing Census 2011 Zila Report: *Bandarban*. Bangladesh Bureau of Statistics (BBS). https://www.bbs.gov.bd/site/page/47856ad0-7e1c-4aab-bd78-892733bc06eb/Population-and-Housing-Census.

BBS, 2011a. Population and Housing Census-2011 *National Volume-2: Union Statistics*. Bangladesh Bureau of Statistics (BBS). http://203.112.218.65:8008/WebTestApplication/userfiles/Image/National%20Reports/Union%20Statistics.pdf.

Blair, L.A.G., Bajón -Fernández, Y., Villa, R., 2022. An exploratory study of the impact and potential of menstrual hygiene management waste in the UK. Clean.Eng. Technol. 7, 100435 https://doi.org/10.1016/j.clet.2022.100435.

Bolarinwa, O., 2015. Principles and methods of validity and reliability testing of questionnaires used in social and health science researches. Niger. Postgrad. Med. J. 22 (4), 195. https://doi.org/10.4103/1117-1936.173959.

Borua, G.R., Islam, S.M.S., Faisel, A.J., 2019. Menstrual hygiene: knowledge and practices among adolescent girls in a selected district of Bangladesh. Police Hospital J.Med.Sci. 87. https://cph.police.gov.bd/images/Others/CPH-Journal-2019.pdf.

Caruso, B.A., Portela, G., McManus, S., Clasen, T., 2020. Assessing women's menstruation concerns and experiences in rural India: development and validation of a menstrual insecurity measure. Int. J. Environ. Res. Publ. Health 17 (10), 3468. https://doi.org/10.3390/ijerph17103468.

Chakma, B., Dewan, J., Chakma, S.B., 2021. Water Scarcity Worsens Hill People's Woes | Prothom Alo. https://en.prothomalo.com/environment/water-scarcity-causes-hill-people-to-suffer.

Chatterjee, P., 2020. Improving menstrual hygiene among adolescent girls in India. Lancet Child Adolescent Health 4 (6), 422–423. https://doi.org/10.1016/S2352-4642(20)30142-5.

Chowdhury, M.Z.I., Turin, T.C., 2020. Variable selection strategies and its importance in clinical prediction modelling. Family Med. Commun. Health 8 (1), e000262. https://doi.org/10.1136/fmch-2019-000262.

Das, P., Baker, K.K., Dutta, A., Swain, T., Sahoo, S., Das, B.S., Panda, B., Nayak, A., Bara, M., Bilung, B., Mishra, P.R., Panigrahi, P., Cairncross, S., Torondel, B., 2015. Menstrual hygiene practices, WASH access and the risk of urogenital infection in women from Odisha, India. PLoS One 10 (6), e0130777. https://doi.org/10.1371/journal.pone.0130777.

Debnath, P., Mamun, M.M.A.A., Karmakar, S., Uddin, M.S., Nath, T.K., 2022. Drinking water quality of Chattogram city in Bangladesh: an analytical and residents' perception study. Heliyon 8 (12), e12247. https://doi.org/10.1016/j.heliyon.2022.e12247.

Downing, S., Benjimen, S., Natoli, L., Bell, V., 2021. Menstrual hygiene management in disasters: the concerns, needs, and preferences of women and girls in Vanuatu. Waterlines 40 (3), 144–159. https://doi.org/10.3362/1756-3488.21-00002.

Dr Shamima Yasmin, D.S.Y., 2013. Menstrual hygiene among adolescent school students: an indepth cross-sectional study in an urban community of WestBengal, India. IOSR J. Dent. Med. Sci. 5 (6), 22–26. https://doi.org/10.9790/0853-0562226.

Elledge, M., Muralidharan, A., Parker, A., Ravndal, K., Siddiqui, M., Toolaram, A., Woodward, K., 2018. Menstrual hygiene management and waste disposal in low and middle income countries—a review of the literature. Int. J. Environ. Res. Publ. Health 15 (11), 2562. https://doi.org/10.3390/ijerph15112562.

Ellis, A., Haver, J., Villasenor, J., Parawan, A., Venkatesh, M., Freeman, M.C., Caruso, B. A., 2016. WASH challenges to girls' menstrual hygiene management in metro manila, masbate, and south central mindanao, Philippines. Waterlines 35 (3), 306–323. https://doi.org/10.3362/1756-3488.2016.022.

Garg, S., Bhatnagar, N., Singh, M.M., Basu, S., Borle, A., Marimuthu, Y., Azmi, F., Dabi, Y., Bala, I., 2022. Menstrual hygiene management and its determinants among adolescent girls in low-income urban areas of Delhi, India: a community-based study. Osong Publ. Health Res. Perspect. 13 (4), 273–281. https://doi.org/10.24171/j.phrp.2022.0127.

Government of the People's Republic of Bangladesh, 2018. National Hygiene Survey 2018. Bangladesh Bureau of Statistics (BBS). https://bbs.portal.gov.bd/sites/default/files/files/bbs.portal.gov.bd/page/b343a8b4_956b_45ca_872f_4cf9b2f1a6e0/2021-02-18-12-34-38806de91fa4ca8d9e70db96ecff4427.pdf.

Ha, Md A.T., Alam, Md Z., 2022. Menstrual hygiene management practice among adolescent girls: an urban–rural comparative study in Rajshahi division, Bangladesh. BMC Wom. Health 22 (1), 86. https://doi.org/10.1186/s12905-022-01665-6.

Harrell, F.E., Lee, K.L., Califf, R.M., Pryor, D.B., Rosati, R.A., 1984. Regression modelling strategies for improved prognostic prediction. Stat. Med. 3 (2), 143–152. https://doi.org/10.1002/sim.4780030207.

Hennegan, J., Sol, L., 2020. Confidence to manage menstruation at home and at school: findings from a cross-sectional survey of schoolgirls in rural Bangladesh. Cult. Health Sex. 22 (2), 146–165. https://doi.org/10.1080/13691058.2019.1580768.

Hossen, Md M., Muhibbullah, Md, Hasnat, M.A., Rahman, Md H., 2016. Water crisis, adaptation practices and management strategies in Chittagong hill Tracts (CHT) area: a case study on naniarchar upazila. Soc. Change 6 (1 & 2), 69–90.

Hussain, S., Ruano, A.L., Rahman, A., Rashid, S.F., Hill, P.S., 2015. From knowing our needs to enacting change: findings from community consultations with indigenous communities in Bangladesh. Int. J. Equity Health 14 (1), 126. https://doi.org/10.1186/s12939-015-0264-x.

Hussein, J., Gobena, T., Gashaw, T., 2022. The practice of menstrual hygiene management and associated factors among secondary school girls in eastern Ethiopia: the need for water, sanitation, and hygiene support. Women's Health 18, 174550572210878. https://doi.org/10.1177/17455057221087871.

Kasiulevičius, V., Šapoka, V., Filipavičiūtė, R., 2006. Sample size calculation in epidemiological studies. Vilnius Univ. Instit.Experiment. Clin. Med. Vilnius Univ. 7 (4). https://www.researchgate.net/publication/254847492_Sample_size_calculation_in_epidemiological_studies?enrichId=rgreq-fa0af9a02bd98e3a78a757b2d962148b-XXX&enrichSource=Y292ZXJQYWdlOzI1NDg0NzQ5MjtBUzoyMTgxNzcxNDY0OTQ5ODBAMTQyOTAyODg5MzUyMQ%3D%3D&el=1_x_2&_esc=publicationCoverPdf.

Krishnan, S., Twigg, J., 2016. Menstrual hygiene: a 'silent' need during disaster recovery. Waterlines 35 (3), 265–276. https://doi.org/10.3362/1756-3488.2016.020.

Kuhlmann, A.S., Henry, K., Wall, L.L., 2017. Menstrual hygiene management in resource-poor countries. Obstet. Gynecol. Surv. 72 (6), 356–376. https://doi.org/10.1097/OGX.0000000000000443.

Leung, S.-O., 2011. A comparison of psychometric properties and normality in 4-, 5-, 6-, and 11-point Likert scales. J. Soc. Serv. Res. 37 (4), 412–421. https://doi.org/10.1080/01488376.2011.580697.

Linacre, J.M., 2017. A User's Guide to FACETS. Rasch-Model Computer Programs. Program Manual 3.80. 0. Hentet, p. 25.

Mahmud, S., Amin, S.M.A., Rashed, A.W., Mahmud, R., 2020. Water, sanitation and hygiene practices among ethnic communities in Chittagong hill tracks, Bangladesh. Int.J. Curr. Res. 12.

Marais, I., Andrich, D., 2008. Formalizing dimension and response violations of local independence in the unidimensional Rasch model. J. Appl. Meas. 9 (3), 200–215.

McCreary, L.L., Conrad, K.M., Conrad, K.J., Scott, C.K., Funk, R.R., Dennis, M.L., 2013. Using the Rasch measurement model in psychometric analysis of the family

effectiveness measure. Nurs. Res. 62 (3), 149–159. https://doi.org/10.1097/NNR.0b013e31828eafe6.
Michael, J., Iqbal, Q., Haider, S., Khalid, A., Haque, N., Ishaq, R., Saleem, F., Hassali, M. A., Bashaar, M., 2020. Knowledge and practice of adolescent females about menstruation and menstruation hygiene visiting a public healthcare institute of Quetta, Pakistan. BMC Wom. Health 20 (1), 4. https://doi.org/10.1186/s12905-019-0874-3.
Mills, J.E., Cumming, O., 2016. The Impact of Water, Sanitation and Hygiene on KeyHealth and Social Outcomes. UNICEF. https://www.researchgate.net/profile/Joanna-Esteves-Mills/publication/319503296_The_impact_of_water_sanitation_and_hygiene_on_key_health_and_social_outcomes_review_of_evidence/links/59afa924458515150e4b0a73/The-impact-of-water-sanitation-and-hygiene-on-key-health-and-social-outcomes-review-of-evidence.pdf.
Mohammed, S., Larsen-Reindorf, R.E., 2020. Menstrual knowledge, sociocultural restrictions, and barriers to menstrual hygiene management in Ghana: evidence from a multi-method survey among adolescent schoolgirls and schoolboys. PLoS One 15 (10), e0241106. https://doi.org/10.1371/journal.pone.0241106.
Muhit, I.B., Chowdhury, S.T., 2013. Menstrual Hygiene Condition Of Adolescent Schoolgirls At Chittagong Division In Bangladesh 2 (6).
Palattiyil, G., Sidhva, D., Seraphia Derr, A., Macgowan, M., 2022. Global trends in forced migration: policy, practice and research imperatives for social work. Int. Soc. Work 65 (6), 1111–1129. https://doi.org/10.1177/00208728211022791.
Parle, J., Khatoon, Z., 2019. Knowledge, attitude, practice and perception about menstruation and menstrual hygiene among adolescent school girls in rural areas of Raigad district. Int.J.Commun.Med.Publ.Health 6 (6), 2490. https://doi.org/10.18203/2394-6040.ijcmph20192310.
Patel, K., Panda, N., Sahoo, K.C., Saxena, S., Chouhan, N.S., Singh, P., Ghosh, U., Panda, B., 2022. A systematic review of menstrual hygiene management (MHM) during humanitarian crises and/or emergencies in low- and middle-income countries. Front. Public Health 10, 1018092. https://doi.org/10.3389/fpubh.2022.1018092.
Peduzzi, P., Concato, J., Kemper, E., Holford, T.R., Feinstein, A.R., 1996. A simulation study of the number of events per variable in logistic regression analysis. J. Clin. Epidemiol. 49 (12), 1373–1379. https://doi.org/10.1016/S0895-4356(96)00236-3.
Raina, D., Balodi, G., 2014. Menstrual hygiene: knowledge, practise and restrictions amongst girls of dehradun, uttarakhand, India. Glob.J. Interdisciplinary.Soc. Sci. 3 (4).
Rossouw, L., Ross, H., 2021. Understanding Period poverty: socio-economic inequalities in menstrual hygiene management in eight low- and middle-income countries. Int. J. Environ. Res. Publ. Health 18 (5), 2571. https://doi.org/10.3390/ijerph18052571.
Roy, R.D., 2012. Country technical notes on indigenous peoples' issues: people's republic of Bangladesh. Int. Fund Agric.Dev. (IFAD) Asia Indigenous Peoples Pact (AIPP). https://www.ifad.org/documents/38714170/40224860/bangladesh_ctn.pdf/ea8bae36-7450-447a-b0e9-4c010ba315bf.
Shumie, Z.S., Mengie, Z.A., 2022. Menstrual hygiene management knowledge, practice and associated factors Among School Girls, Northeast Ethiopia. PLoS One 17 (7), e0271275. https://doi.org/10.1371/journal.pone.0271275.
Souza, M.A.P., Coster, W.J., Mancini, M.C., Dutra, F.C.M.S., Kramer, J., Sampaio, R.F., 2017. Rasch analysis of the participation scale (P-scale): usefulness of the P-scale to a rehabilitation services network. BMC Publ. Health 17 (1), 934. https://doi.org/10.1186/s12889-017-4945-9.
Sultana, F., 2011. Water, culture, and gender: an analysis from Bangladesh. In: Johnston, B.R., Hiwasaki, L., Klaver, I.J., Ramos Castillo, A., Strang, V. (Eds.), Water, Cultural Diversity, and Global Environmental Change. Springer Netherlands, pp. 237–252. https://doi.org/10.1007/978-94-007-1774-9_18.
Tegegne, T.K., Sisay, M.M., 2014. Menstrual hygiene management and school absenteeism among female adolescent students in Northeast Ethiopia. BMC Publ. Health 14 (1), 1118. https://doi.org/10.1186/1471-2458-14-1118.
Thakre, S.B., Thakre, S.S., Reddy, M., Rathi, N., Pathak, K., Ughade, S., 2011. Menstrual hygiene: knowledge and practice among adolescent school girls of saoner, nagpur district. J. Clin. Diagn. Res. 5.
Torondel, B., Sinha, S., Mohanty, J.R., Swain, T., Sahoo, P., Panda, B., Nayak, A., Bara, M., Bilung, B., Cumming, O., Panigrahi, P., Das, P., 2018. Association between unhygienic menstrual management practices and prevalence of lower reproductive tract infections: a hospital-based cross-sectional study in Odisha, India. BMC Infect. Dis. 18 (1), 473. https://doi.org/10.1186/s12879-018-3384-2.
Van Eijk, A.M., Sivakami, M., Thakkar, M.B., Bauman, A., Laserson, K.F., Coates, S., Phillips-Howard, P.A., 2016. Menstrual hygiene management among adolescent girls in India: a systematic review and meta-analysis. BMJ Open 6 (3), e010290. https://doi.org/10.1136/bmjopen-2015-010290.
VanLeeuwen, C., Torondel, B., 2018. Improving menstrual hygiene management in emergency contexts: literature review of current perspectives. Int. J. Wom. Health 10, 169–186. https://doi.org/10.2147/IJWH.S135587.
Wali, N., Georgeou, N., Simmons, O., Gautam, M.S., Gurung, S., 2020. Women and WASH in Nepal: a scoping review of existing literature. Water Int. 45 (3), 222–245. https://doi.org/10.1080/02508060.2020.1754564.
World Bank, 2018. Bangladesh: Access to Clean Water Will Reduce Poverty Faster [Text/HTML]. World Bank, 10/11/bangladesh-access-to-clean-water-will-reduce-poverty-faster.
Wu, H., Leung, S.-O., 2017. Can Likert scales be treated as interval scales?—a simulation study. J. Soc. Serv. Res. 43 (4), 527–532. https://doi.org/10.1080/01488376.2017.1329775.

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Prenatal exposure to nitrosatable drugs and timing of puberty in sons and daughters: A nationwide cohort study

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ABSTRACT

Background: N-nitroso compounds (NOCs) can be formed by endogenous reactions between nitrosatable drugs and nitrite. Animal studies have found that several NOCs are teratogenic, and epidemiological studies report associations between prenatal exposure to nitrosatable drugs and adverse birth outcomes. It is unknown whether prenatal exposure to nitrosatable drugs is harmful to the child's reproductive health, including pubertal development.

Objectives: We investigated whether prenatal exposure to nitrosatable drugs was associated with timing of puberty and whether nitrate, nitrite and antioxidant intake modified any association.

Methods: The population-based Danish National Birth Cohort (DNBC) Puberty Cohort, which includes 15,819 children, was used to investigate the association between prenatal exposure to nitrosatable drugs and timing of puberty. Around gestational week 11 and gestational week 18, mothers provided information about drug use during pregnancy. The children's self-reported information on onset of pubertal milestones was collected every six months from 11 years of age and throughout puberty. To investigate potential effect modification by nitrite, nitrate and antioxidant intake, information on these factors was obtained from a food frequency questionnaire completed by the mothers in gestational week 25, and information on nitrate concentration in maternal drinking water at her residential address was obtained from monitoring data from public waterworks. Data were analysed using a multivariable regression model for interval-censored data estimating difference in months in timing of puberty between exposure groups.

Results: A total of 2,715 children were prenatally exposed to nitrosatable drugs. We did not find an association between prenatal exposure to nitrosatable drugs and timing of puberty. This finding was supported by null-findings in the following sub-analyses investigating: 1. subtypes of nitrosatable drugs (secondary and tertiary amines and amides), 2. dose-dependency (duration of drug intake), 3. effect modification by maternal intake of nitrate, nitrite, and antioxidants. 4. confounding by indication.

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Conclusions: Prenatal exposure to nitrosatable drugs was not associated with timing of puberty. Nitrosatable drugs are commonly used drugs in pregnancy, and further research is needed to allow firm conclusions on the potential effect of prenatal exposure to nitrosatable drugs on the child's reproductive health.

1. Introduction

Nitrosatable drugs, such as penicillin, beta2-agonists, codeine, and several antihistamines, are commonly used during pregnancy (Brender et al., 2011a). The use of nitrosatable drugs has been reported in up to 24% of pregnancies (Brender et al., 2011a). In reaction with nitrite, nitrosatable drugs form N-nitroso-compounds (NOCs) in the acidic environment in the stomach (Brambilla and Martelli, 2007), and these NOCs may act as teratogens through DNA-alkylation (Bochert et al., 1985). Studies in Syrian golden hamsters show that NOCs passed the placental barrier (Alaoui-Jamali et al., 1989), and studies in mice found prenatal exposure to NOCs to be associated with brain malformations (Diwan, 1974; Nagao et al., 1991). Epidemiological studies have reported an association between prenatal exposure to nitrosatable drugs and adverse birth outcomes such as stillbirth, preterm birth, and malformations. Malformations of various sites have been reported, and several studies find associations with brain malformations and neural tube defects (Olshan and Faustman, 1989; Brender et al., 2004, 2011b, 2012; Vuong et al., 2015; Thomsen et al., 2019). It is unknown whether prenatal exposure to NOCs affects specific areas in the brain such as the hypothalamus and pituitary gland crucial for initiation of puberty.

Puberty is initiated when gonadotropin-releasing hormone starts to be secreted from the hypothalamus which then stimulates the pituitary gland that finally stimulates the secretion of testosterone and estrogen from the gonads (Styne et al., 2011). Much is still unknown about which factors influence timing of puberty; though, the gestational period is pointed out as a time window where external exposures may interfere with the developing reproductive system, including the hypothalamic-pituitary-gonadal (HPG) axis, and hereby timing of puberty (Parent et al., 2003; Juul, 2011; Greenspan and Lee, 2018).

Identifying causes of earlier age at puberty is of public health interest, since an overall decline in the age of puberty among girls in Western countries is considered evident and for boys suggestive (Ong et al., 2006; Euling et al., 2008; Brix et al., 2019a; Benedum et al., 2015; Prentice and Viner, 2013). Furthermore, children with earlier puberty may be at increased risk for mental health impairment and diseases in adulthood, such as metabolic syndrome, breast, and testicular cancer (Ong et al., 2006; Golub et al., 2008; Graber, 2013). Prenatal exposure to nitrosatable drugs has not yet been investigated with regard to a potential impact on timing of puberty even though the drugs cover a wide variety of medications used to treat common both acute and chronic conditions in pregnancy such as infectious and allergic diseases, pain, asthma, psychiatric disorders, and nausea (Brender et al., 2011a). Nitrosatable drugs contain a nitrosatable group (amides, secondary or tertiary amines) that can undergo nitrosation, and the level of this reaction depends on factors like pH in the stomach, concurrent intake of inhibitors of nitrosation (antioxidants), and the level of nitrate and nitrite intake from diet and drinking water (Brambilla and Martelli, 2007; Ward et al., 2018; World Health Organization, 2016).

The aim of this study was to investigate if nitrosatable drug exposure during gestation was associated with timing of puberty in participants in a large Danish puberty cohort. Furthermore, the large amount of data allowed us to explore heterogeneity of effect by concurrent intake of nitrate, nitrite, and antioxidants on timing of puberty.

2. Methods

2.1. Study design and population

In this population-based cohort study, we included children participating in the Puberty Cohort (Ernst et al., 2020) nested within the Danish National Birth Cohort (DNBC) (Olsen et al., 2001). In the DNBC, Danish-speaking pregnant women were invited to participate at their first visit to the general practitioner (Andersen and Olsen, 2011); in the period from 1996 to 2002, around 31–35% of all pregnant women in Denmark were enrolled (Olsen et al., 2001; Jacobsen et al., 2010). To be eligible for participation in the follow-up in the Puberty Cohort, the children had to be singletons, born from 2000 to 2003, and have a mother that responded to the first DNBC pregnancy questionnaire around gestational week 18 about lifestyle, drug use, and health (Ernst et al., 2020). Out of 56,641 eligible children, 22,439 were invited to participate. Children were sampled for invitation to the Puberty Cohort based on 15 predefined exposures suggested to affect timing of puberty together with a group of 8000 randomly selected children (Brix et al.,

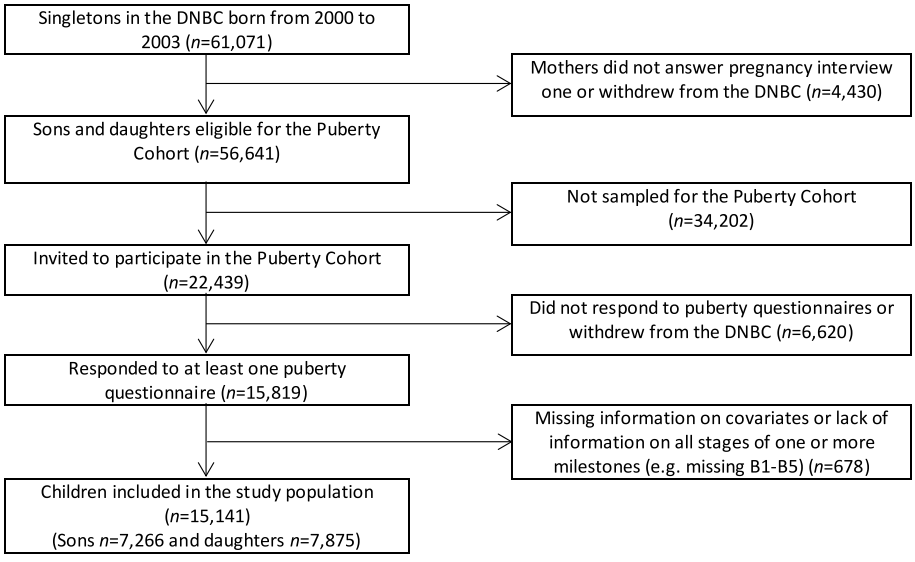

Fig. 1. Selection of the study population, the Puberty Cohort, Denmark, 2000–2021.

2019b). In total, 15,819 children (70% of the invited) participated in the Puberty Cohort either by replying to 1: an 11-year questionnaire that was sent out to all children in the DNBC and included questions about current pubertal stage (n = 1,063), 2: at least one questionnaire with similar questions on pubertal timing sent out to the Puberty Cohort (n = 5,154) or 3: both the 11-year questionnaire and the Puberty Cohort questionnaires (n = 9,602) (Ernst et al., 2020). For this study, 678 children were excluded due to a lack of information on covariates or a lack of information on all pubertal milestones. The final study population included 15,141 (67% of the invited) (see Fig. 1).

2.2. Assessment of nitrosatable drug exposure

Information about maternal drug use (including both over-the-counter drugs and prescribed drugs) during early pregnancy was obtained from the DNBC enrolment form completed around gestational week 11 and the first pregnancy interview completed around gestational week 18. Two versions of the enrolment form were used, and the latter was introduced to obtain more details on maternal drug use. In the first version (n = 4,349), the pregnant women were asked about their intake of drugs and supplements up to three months before enrolment. In the second version (n = 10,792), the pregnant women provided information on the specific weeks of intake with the earliest intake occurring four weeks before the first day of the last menstrual period. In the first pregnancy interview (n = 15,141), the pregnant women were asked if they had had further intake of drugs and, if so, which drug intake since filling out the enrolment form. This information was added to the drug information from the enrolment form; thus, the period with information on nitrosatable drug use ranged from around four weeks before the first day of the last menstrual period until around gestational week 18.

All drugs used were classified according to their nitrosatable status. Drugs previously classified as nitrosatable by Brender et al. and Brambilla et al. were classified as nitrosatable in this study (Brender et al., 2011a; Brambilla and Martelli, 2007), while drugs comparable with the classified drugs but not tested regarding their nitrosatable status were classified as probably nitrosatable. Only orally administered drugs or inhaled drugs were considered nitrosatable. The nitrosatable drugs were further subdivided into three groups based on their chemical structure: secondary amine, tertiary amine, and amide. Furthermore, the nitrosatable drugs were classified according to the main indication for use of the specific drug.

2.3. Assessment of timing of puberty

From 2012 to 2021, children sampled for the Puberty Cohort received invitations every six months to complete a web-based questionnaire about their pubertal stage from the age of 11.5 years. No more invitations were sent when the child turned 18 years; if completion of puberty (Tanner stage 5 for breast or genitals) was reached before the age of 18 the invitations also stopped. Questions about current breast (girls), genital (boys), and pubic hair Tanner stage (from 1 to 5) were supplemented by illustrations of the specific Tanner stage to guide the child to a more precise evaluation of the pubertal stage. Current axillary hair and acne were asked as yes/no. Voice break (boys) was asked as "is the voice sometimes changed", "completely changed" or "not changed". The first ejaculation (boys) and menarche (girls) were asked as yes/no, followed by a question about the specific age of the event (Ernst et al., 2020). If the children had responded to similar questions about pubertal development in an 11-year questionnaire sent out to all children in the DNBC, this information was included in the analyses.

2.4. Confounding variables and effect modifiers

We used directed acyclic graphs to identify confounding variables (Supplementary Material, Figure S1) (Pearl, 1995; Greenland et al., 1999). The analyses were adjusted for the following variables: parental socioeconomic status using the highest educational level of the parents, maternal pre-pregnancy body mass index (BMI), maternal smoking in first trimester, cohabitation of the parents during pregnancy, maternal age of menarche, and maternal age at delivery. After this adjustment, a confounding back door path through the underlying diseases persisted; however, due to the risk of bias amplification from uncontrolled confounding as well as loss of precision when adjusting for diseases that are strongly associated with the exposure but has uncertain associations with the outcome, we refrained from adjusting for the underlying disease (Cinelli et al., 2022). Instead, confounding by indication was investigated in a sub-analysis by using an active comparator design with asthma medication. The covariates were available from the pregnancy interviews in the DNBC, except for maternal age at delivery, which was obtained from the Danish Medical Birth Register (MBR) (Bliddal et al., 2018).

In a sub-analysis, the following factors with a potential modifiable effect on the association between nitrosatable drug exposure and timing of puberty were investigated: maternal exposure to nitrate from drinking water (n = 13,140), maternal nitrate and nitrite intake from diet, and maternal intake of the antioxidants C and E vitamins from supplements and diet (n = 11,284). Details on the assessment of these exposures have previously been described in detail (Jul Clemmensen et al., 2022), but in summary, information on nitrate concentration in maternal drinking water during pregnancy was obtained using data from the Danish geodatabase Jupiter on Danish public waterworks. These nitrate concentrations were then linked to the mother's residential address during pregnancy. Information on maternal intake of supplements and nitrate, nitrite and vitamins from diet was calculated from a food frequency questionnaire (FFQ) that the mother completed in gestational week 25. National food monitoring data were used to estimate the nitrate and nitrite level in each food item and based on this the mother's total intake of nitrate and nitrite was calculated covering the period four weeks before completion of the FFQ. The analyses with information from the FFQ were restricted to mothers with an energy intake of >4.2 MJ (MJ) or <16.7 MJ.

2.5. Data analysis

A multivariable regression model for interval-censored data was used to obtain mean age differences in months at reaching each of the specific pubertal milestones for the different exposure categories (exposed and probably exposed to nitrosatable drugs) compared to the reference group. The estimates are presented as crude and adjusted estimates with 95% confidence intervals (CI). Confounding variables were categorized as presented in Table 1 except for maternal age at delivery which was modeled as a second-order polynomial. Age at reaching each pubertal milestone was interval-censored since reaching a pubertal milestone occurred in the period between two questionnaires and only for menarche and first ejaculation, the children were asked about their specific age at the event. If a milestone was reached before replying to the first questionnaire, the age was left-censored, and if a milestone was not reached at the last questionnaire, the age was right-censored. A model assumption was that for each milestone, the residuals for age at reaching the milestone are normally distributed. The statistical model allowed us to include all participants regardless of the number of questionnaire responses. The assumption of normally distributed residuals was checked for the exposure and each level of the covariates by comparing the nonparametric distribution function of the residuals with the parametric distribution function. The data were compatible with this assumption. To assess these model assumptions, we used the IcenReg package in R (R Core Team, 2020) and for all other statistics, we used Stata 17.0 software (Stata Corporation, College Station, TX).

Invitation for the Puberty Cohort was based on oversampling of children of mothers with specific exposures. To account for this sampling strategy, all invited children were assigned a weight (1/their probability of being sampled) (Brix et al., 2019b). Furthermore, each

Table 1
Baseline characteristics of mothers and children in the Puberty Cohort according to exposure to nitrosatable drugs, n = 15,141.

	Not exposed to nitrosatable drugs	Exposed to nitrosatable drugs	Exposed to drugs classified as probably nitrosatable
N (%)	11,380 (75.2)	2,715 (17.9)	1,046 (6.9)
Characteristics			
Age at delivery in years			
Mean (±SD)	31 (4)	31 (4)	31 (4)
Highest social class of parents, n (%)			
High grade professional	2,609 (22.9)	658 (24.2)	285 (27.2)
Low grade professional	3,725 (32.7)	924 (34.0)	345 (33.0)
Skilled worker	3,222 (28.3)	695 (25.6)	258 (24.7)
Unskilled worker	1,550 (13.6)	372 (13.7)	119 (11.4)
Student	216 (1.9)	51 (1.9)	28 (2.7)
Economically inactive	58 (0.5)	15 (0.6)	11 (1.1)
Daily number of cigarettes in first trimester, n (%)			
Non-smoker	8,248 (72.5)	1,918 (70.6)	763 (72.9)
≤10	2,516 (22.1)	613 (22.6)	218 (20.8)
>10	616 (5.4)	184 (6.8)	65 (6.2)
Pre-pregnancy BMI, n (%)			
<18.5	785 (6.9)	165 (6.1)	80 (7.6)
18.5 - <25	7,122 (62.6)	1,588 (58.5)	642 (61.4)
25 - <30	2,385 (21.0)	622 (22.9)	207 (19.8)
30+	1,088 (9.6)	340 (12.5)	117 (11.2)
Maternal age at menarche, n (%)			
Earlier than peers	2,842 (25.0)	732 (27.0)	291 (27.8)
Same time as peers	6,517 (57.3)	1,559 (57.4)	588 (56.2)
Later than peers	2,021 (17.8)	424 (15.6)	167 (16.0)
Cohabitation of parents, n (%)			
Do not live together	208 (1.8)	75 (2.8)	26 (2.5)
Live together	11,172 (98.2)	2,640 (97.2)	1,020 (97.5)
Sex, n (%)			
Sons	5,446 (47.9)	1,305 (48.1)	515 (49.2)
Daughters	5,934 (52.1)	1,410 (51.9)	531 (50.8)

Abbreviations: BMI, body mass index.

participant was assigned a weight to account for a potential selection bias caused by selective nonparticipation. The weight was calculated based on the probability of participation given covariates assumed to affect the willingness to participate (1/probability of participating given covariates) estimated using a multivariable logistic regression model. The two weights were combined by multiplication.

To account for multiple testing due to testing of multiple correlated outcomes, a single marker of pubertal development was calculated. This marker was calculated for each sex separately as the average of all the single pubertal milestones and used as the only outcome in sub-analyses (except the sub-analysis on nitrosatable drug types) and sensitivity analyses. This decision was made based on no observed overall differences between adrenarche and gonadarche milestones in the main analysis and the sub-analysis of different types of nitrosatable drugs. To account for the weighting strategy, clustering of siblings, and interdependence in reaching the pubertal milestones in the same individual when calculating a single marker of pubertal development, robust standard errors were used (Huber, 1967; White, 1980).

In the main analysis, children prenatally exposed to nitrosatable drugs and children prenatally exposed to drugs classified as probably nitrosatable were compared with children of mothers with no drug intake or only supplement intake or drug intake not classified as nitrosatable/probably nitrosatable. In a sensitivity analysis, we restricted the analyses to participants with information on specific weeks of drug exposure and excluded those with a nitrosatable drug intake reported to be before pregnancy (n = 79). In a second sensitivity analysis, we removed other drug users from the reference group (n = 5,100) to meet the risk of misclassification of nitrosatable drugs as non-nitrosatable.

We performed four sub-analyses. First, drugs classified as nitrosatable were divided into three groups according to chemical structure: secondary amines, tertiary amines, and amides. Some drugs belonged to more than one group and are therefore located in more than one exposure group, and some children were exposed to several drugs and are therefore included in more than one exposure group. The reference group included children without any intake of nitrosatable drugs. The analyses were conducted as three separate regression analyses.

In the second sub-analysis, we explored dose-dependency. We did not have information about the dose of intake. Hence, weeks of use was used as a proxy of dose among those with a reported intake of nitrosatable drugs in the enrollment form. The duration of drug exposure was categorized as ≤ 1 week; 2–4 weeks; >4 weeks and if the mother had an intake of more than one drug, the period of the longest use was used. The reference group included children of mothers without a reported intake of nitrosatable drugs at enrolment. In a sensitivity analysis, we excluded children of mothers who reported a nitrosatable drug intake in the first pregnancy interview from the reference group.

In the third sub-analysis, effect modification by exposure to nitrate from maternal drinking water, maternal dietary intake of nitrate, nitrite, C and E vitamins, and supplement intake of C and E vitamins were investigated. The potential effect modifiers were divided into two groups separated at the median except for supplement intake which was grouped as yes/no and nitrate concentration in maternal drinking water which was separated at a concentration of 25 mg/L (half of the World Health Organization drinking water standard for nitrate) (World Health Organization, 2016). The association between intake of nitrosatable drugs and timing of puberty was investigated within the different nitrate/nitrite and vitamin exposure levels.

In the fourth sub-analysis, confounding by indication was investigated by restricting the study population to participants with an intake of asthma medication. Asthma medications were chosen because the indication for intake of these drugs was common and the drug group included both nitrosatable (some beta2 agonists) and non-nitrosatable drugs (glucocorticoids). Within this sub-population, difference in timing of puberty was investigated with the group not using nitrosatable drugs as the reference. This was done to investigate any difference in pubertal timing, that was not caused by the underlying disease and the analysis was based on the assumption that the drugs were used for the same severity of the underlying disease.

3. Results

Out of 15,141 study participants, 2,715 (17.9%) were prenatally exposed to nitrosatable drugs and 1,046 (6.9%) were prenatally exposed to drugs classified as probably nitrosatable. Exposed children were in general born of higher educated mothers, mothers who smoked more, had a higher BMI, and an earlier age at menarche compared to unexposed children. This was also the case for the probably exposed apart from maternal smoking status (Table 1).

When grouping drugs in relation to the main indication for the use, the drugs used belonged mainly to four indication groups: asthma (14%), gastrointestinal disorder including nausea (15%), infection (30%) and pain (29%) (Supplementary Material, Table S1). In the secondary amine group, asthma was the main indication, in the tertiary amine group, pain was the main indication, and in the amide group, infection was the main indication.

No overall association was observed between prenatal exposure to nitrosatable drugs and timing of puberty (Table 2). This was also the case when looking at each milestone separately. The mean age difference for all pubertal milestones for exposed sons was −0.3 months (−1.1,0.6) and for daughters −0.3 months (−1.1,0.6) compared to the reference group. When restricting the analyses to those with information

Table 2
Average age difference (in months) at reaching each of the specific pubertal milestones and an average of all pubertal milestones together for sons (n = 7,266) and daughters (n = 7,875) exposed to nitrosatable drugs in early pregnancy compared to sons and daughters with no exposure.

	Reference	Exposed to nitrosatable drugs		Exposed to drugs classified as probably nitrosatable	
	Crude mean age in years	Mean age difference in months		Mean age difference in months	
Pubertal milestones		Crude (95% CI)	Adjusted (95% CI)[a]	Crude (95% CI)	Adjusted (95% CI)[a]
Sons					
Genitals					
Tanner stage 2	10.9	−1.8 (−3.1,-0.4)	−1.7 (−3.0,-0.3)	0.6 (−1.3,2.6)	0.6 (−1.3,2.6)
Tanner stage 3	12.5	−0.8 (−2.1,0.4)	−0.6 (−1.9,0.7)	0.3 (−1.6,2.2)	0.3 (−1.5,2.2)
Tanner stage 4	13.7	−1.0 (−2.3,0.4)	−0.7 (−2.0,0.6)	−0.2 (−2.0,1.7)	−0.1 (−2.0,1.7)
Tanner stage 5	15.8	1.2 (−1.0,3.4)	1.6 (−0.5,3.7)	−0.1 (−3.0,2.9)	0.0 (−2.9,2.9)
Pubic hair					
Tanner stage 2	11.3	−1.0 (−2.2,0.3)	−0.7 (−1.9,0.5)	0.6 (−1.2,2.5)	0.7 (−1.2,2.5)
Tanner stage 3	12.7	−0.8 (−1.9,0.3)	−0.5 (−1.6,0.6)	0.5 (−1.2,2.2)	0.5 (−1.1,2.2)
Tanner stage 4	13.5	−0.3 (−1.4,0.8)	0.1 (−1.0,1.1)	0.6 (−1.0,2.1)	0.6 (−0.9,2.2)
Tanner stage 5	14.8	0.5 (−1.0,2.0)	0.9 (−0.5,2.3)	0.4 (−1.8,2.5)	0.4 (−1.7,2.6)
Ejaculation	13.4	−1.5 (−2.7,-0.2)	−1.3 (−2.5,0.0)	−1.2 (−3.0,0.6)	−1.1 (−2.8,0.7)
Voice break	13.0	−0.9 (−2.3,0.4)	−0.6 (−1.9,0.8)	1.4 (−0.4,3.2)	1.4 (−0.4,3.1)
Adult voice	15.2	0.6 (−1.7,3.0)	1.2 (−1.1,3.5)	1.1 (−2.1,4.4)	1.2 (−2.0,4.5)
Acne	12.2	−0.3 (−1.6,0.9)	−0.1 (−1.4,1.1)	−0.5 (−2.4,1.3)	−0.6 (−2.5,1.2)
Axillary hair	13.3	−0.2 (−1.6,1.3)	0.3 (−1.2,1.7)	1.1 (−1.0,3.1)	1.0 (−1.0,3.1)
Average of all pubertal milestones	–	−0.6 (−1.5,0.2)	−0.3 (−1.1,0.6)	0.3 (−1.0,1.6)	0.3 (−0.9,1.6)
Daughters					
Breast					
Tanner stage 2	9.9	−0.2 (−2.0,1.5)	0.3 (−1.4,2.0)	2.3 (−0.3,4.9)	2.4 (−0.1,4.9)
Tanner stage 3	11.7	−0.5 (−1.7,0.6)	−0.9 (−1.2,1.0)	0.6 (−1.0,2.3)	0.8 (−0.9,2.4)
Tanner stage 4	13.1	−0.7 (−1.9,0.5)	−0.4 (−1.6,0.8)	0.8 (−1.0,2.7)	1.0 (−0.8,2.8)
Tanner stage 5	16.1	−0.6 (−2.9,1.7)	−0.1 (−2.4,2.1)	2.0 (−1.5,5.4)	2.2 (−1.2,5.5)
Pubic hair					
Tanner stage 2	11.2	0.4 (−0.5,1.4)	0.6 (−0.3,1.6)	0.4 (−0.9,1.8)	0.6 (−0.8,2.0)
Tanner stage 3	12.5	−0.4 (−1.3,0.5)	−0.2 (−1.1,0.7)	1.1 (−0.3,2.4)	1.2 (−0.1,2.6)
Tanner stage 4	13.5	−0.8 (−2.0,0.4)	−0.6 (−1.8,0.6)	1.1 (−0.8,2.9)	1.3 (−0.5,3.2)
Tanner stage 5	15.6	−1.3 (−3.1,0.6)	−1.0 (−2.9,0.8)	1.3 (−1.4,4.0)	1.5 (−1.2,4.2)
Menarche	13.0	−0.2 (−1.2,0.7)	0.0 (−0.9,1.0)	1.8 (0.3,3.4)	2.0 (0.5,3.5)
Acne	11.4	−0.8 (−2.4,0.7)	−0.6 (−2.0,0.9)	−0.6 (−2.9,1.6)	−0.5 (−2.7,1.8)
Axillary hair	11.9	−0.9 (−2.1,0.4)	−0.6 (−1.9,0.7)	0.7 (−1.3,2.7)	0.9 (−1.0,2.9)
Average of all pubertal milestones	–	−0.6 (−1.5,0.3)	−0.3 (−1.1,0.6)	0.9 (−0.4,2.3)	1.2 (−0.1,2.5)

[a] Adjusted for parental socioeconomic status, maternal pre-pregnancy body mass index, maternal smoking in first trimester, cohabitation of parents, maternal age at menarche and maternal age at delivery.

on specific weeks of drug intake (n = 10,792), the results remained similar and no overall differences were observed when excluding participants whose mothers had an intake only before the first day of the last menstrual period (n = 79) (Supplementary Material, Table S2). In the second sensitivity analysis, mothers with a non-nitrosatable drug intake were removed (n = 5,100) from the reference group. This did not change the overall interpretation of the results (Supplementary Material, Table S3). In the main analysis, the level of left-censoring for the earliest milestones was 85% for Tanner B2 and 54% for Tanner PH2 for daughters and 64% for Tanner G2 and 52% for Tanner PH2 for sons.

In the first sub-analysis, a tendency towards earlier age at timing of puberty among children exposed to secondary amines was observed in the crude analyses, especially for daughters. However, after adjustment, this association attenuated with a mean age difference for all pubertal milestones for sons of −0.6 months (−2.2,1.1) and daughters of −0.8 months (−2.3,0.7) (Table 3).

In the second sub-analysis, no dose-response relationship was observed with increasing weeks of exposure (Supplementary Material, Table S4). When restricting the analyses to participants without exposure in interview 1, the results did not change.

In the third sub-analysis, stratification based on different levels of nitrate concentration in maternal drinking water, maternal intake of nitrate, nitrite, C and E vitamins from diet, and C and E vitamins from supplements during pregnancy, did not identify any vulnerable subgroups (Table 4).

In the fourth sub-analysis, confounding by indication was investigated using an active comparator design. The mean age difference for all pubertal milestones for sons of mothers treated with asthma medication was 1.7 (−4.0,7.3) when comparing those exposed to nitrosatable asthma drugs with those exposed to non-nitrosatable asthma drugs (Supplementary Material, Table S5).

4. Discussion

We did not find that prenatal exposure to nitrosatable drugs was associated with timing of puberty in this population-based cohort study. This conclusion was supported by several sensitivity analyses and sub-analyses. In total, 17.9% of the study population was exposed to at least one nitrosatable drug in the first part of pregnancy; this exposure level was in line with previously performed studies (Brender et al., 2011a; Thomsen et al., 2019).

For the probably exposed group, we neither found any association with timing of puberty; on the contrary, for some of the milestones timing of puberty went in the opposite direction compared to the exposed group. A priori, we expected the two groups to be comparable besides drugs in the exposed group had been tested regarding nitrosability, and drugs in the probably exposed group had not been tested. Since previously performed studies have found that only minor differences in the chemical structure of a drug can lead to changes in nitrosability (Brambilla and Martelli, 2007), we decided to keep the probably exposed in a separate exposure group. The observed differences in timing of puberty between the two exposure groups could be due to differences in prescription indication, differences in chemical structure, or random differences. The reference group included children of mothers without drug intake, only with an intake of supplements, or with an intake of drugs not closely related to the drugs tested as nitrosatable. If some of these drugs were in fact nitrosatable, there is a risk of bias that would lead the results toward the null. Therefore, in a

Table 3
Average age difference (in months) at reaching each of the specific pubertal milestones and an average of all pubertal milestones together for sons (n = 7,266) and daughters (n = 7,875) according to type of nitrosatable drug exposure compared to sons and daughters with no exposure (n = 11,380).

	Secondary amine exposure n = 728		Tertiary amine exposure n = 1,557		Amide exposure n = 1,486	
	Mean age difference in months		Mean age difference in months		Mean age difference in months	
Pubertal milestones	Crude (95% CI)	Adjusted (95% CI)[a]	Crude (95% CI)	Adjusted (95% CI)[a]	Crude (95% CI)	Adjusted (95% CI)[a]
Sons						
Genitals						
Tanner stage 2	−1.9 (−4.8,0.8)	−1.8 (−4.6,1.1)	−1.4 (−3.0,0.2)	−1.3 (−2.9,0.3)	−1.8 (−3.6,-0.1)	−1.8 (−3.5,0.0)
Tanner stage 3	−2.1 (−4.5,0.3)	−1.6 (−4.1,0.8)	−0.6 (−2.2,0.9)	−0.5 (−2.0,1.1)	−0.7 (−2.4,0.9)	−0.6 (−2.3,1.1)
Tanner stage 4	−2.0 (−4.4,0.4)	−1.5 (−3.9,1.0)	−0.5 (−2.2,1.2)	−0.3 (−2.0,1.3)	−1.0 (−2.8,0.7)	−0.9 (−2.7,0.9)
Tanner stage 5	1.1 (−2.9,5.1)	1.8 (−2.2,5.9)	2.8 (0.1,5.4)	3.1 (0.5,5.6)	1.8 (−1.2,4.8)	2.0 (−0.9,4.9)
Pubic hair						
Tanner stage 2	−2.0 (−4.6,0.5)	−1.7 (−4.3,0.8)	−0.1 (−1.7,1.4)	0.0 (−1.5,1.5)	−0.6 (−2.3,0.9)	−0.5 (−2.1,1.1)
Tanner stage 3	−3.0 (−4.0,-1.0)	−2.5 (−4.5,-0.4)	−0.1 (−1.5,1.3)	0.1 (−1.2,1.5)	−0.5 (−1.9,1.0)	−0.3 (−1.7,1.2)
Tanner stage 4	−1.3 (−3.2,0.5)	−0.7 (−2.7,1.2)	0.1 (−1.2,1.4)	0.4 (−0.9,1.7)	−0.4 (−1.8,1.0)	−0.1 (−1.5,1.2)
Tanner stage 5	0.1 (−2.6,2.9)	1.0 (−1.8,3.7)	1.8 (0.0,3.6)	2.1 (0.3,3.8)	0.3 (−1.6,2.2)	0.6 (−1.3,2.4)
Ejaculation	−2.0 (−4.3,0.3)	−1.6 (−3.9,0.7)	−1.0 (−2.6,0.6)	−0.8 (−2.4,0.8)	−1.7 (−3.3,0.0)	−1.5 (−3.2,0.2)
Voice break	−0.4 (−2.8,2.0)	0.6 (−2.0,2.9)	−0.3 (−2.0,1.4)	−0.1 (−1.7,1.6)	−0.9 (−2.7,0.8)	−0.7 (−2.4,1.0)
Adult voice	4.1 (−0.3, 8.5)	5.1 (0.8,9.4)	−0.2 (−3.0,2.7)	0.2 (−2.6,3.1)	−0.5 (−3.4,2.3)	−0.1 (−2.9,2.7)
Acne	0.0 (−2.4,2.4)	0.5 (−1.9,2.9)	−0.3 (−1.9,1.2)	−0.1 (−1.7,1.4)	−0.6 (−2.2,1.0)	−0.5 (−2.1,1.1)
Axillary hair	−1.8 (−4.4,0.9)	−1.0 (−3.7,1.7)	0.8 (−0.9,2.5)	1.1 (−0.6,2.9)	0.7 (−1.2,2.5)	1.0 (−0.8,2.9)
Average of all pubertal milestones	−1.3 (−2.9,0.3)	−0.6 (−2.2,1.1)	0.0 (−1.1,1.0)	0.2 (−0.8,1.3)	−0.6 (−1.7,0.6)	−0.3 (−1.4,0.8)
Daughters						
Breast						
Tanner stage 2	−3.2 (−6.3,-0.2)	−1.2 (−4.3,1.8)	0.5 (−1.8,2.8)	0.6 (−1.6,2.8)	−0.5 (−2.8,1.8)	−0.2 (−2.4,2.0)
Tanner stage 3	−2.4 (−4.4,-0.5)	−0.9 (−2.8,1.1)	0.0 (−1.4,1.5)	0.1 (−1.3,1.5)	−0.9 (−2.4,0.6)	−0.5 (−2.0,0.9)
Tanner stage 4	−1.9 (−3.8,0.0)	−0.8 (−2.7,1.2)	0.3 (−1.3,1.9)	0.4 (−1.2,2.0)	−1.2 (−2.8,0.4)	−0.9 (−2.4,0.7)
Tanner stage 5	−1.5 (−5.6,2.5)	0.3 (−3.7,4.3)	0.2 (−2.7,3.2)	0.3 (−2.7,3.2)	−1.0 (−3.9,2.0)	−0.7 (−3.6,2.2)
Pubic hair						
Tanner stage 2	−0.8 (−2.4,0.7)	−0.1 (−1.7,1.4)	0.8 (−0.4,2.1)	0.8 (−0.4,2.0)	0.2 (−1.1,1.5)	0.4 (−0.9,1.7)
Tanner stage 3	−0.9 (−2.4,0.6)	−0.1 (−1.7,1.4)	0.0 (−1.1,1.2)	0.0 (−1.2,1.1)	−0.6 (−1.8,0.6)	−0.4 (−1.6,0.8)
Tanner stage 4	−1.4 (−3.5,0.8)	−0.7 (−2.9,1.5)	0.0 (−1.7,1.6)	−0.1 (−1.7,1.5)	−0.9 (−2.5,0.6)	−0.8 (−2.4,0.8)
Tanner stage 5	−3.0 (−6.1,0.1)	−1.9 (−5.1,1.3)	−0.8 (−3.2,1.6)	−0.9 (−3.3,1.4)	−1.7 (−4.0,0.7)	−1.5 (−3.8,0.8)
Menarche	−1.9 (−3.4,-0.3)	−0.8 (−2.3,0.7)	0.4 (−0.9,1.7)	0.4 (−0.8,1.6)	−0.6 (−1.8,0.7)	−0.4 (−1.5,0.8)
Acne	−1.8 (−4.5,0.8)	−0.9 (−3.5,1.8)	0.6 (−1.4,2.6)	0.6 (−1.4,2.5)	−1.9 (−3.7,-0.2)	−1.8 (−3.6,0.0)
Axillary hair	−2.7 (−4.9,-0.5)	−1.7 (−3.9,0.5)	−0.5 (−2.1,1.2)	−0.5 (−2.1,1.2)	−1.1 (−2.7,0.4)	−1.0 (−2.6,0.6)
Average of all pubertal milestones	−2.0 (−3.5,-0.5)	−0.8 (−2.3,0.7)	0.2 (−1.0,1.4)	0.2 (−1.0,1.3)	−1.0 (−2.1,0.2)	−0.7 (−1.8,0.4)

[a] Adjusted for parental socioeconomic status, maternal pre-pregnancy body mass index, maternal smoking in first trimester, cohabitation of parents, maternal age at menarche, and maternal age at delivery.

sensitivity analysis, we excluded mothers from the reference group who had a drug intake. The interpretation of the results remained comparable with the main analysis and misclassification of the drugs did not seem to explain the finding of no association. In sub-analyses, we kept children of mothers exposed to other drugs in the reference group. This was done to sustain exchangeability between the exposed group and the reference group since the exposed group could also be exposed to non-nitrosatable drugs. Furthermore, the use of data on drug use collected during pregnancy reduced the risk of recall bias.

We used information from the enrolment form to obtain knowledge on drug use in the exposure window of organogenesis that occurs from week 3–8 (Pryor et al., 2000). The HPG-axis is a central axis in initiation of puberty and the formation and programming of this axis continues to around mid-pregnancy (Styne et al., 2011; edited by Rodney A, 2009); therefore, information on drug use from the first interview in the DNBC around gestational week 18 was further included. The data did not allow us to explore various exposure windows, which may mask a potential effect if this is related to specific exposure windows outside of the investigated exposure window. The mothers reported both over-the-counter drugs, and prescribed drugs, which is a strength since a study in the U.S. on pregnant women found that around half of the nitrosatable drugs used were over-the-counter drugs (Brender et al., 2011a). The DNBC mothers were asked about their intake around four weeks before gestational week one. This approach was used to include all drugs taken around pregnancy start and accounted for a potential wash-out period some drugs may have. However, there is a risk that some mothers were classified as exposed even though their intake was before pregnancy. This would categorize some unexposed participants in the exposed group which would have blurred the exposure contrast, making it more difficult to find a difference between the two groups. This would have led to bias towards the null. When removing mothers with a reported intake of nitrosatable drugs before pregnancy from the analyses, the results remained comparable with the main analysis and including mothers with an intake before pregnancy has probably not biased our results. We did not have information on the actual dose of the intake and weeks of intake were used as a rough marker of dose. A more precise indication of the dose would have led to a more precise analysis of potential dose-response relationships.

The nitrosatable drugs were grouped as secondary amines, tertiary amines, and amides according to their chemical structure. Only a limited number of NOCs have been tested regarding their teratogen potential (McKean-Cowdin et al., 2003); however, differences in the level and type of formed NOCs have been described in relation to the type of nitrosatable drug (Brambilla and Martelli, 2007; McKean-Cowdin et al., 2003). There is a chance that even though NOCs are formed they might not be teratogenic. We used the same definition of nitrosatable drugs as previously performed studies that found an association with several negative birth outcomes (Brender et al., 2011b, 2012; Vuong et al., 2015; Thomsen et al., 2019). If the findings of these studies were causally linked to nitrosatable drug exposure, we may assume that teratogenic compounds were formed from the drugs included in our study.

The level of endogenous NOC formation depends on several factors, such as stomach pH and the level of amines and amides, nitrite, and inhibitors of NOC formation, such as vitamin C and E (Brambilla and Martelli, 2007; Ward et al., 2018). Epidemiological studies have previously found a stronger association with neural tube defects and other birth defects and preterm birth among children of mothers with

Table 4
Investigation of effect modification by intake of nitrate, nitrite, C and E vitamins from diet and C and E vitamins from supplements. Average age difference (in months) for all pubertal milestones together for sons and daughters exposed to nitrosatable drugs compared to sons and daughters with no exposure. The association was investigated within each group of the potential effect modifier.

	Mean age difference in months	
	Crude (95% CI)	**Adjusted (95% CI)[a]**
Sons		
Nitrate concentration in drinking water (mg/L)		
≤25	−0.5 (−1.3,0.4)	−0.1 (−1.0,0.7)
>25	−1.6 (−8.4,5.2)	1.0 (−3.7,5.6)
Nitrate intake from diet (mg)[b]		
≤61	−0.1 (−1.5,1.3)	0.4 (−0.9,1.7)
>61	−1.1 (−2.4,0.2)	−0.8 (−2.1,0.5)
Nitrite intake from diet (mg/day)[b]		
≤0.3	0.0 (−1.7,1.7)	0.7 (−1.0,2.3)
>0.3	−0.9 (−2.0,0.2)	−0.7 (−1.8,0.4)
C vitamin from diet (mg/day)[b]		
≤119	−0.3 (−1.7,1.0)	0.1 (−1.3,1.4)
>119	−0.9 (−2.2,0.5)	−0.6 (−2.0,0.7)
E vitamin from diet (a-TE/day)[b]		
≤8	−0.1 (−1.6,1.3)	0.5 (−0.9,2.0)
>8	−1.0 (−2.6,0.2)	−0.8 (−2.0,0.5)
Supplement intake of C vitamin		
Yes	−0.4 (−1.5,0.6)	−0.1 (−1.2,0.9)
No	−1.8 (−4.0,0.5)	−0.5 (−2.6,1.7)
Supplement intake of E vitamin		
Yes	−0.6 (−1.6,0.5)	−0.3 (−1.3,0.8)
No	−0.6 (−2.7,1.5)	0.5 (−1.5,2.5)
Daughters		
Nitrate concentration in drinking water (mg/L)		
≤25	−0.5 (−1.5,0.4)	−0.2 (−1.1,0.7)
>25	−2.0 (−6.6,2.5)	−1.7 (−6.7,3.4)
Nitrate intake from diet (mg)[b]		
≤61	−1.0 (−2.4,0.3)	−0.7 (−2.0,0.6)
>61	−0.6 (−2.1,0.9)	−0.2 (−1.7,1.2)
Nitrite intake from diet (mg/day)[b]		
≤0.3	−1.2 (−2.8,0.4)	−1.1 (−2.6,0.5)
>0.3	−0.6 (−1.8,0.7)	−0.1 (−1.3,1.2)
C vitamin from diet (mg/day)[b]		
≤119	−1.8 (−3.1,-0.6)	−1.3 (−2.6,0.0)
>119	0.2 (−1.3,1.7)	0.3 (−1.2,1.7)
E vitamin from diet (a-TE/day)[b]		
≤8	−1.2 (−2.7,0.3)	−0.8 (−2.3,0.6)
>8	−0.5 (−1.8,0.9)	−0.2 (−1.5,1.1)
Supplement intake of C vitamin		
Yes	−1.0 (−2.1,0.1)	−0.8 (−1.7,0.3)
No	1.0 (−1.7,3.7)	0.7 (−2.3,3.6)
Supplement intake of E vitamin		
Yes	−1.0 (−2.1,0.1)	−0.8 (−1.8,0.3)
No	0.4 (−2.2,3.0)	0.8 (−1.7,3.2)

[a] Adjusted for parental socioeconomic status, maternal pre-pregnancy body mass index, maternal smoking in first trimester, cohabitation of parents, maternal age at menarche, and maternal age at delivery.

[b] Divided at the median. Reported as pseudo medians following local regulations (GDPR, Regulation (EU), 2016/679 of May 25, 2018).

nitrosatable drug intake and a higher intake of nitrite from diet compared to a lower intake (Brender et al., 2004, 2011b, 2012; Vuong et al., 2016). This was also observed with a higher intake of nitrate from drinking water in two studies investigating stillbirth and neural tube defects, but not in a third study investigating other birth defects (Brender et al., 2004, 2013; Thomsen et al., 2021). Weaker associations between nitrosatable drug exposure and several adverse birth outcomes were observed in mothers with higher vitamin C intake (Brender et al., 2011b, 2012; Shinde et al., 2013). In our study, we did not identify any vulnerable subgroups. Besides endogenously formed NOCs, these compounds may also be formed exogenously (Brambilla and Martelli, 2007). Information on intake of preformed NOCs from diet was not accounted for in this study. The level of preformed NOCs in food has decreased and NOCs is only present in a few food items like preserved or smoked fish and meat (Brambilla and Martelli, 2007; Jakszyn and Gonzalez, 2006). If an intake of preformed NOCs is related to socioeconomic factors, we have at least partly adjusted for it in our analyses.

Drug intake is closely related to the appearance of an underlying disease and confounding by indication is of concern in observational studies investigating the negative effects of drug exposure (Yoshida et al., 2015). The association between maternal disease and timing of puberty has only been investigated for a small range of diseases, and only minor associations were observed (Lunddorf et al., 2020, 2022; Subramanian et al., 2021). Preliminary results from our research group on maternal asthma did not find an association with timing of puberty and another study on nausea and timing of puberty also did not find an association (Bruun et al., 2021). Studies on the other main indications for nitrosatable drug use have not been identified. The weak associations observed between maternal disease and timing of puberty could indicate a low risk of confounding by indication in this study. However, in a sub-analysis, we used an active comparator design to investigate if timing of puberty differed among asthma patients who had an intake of nitrosatable drugs and those with an intake of non-nitrosatable drugs. If the indication for drug use was the same and there was an association with nitrosatable drug exposure, we would expect a difference between the two groups. Only minor differences were observed for the sons. In other studies on prenatal exposure to nitrosatable drugs, confounding by indication has also been investigated. In a case-control study investigating the risk of childhood cancer from prenatal exposure to nitrosatable drugs, the risk of confounding by indication from underlying infectious disease was investigated by presenting odds ratios for children exposed to non-nitrosatable antibiotics and odds ratios for children exposed to nitrosatable antibiotics. Confounding by underlying infections was only suggested to explain the association observed for one outcome (Sirirungreung et al., 2023). In another case-control study, confounding by indication was handled by adjusting for fever. This only slightly lowered the association between nitrosatable drug exposure and neural tube defects (Brender et al., 2004).

Detailed information on pubertal development was reported every six months at the time of development. In a validation study in the Puberty Cohort, the agreement between self-reported information and a clinical examination was evaluated and found to be moderate to fair (Ernst et al., 2018), and a potential misclassification of the outcome is expected to be non-differential. The participation rate in the Puberty Cohort was high, and the risk of selection bias was further reduced by introducing selection weights in the analyses. All mothers in the Puberty Cohort had responded to detailed questionnaires allowing us to adjust for several potential confounding factors; however, baseline characteristics revealed differences between exposure groups and the risk of residual confounding may still be present. Due to the risk of over-adjustment from adjustment for a mediator, we did not include childhood factors in our adjusted analyses. The children in the Puberty Cohort had turned 11 years when the Puberty Cohort started, and many of the children had already reached the first pubertal milestones. This was handled by the analytic strategy that allowed us to include children who had already started puberty under the assumption of normal distribution of age at reaching the pubertal milestones.

In conclusion, this large population-based cohort study with detailed information on nitrosatable drug exposure in pregnancy, potential effect modifiers, and covariates, together with continuously collected information on pubertal development, allowed us to investigate whether prenatal exposure to nitrosatable drugs was associated with timing of puberty. We did not find an association between prenatal exposure to nitrosatable drugs and timing of puberty. Several sensitivity analyses and sub-analyses supported this main finding.

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Ethical approvals

The DNBC is approved by the Danish Data Protection Agency and the Committee on Health Research Ethics. The DNBC participants provided informed consent at enrolment. This study was registered by the Danish Data Protection Agency (2016-051-000001, rec no 1643) and approved by the Steering Committee of the DNBC (Ref. no. 2018–27).

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Access to data from the DNBC can be obtained. The procedure for application for data access is available at the website: https://www.dnbc.dk/access-to-dnbc-data.

List of abbreviations

FFQ	Food frequency questionnaire
DNBC	The Danish National Birth Cohort
NOCs	N-nitroso compounds (NOCs)
CI	Confidence interval
BMI	Body mass index
MBR	The Danish Medical Birth Register
MJ	Megajoules

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2023.114271.

References

Alaoui-Jamali, M.A., Rossignol, G., Schuller, H.M., Castonguay, A., 1989. Transplacental genotoxicity of a tobacco-specific N-nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, in Syrian golden hamster. Mutat. Res. Genet. Toxicol. 223 (1), 65–72.

Andersen, A.M., Olsen, J., 2011. The Danish National Birth Cohort: selected scientific contributions within perinatal epidemiology and future perspectives. Scand. J. Publ. Health 39 (7 Suppl. l), 115–120.

Benedum, CM, Yazdy, MM, Mitchell, AA, Werler, MM, 2015. Impact of Periconceptional Use of Nitrosatable Drugs on the Risk of Neural Tube Defects. American journal of epidemiology. 2015/10// 182 (8), 675–684.

Bliddal, M., Broe, A., Pottegard, A., Olsen, J., Langhoff-Roos, J., 2018. The Danish medical birth register. Eur. J. Epidemiol. 33 (1), 27–36.

Bochert, G., Platzek, T., Blankenburg, G., Wiessler, M., Neubert, D., 1985. Embryotoxicity induced by alkylating agents: left-sided preponderance of paw malformations induced by acetoxymethyl-methylnitrosamine in mice. Arch. Toxicol. 56 (3), 139–150.

Brambilla, G., Martelli, A., 2007. Genotoxic and carcinogenic risk to humans of drug–nitrite interaction products. Mutat. Res. Rev. Mutat. Res. 635 (1), 17–52.

Brender, J.D., Olive, J.M., Felkner, M., Suarez, L., Marckwardt, W., Hendricks, K.A., 2004. Dietary nitrites and nitrates, nitrosatable drugs, and neural tube defects. Epidemiology 15 (3).

Brender, J.D., Kelley, K.E., Werler, M.M., Langlois, P.H., Suarez, L., Canfield, M.A., et al., 2011a. Prevalence and patterns of nitrosatable drug use among U.S. women during early pregnancy. Birth Defects Res A Clin Mol Teratol 91 (4), 258–264.

Brender, J.D., Werler, M.M., Kelley, K.E., Vuong, A.M., Shinde, M.U., Zheng, Q., et al., 2011b. Nitrosatable drug exposure during early pregnancy and neural tube defects in offspring: national Birth Defects Prevention Study. Am. J. Epidemiol. 174 (11), 1286–1295.

Brender, J.D., Werler, M.M., Shinde, M.U., Vuong, A.M., Kelley, K.E., Huber Jr., J.C., et al., 2012. Nitrosatable drug exposure during the first trimester of pregnancy and selected congenital malformations. Birth Defects Res A Clin Mol Teratol 94 (9), 701–713.

Brender, J.D., Weyer, P.J., Romitti, P.A., Mohanty, B.P., Shinde, M.U., Vuong, A.M., et al., 2013. Prenatal nitrate intake from drinking water and selected birth defects in offspring of participants in the national birth defects prevention study. Environ. Health Perspect. 121 (9), 1083.

Brix, N., Ernst, A., Lauridsen, L.L.B., Parner, E., Stovring, H., Olsen, J., et al., 2019a. Timing of puberty in boys and girls: a population-based study. Paediatr. Perinat. Epidemiol. 33 (1), 70–78.

Brix, N., Ernst, A., Lauridsen, L.L.B., Parner, E.T., Olsen, J., Henriksen, T.B., et al., 2019b. Maternal smoking during pregnancy and timing of puberty in sons and daughters: a population-based cohort study. Am. J. Epidemiol. 188 (1), 47–56.

Bruun, M.R., Ernst, A., Arendt, L.H., Ramlau-Hansen, C.H., Brix, N., 2021. Nausea during pregnancy and timing of pubertal development in sons and daughters: a population-based cohort study. Clin. Epidemiol. 13, 895–905 (1179-1349 (Print)).

Cinelli, C., Forney, A., Pearl, J., 2022. A crash course in good and bad controls. Socio. Methods Res., 00491241221099552

Diwan, B.A., 1974. Strain-dependent teratogenic effects of 1-Ethyl-1-nitrosourea in inbred strains of Mice1. Cancer Res. 34 (1), 151–157.

Rodney A. Rhoades DRB. Medical Physiology : Principles for Clinical Medicine, third ed., 2009. Wolters Kluwer Health/Lippincott Williams & Wilkins, [2009] ©2009, Philadelphia. edited by.

Ernst, A., Lauridsen, L.L.B., Brix, N., Kjersgaard, C., Olsen, J., Parner, E.T., et al., 2018. Self-assessment of pubertal development in a puberty cohort. J. Pediatr. Endocrinol. Metabol. : JPEM (J. Pediatr. Endocrinol. Metab.) 31 (7), 763–772.

Ernst, A., Brix, N., Lauridsen, L.L.B., Strandberg-Larsen, K., Bech, B.H., Nohr, E.A., et al., 2020. Cohort profile: the puberty cohort in the Danish national birth cohort (DNBC). Int. J. Epidemiol. 49 (2), 373. -4g.

Euling, S.Y., Herman-Giddens, M.E., Lee, P.A., Selevan, S.G., Juul, A., Sorensen, T.I.A., et al., 2008. Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings. Pediatrics 121 (Suppl. 3), 172–191.

Golub, M.S., Collman, G.W., Foster, P.M., Kimmel, C.A., Rajpert-De Meyts, E., Reiter, E. O., et al., 2008. Public health implications of altered puberty timing. Pediatrics 121 (Suppl. 3), S218–S230.

Graber, J.A., 2013. Pubertal timing and the development of psychopathology in adolescence and beyond. Horm. Behav. 64 (2), 262–269.

Greenland, S., Pearl, J., Robins, J.M., 1999. Causal diagrams for epidemiologic research. Epidemiology 10 (1), 37–48.

Greenspan, L.C., Lee, M.M., 2018. Endocrine disrupters and pubertal timing. Curr. Opin. Endocrinol. Diabetes Obes. 25 (1), 49–54.

Huber, P.J., 1967. The Behavior of Maximum Likelihood Estimates under Nonstandard Conditions. Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics and Probability. Statistics, vol. 1. University of California Press, Berkeley, Calif, pp. 221–233.

Jacobsen, T.N., Nohr, E.A., Frydenberg, M., 2010. Selection by socioeconomic factors into the Danish national birth cohort. Eur. J. Epidemiol. 25 (5), 349–355.

Jakszyn, P., Gonzalez, C.A., 2006. Nitrosamine and related food intake and gastric and oesophageal cancer risk: a systematic review of the epidemiological evidence. World J. Gastroenterol. 12 (27), 4296–4303.

Jul Clemmensen, P., Brix, N., Schullehner, J., Lunddorf, L.L.H., Ernst, A., Ebdrup, N.H., et al., 2022. Prenatal nitrate exposure from diet and drinking water and timing of puberty in sons and daughters: a nationwide cohort study. Environ. Int. 170, 107659.

Juul, A., 2011. In utero programming of pubertal development? *Archives of Disease in.* Childhood 96 (8), 703.

Lunddorf, L.L.H., Brix, N., Ernst, A., Arendt, L.H., Støvring, H., Clemmensen, P.J., et al., 2020. Hypertensive disorders in pregnancy and timing of pubertal development in daughters and sons. Hum. Reprod. 35 (9), 2124–2133.

Lunddorf, L.L.H., Ernst, A., Brix, N., Arendt, L.H., Andersen, S.L., Olsen, J., et al., 2022. Maternal thyroid disease in pregnancy and timing of pubertal development in sons and daughters. Fertil. Steril. 118 (1), 136–146.

McKean-Cowdin, R., Pogoda, J.M., Lijinsky, W., Holly, E.A., Mueller, B.A., Preston-Martin, S., 2003. Maternal prenatal exposure to nitrosatable drugs and childhood brain tumours. Int. J. Epidemiol. 32 (2), 211–217.
Nagao, T., Morita, Y., Ishizuka, Y., Wada, A., Mizutani, M., 1991. Induction of fetal malformations after treatment of mouse embryos with methylnitrosourea at the preimplantation stages. Teratog. Carcinog. Mutagen. 11 (1), 1–10.
Olsen, J., Melbye, M., Olsen, S.F., Sorensen, T.I.A., Aaby, P., Andersen, A.M.N., et al., 2001. The Danish National Birth Cohort - its background, structure and aim. Scand. J. Publ. Health 29 (4), 300–307.
Olshan, A.F., Faustman, E.M., 1989. Nitrosatable drug exposure during pregnancy and adverse pregnancy outcome. Int. J. Epidemiol. 18 (4), 891–899.
Ong, K.K., Ahmed, M.L., Dunger, D.B., 2006. Lessons from large population studies on timing and tempo of puberty (secular trends and relation to body size): the European trend. Mol. Cell. Endocrinol. 254, 8–12.
Parent, A.-S., Teilmann, G., Juul, A., Skakkebaek, N.E., Toppari, J., Bourguignon, J.-P., 2003. The timing of normal puberty and the age limits of sexual precocity: variations around the World, secular trends, and changes after migration. Endocr. Rev. 24 (5), 668–693.
Pearl, J., 1995. Causal diagrams for empirical research. Biometrika 82 (4), 669–688.
Prentice, P., Viner, R.M., 2013. Pubertal timing and adult obesity and cardiometabolic risk in women and men: a systematic review and meta-analysis. Int. J. Obes. 37 (8), 1036–1043.
Pryor, J.L., Hughes, C., Foster, W., Hales, B.F., Robaire, B., 2000. Critical windows of exposure for children's health: the reproductive system in animals and humans. Environ. Health Perspect. 108 (Suppl. 3), 491–503. Suppl 3.
R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.
Shinde, M.U., Vuong, A.M., Brender, J.D., Werler, M.M., Kelley, K.E., Huber Jr., J.C., et al., 2013. Prenatal exposure to nitrosatable drugs, vitamin C, and risk of selected birth defects. Birth Defects Res A Clin Mol Teratol 97 (8), 515–531.
Sirirungreung, A., Hansen, J., He, D., Huang, X., Ritz, B., Heck, J.E., 2023. Exposure to nitrosatable drugs during pregnancy and childhood cancer: a matched case-control study in Denmark, 1996-2016. Pharmacoepidemiol. Drug Saf. 32 (4), 496–505.
Styne, D.M., Grumbach, M.M., 2011. Chapter 25 - puberty: ontogeny, neuroendocrinology, physiology, and disorders. In: Melmed, S., Polonsky, K.S., Larsen, P.R., Kronenberg, H.M. (Eds.), Williams Textbook of Endocrinology, twelfth ed. W.B. Saunders, Philadelphia, pp. 1054–1201.
Subramanian, A., Idkowiak, J., Toulis, K.A., Thangaratinam, S., Arlt, W., Nirantharakumar, K., 2021. Pubertal timing in boys and girls born to mothers with gestational diabetes mellitus: a systematic review. Eur. J. Endocrinol. 184 (1), 51–64.
Thomsen, A.M.L., Liew, Z., Riis, A.H., Stayner, L.T., Ramlau-Hansen, C.H., Sigsgaard, T., et al., 2019. Nitrosatable drug exposure during pregnancy and risk of stillbirth. Pharmacoepidemiol. Drug Saf. 28 (9), 1204–1210.
Thomsen, A.M.L., Ramlau-Hansen, C.H., Schullehner, J., Ebdrup, N.H., Liew, Z., Coffman, V., et al., 2021. Prenatal nitrosatable prescription drug intake, drinking water nitrate, and the risk of stillbirth: a register- and population-based cohort of Danish pregnancies, 1997-2017. Environ. Health 20 (1), 118.
Vuong, A.M., Shinde, M.U., Brender, J.D., Shipp, E.M., Huber Jr., J.C., Zheng, Q., et al., 2015. Nitrosatable drug exposure during pregnancy and preterm and small-for-gestational-age births. Paediatr. Perinat. Epidemiol. 29 (1), 60–71.
Vuong, A.M., Shinde, M.U., Brender, J.D., Shipp, E.M., Huber, J.J.C., Sharkey, J.R., et al., 2016. Prenatal exposure to nitrosatable drugs, dietary intake of nitrites, and preterm birth. Am. J. Epidemiol. 183 (7), 634–642.
Ward, M.H., Jones, R.R., Brender, J.D., de Kok, T.M., Weyer, P.J., Nolan, B.T., et al., 2018. Drinking water nitrate and human health: an updated review. Int. J. Environ. Res. Publ. Health 15 (7), 1557.
White, H., 1980. A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. Econometrica 48 (4), 817–838.
World Health Organization, 2016. Nitrate and Nitrite in Drinking-Water. Background Document for Development of WHO Guidelines for Drinking-Water Quality reference number WHO/SDE/WSH/7.01/16/Rev/1.
Yoshida, K., Solomon, D.H., Kim, S.C., 2015. Active-comparator design and new-user design in observational studies. Nat. Rev. Rheumatol. 11 (7), 437–441.