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Characterization of the aerosol release from spray cleaning and disinfection products – Spray scenarios in a climate chamber

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ABSTRACT

Cleaning work using spray products has been associated with adverse respiratory effects but little is known of the exposure concentrations. The purpose of this study was to characterize aerosol generation at spray scenarios in a controlled climate chamber. Spraying on vertically and horizontally oriented surfaces, as well as spraying on a cloth, was investigated. Furthermore, the effect of nozzle geometry was tested. The average mass generation rates of six pressurized spray cans and 13 trigger sprays were about 1.7 g/s and did not differ significantly, but the average values of the individual sprays had large variations (0.5–3.1 g/s). The time required to halve the air concentration of aerosol particles, the *half-life time*, was determined for all spray products. The average *half-life time* of the total particle *mass* concentration (TPMC) of the pressurized spray cans was 0.5 h versus 0.25 h for trigger sprays. Gravimetrically determined airborne fractions of pressurized spray cans tended to be higher than those of trigger sprays. However, airborne fractions based on the measured peak TPMC were up to three orders of magnitude smaller. A comparison of different trigger spray nozzles when spraying the same product showed that the TPMC can be up to 18 times higher for the largest emitting nozzle. The distance of the nozzle to a cloth should be 1 cm to significantly reduce the concentration of the generated aerosols. ConsExpo modeling predicted the measured peak TPMC well but less well the decay.

1. Introduction

Cleaning and disinfection products have been associated with adverse respiratory effects in professional cleaners and among residents doing domestic cleaning (Folletti et al., 2014; Gonzalez et al., 2013; Vandenplas et al., 2014). The causative cleaning agents and the associated mechanisms have not been adequately identified. Spray products are considered potential candidates because they "may facilitate the inhalation of respiratory irritants and sensitizers and contribute to the burden of asthma" (Quirce and Barranco, 2010; Zock et al., 2010). Thus, users are exposed to the generated aerosols that contain all substances in the spray product (Zock et al., 2007).

Clausen et al. (2020a) identified only five studies about exposure measurements of spray cleaning and disinfection products used in workplace settings. These are Vincent et al. (1993), who studied exposure to 2-butoxyethanol from surface and glass cleaning sprays and measured concentrations from <480 to 35,400 μg/m^3; Garrod et al. (1998), who measured quaternary ammonium compounds (QACs) from remedial in-situ masonry treatment using a more industrial type of spraying and measured concentration from 130 to 12900 μg/m^3; Fedoruk et al. (2005) who measured ammonia from spray-on glass cleaners while washing several large windows in an office setting and measured concentrations around 450 μg/m^3; Vincent et al. (2007), who studied exposure to QACs during spray disinfection in a hospital, measured concentration <187 μg/m^3; and Gerster et al. (2014), who studied exposure to mono-ethanolamine, glycol ethers, and benzyl alcohol during professional indoor cleaning where spraying was only one of several cleaning activities. They measured concentrations of mono-ethanolamine, glycol ethers, and benzyl alcohol of 5–559 μg/m^3, 9–58700 μg/m^3; and 864–4300 μg/m^3, respectively. Furthermore, a study by Shin et al. (2020) used questionnaires on the number of times sprayed in combination with laboratory measurements of the mass

Abbreviations: AER, air exchange rate; ANOVA, analysis of variance; APS, aerodynamic particle sizer; ELPI, electrical low-pressure impactor; MGR, mass generation rate; NS, nano scan; OPS, optical particle sizer; QAC, quaternary ammonium compounds; TVOC, total volatile organic compounds; TPMC, total particle *mass* concentration.

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generation rates (MGRs) to estimate the exposure of the Korean population to i.a. spray cleaning and disinfection products. Few studies have dealt with exposure to spray cleaning and disinfection products in simulated exposure scenarios in climate chambers, gloveboxes, or simulated bathrooms, often combined with modeling. Bello et al. (2013) measured 2-butoxyethanol and TVOC (total volatile organic compounds) after spraying as a function of the room air exchange rate (AER), bathroom volume, type of cleaning task, and product type; Singer et al. (2006) measured 2-butoxyethanol and 2-hexyloxyethanol in a climate chamber after cleaning a tabletop with a trigger spray cleaning product followed by wiping; Rogers et al. (2005) simulated post-application inhalation levels of fragrances from a surrogate air freshener formulation expelled from a pressurized spray can in an environmentally controlled room; finally, Clausen et al. (2020b) studied the exposure concentrations of an aqueous solution of benzalkonium chloride after 6 s spraying in a climate chamber. A study of aerosols from pressurized spray cans containing nanoparticles (propellant driven) observed higher exposure concentrations of nanoparticles compared to aerosols from trigger sprays with nanoparticles (Lorenz et al., 2011). Park et al. (2017) made similar observations when comparing pressurized cans and trigger sprays. A Norwegian study (Olsen, 2017) measured aerosols from 17 products using seven different trigger spray cans in a bathroom-like location. Chen et al. (2010) studied nanoparticle release during the typical use of a single consumer spray bathroom cleaner in a well-controlled indoor environment.

It is not possible, based on these stand-alone studies, to draw general conclusions about inhalation exposure. Many fundamental issues related to the work process and associated exposure are still lacking. Thus, the aim of the present study was to answer four research questions not previously systematically investigated, but important for understanding inhalation exposure of aerosols from cleaning spray products, and identify issues that increase the exposure. Different spray cans and trigger sprays of professional cleaning and disinfection products were selected from the Danish market. Exposure scenarios in a controlled climate chamber were carried out to investigate: 1) differences in emission of spraying on vertically and horizontally oriented surfaces, 2) exposure differences when spraying on a cloth at a distance of 1 versus 10 cm followed by wiping a vertical surface, 3) how nozzle geometry of a trigger spray alters the formation of aerosols from the same product. Finally, 4) Consexpo modeling was compared with the measured aerosol data.

2. Materials and method

2.1. Selection of spray products

Products were selected from the inventory of 101 professional spray cleaning and disinfection products identified by Clausen et al. (2020a), which was based on searches in the Danish Product Registry. The Product Registry is a database with information on the content of chemicals in products and amounts sold in Denmark. The 101 products consisted of 72 trigger sprays, 24 pressurized can sprays, and 5 non-aerosol forming sprays (foam sprays); the most prevalent product types were glass cleaners, disinfectants, and sanitary/bathroom cleaners (Clausen et al., 2020a).

From the list of 101 spray cleaning and disinfection products, 19 were selected for pre-testing of their aerosol-releasing potential in a climate chamber (Table 1), however, products 11, 13, and 20 had been taken off the market by the producers and therefore were not available for testing, resulting in 19 selected spray products. Thus, the selection was based on product amount (largest quantities sold), spray type, product category, and content of chemicals that in the scientific literature have been suspected of causing asthma, as assessed by Clausen et al. (2020a) and Hadrup et al. (2022). These chemicals included enzymes, isothiazolinones, ethanolamines, morpholine, Bronopol, EDTA, QACs, chlorine (hypochlorite), hydrogen peroxide, ammonia, bases, volatile acids, semi-volatile acids, non-volatile acids, and VOCs.

Table 1
Product category, spray type, and particle release in the pre-test of 19 selected spray products.

Product#	Product category	Spray type	Maximum released number of particles	The 8 products selected for further experiments
1	Multipurpose	Pressurized can	++	*
2	Oven + grill	Pressurized can	+++++	*
3	Glass cleaning	Pressurized can	+++	*
4	Disinfectant	Pressurized can	+	
5	Disinfectant	Pressurized can	+++	*
6	Multipurpose	Pressurized can	++	
7	Disinfectant	Trigger	+	
8	Glass cleaning	Trigger	++	
9	Multipurpose + disinfectant	Trigger	+++++	
10	Glass cleaning	Trigger	++	
12	Glass cleaning	Trigger	+++	
14[a]	Disinfectant	Trigger	++	*
15	Sanitary + bathroom cleaner	Trigger	+	
16	Disinfectant	Trigger	+	
17	Glass cleaning	Trigger	+	*
18	Oven + grill	Trigger	++	
19	Oven + grill	Trigger	++++	*
21	Multipurpose	Trigger	+	
22	Glass cleaning	Trigger	++	*

[a] Reference spray cleaning product used in all further detailed experiments.

Based on the pre-test, eight products were selected for further experiments according to the four research questions. As shown in Table 1, the eight products represented a broad range of different types of products (category) and different spray types (trigger or pressurized can spray products).

2.2. Controlled climate test chamber

The experiments were conducted in a well-controlled steel chamber (Fig. 1) with dimensions 2.56 m × 3.46 m x 2.29 m (W x L x H), corresponding to a total volume of 20.3 m^3 at the average experimental conditions of 25.6 °C, 42% RH, and AER of 0.51 h^{-1} (full details in Table S1). The AER was selected using European building standards for ventilation in dwellings (Dimitroulopoulou, 2012). The conditions were monitored during each experiment. The air velocity in the chamber was continuously monitored by the airflow in the inlet tubes and electronically converted to air velocity in the chamber which is shown on a display. The air velocity was verified by the instant release of a tracer gas (isobutane) and following the decay by a photoionization detector. The chamber was cleaned before each test, first with 50/50% ethanol/water followed by water, and subsequently with 24 h ventilation.

2.3. Application of the spray products to the test surface in the climate chamber

A whiteboard (91 cm × 65 cm) was used as a spray target to simulate a hard and uniform surface. The board was placed vertically and the surface was parallel to the airflow in the chamber. Twelve spots were marked on the surface of the whiteboard (Fig. 1). Four spots were placed equidistantly (17 cm) in three rows each. The upper row was 145 cm above the floor. Products in pressurized cans were applied to the surface by continuous spraying for approximately 10 s from the top left spot

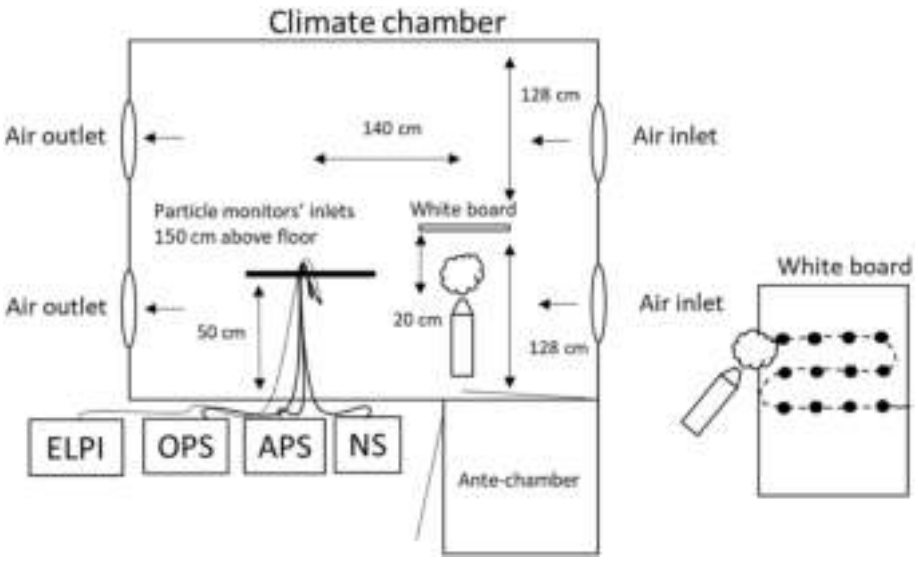

Fig. 1. Climate chamber and spray scenario setup. ELPI = Electrical Low-pressure Impactor, OPS = Optical Particle Sizer, APS = Aerodynamic Particle Sizer, NS = NanoScan (Scanning Mobility Particle Sizer).

through the twelve spots in a snake-like movement (Fig. 1). Products with trigger sprays were similarly applied for 10 s with one activation of the trigger at each of the twelve spots starting with the top left spot. The nozzle-to-surface distance was approximately 20 cm. The instructions for application given on the product containers were followed, e.g., finishing by wiping with a dry cloth or washing off with water. The whiteboard was cleaned before each test, first with 50/50% ethanol/water and subsequently with water.

2.4. Aerosol monitoring

Aerosol particle *number* and size distributions were measured with a 60 s time resolution using aerosol instrumentation covering sizes between 10 nm and 20 μm. An optical particle sizer (OPS) was used to detect particles between 0.3 and 10 μm (TSI model 3330, TSI Inc., Shoreview, MN, USA). Particles between 0.5 and 20 μm were measured using an aerodynamic particle sizer (APS) (TSI model 3321, TSI Inc., Shoreview, MN, USA); finally, a NanoScan (NS) was measuring particle mobility size distributions of particles between 0.01 and 0.42 μm (TSI NanoScan SMPS model 3910, TSI Inc., Shoreview, MN, USA). The inlets of the instruments were placed at the same location in the chamber 150 cm above the floor (Fig. 1). Background particle concentrations were determined before each spray experiment.

For estimation of aerodynamic particle size distributions, data from an Electrical Low-Pressure Impactor (ELPI) in normal mode with 14 size channels between 6 nm and 10 μm with 1 s intervals, was used (ELPI; Dekati model ELPI+/HR-ELPI+, Dekati Ltd., Tampere, Finland).

2.5. Pre-test of 19 products for further investigation

Of the 19 products identified (Table 1), eight products were selected for further investigation based on a pre-test of the aerosol release potential. Two experiments with different doses were carried out in the pre-test, "low dose" where the products were applied once, and "high dose" where the spray procedure was carried out in triplicate. Products with the highest resulting maximum particle *number* concentrations were selected for maximum data collection. In the initial experiments, Product#14 showed relatively high concentrations of particles in all three aerosol monitors and was therefore chosen as the reference product that would be used in all subsequent experiments.

2.6. Determination of mass generation rate

Each spray can was weighed using a Sartorius BP 610 scale (d = 0.01 g) before and after spraying. The spray time was determined using the stopwatch function in an iPhone 8. Spray time for pressurized spray cans was defined as the time for activation of the spray. For trigger sprays, spray time was considered as the total time for 12 activations of the trigger for the low dose and 36 times for the high dose; the time between each activation was considered negligible. The MGR (g/s) was calculated for all experiments as the mass of sprayed product divided by the spray time.

2.7. Gravimetric determination of the airborne fraction

A gravimetric method similar to a previously described method (Olsen, 2017) was used to estimate the airborne fraction. A piece of aluminum foil (w x h approximately 28 × 40 cm) was hung vertically on and covering the whiteboard (spray target). The aluminum foil was bent so it had a gutter at the bottom to collect the product that ran down after spraying. The spray products were applied as described above. The foil and the spray can was weighed before and after application of the product. The spray time, the amount of product collected on the foil, and the sprayed amount of product were noted. The difference between the sprayed amount of product and the remaining non-aerosolized amount of the product collected on the aluminum foil was assumed to be equal to the airborne amount. However, as discussed in Section 3.3, it appears that large particles sediments before they reach the aluminum foil target, and that evaporation from the aerosols takes place simultaneously. Both mass losses are erroneously included in the airborne fraction, which was then overestimated. The airborne fraction was calculated as the airborne amount relative to the sprayed amount in %. The experiments were repeated 3–5 times. Aerosol particle concentrations were not monitored during these experiments.

2.8. Testing of the effect of different nozzles on aerosol formation with the same product

To test the effect of nozzle geometry on aerosol formation Product#14 was transferred to five bottles with different trigger nozzles. Photos of the spray bottles are shown in Figs. S6–S11. The names, dealers, manufacturers, and/or countries are given to enable identification of the trigger sprays by an internet search. The nozzles were

named Nozzle#A (N#A, the original nozzle on Product#14 (a foam spray), N#B (Gloria Hobby 05), N#C (Kiehl-SanEco, empty trigger foam spray bottle), N#D (Industrial spray bottle with blue trigger (empty), 630 ml, NOWAS, Denmark), N#E (Mercury Pro 0.5 l (empty), Kwasar), and N#F (Vikan spray bottle 0.25 l (empty), Vikan A/S, Denmark). The product was sprayed in a pattern toward the whiteboard as described in section 2.3.

2.9. Testing of the effect on the aerosol formation when spraying on a vertical versus horizontal surface and on a cloth

The difference in particle concentrations by applying the product to a vertical versus a horizontal surface was investigated. One product with a trigger nozzle (Product#14) and one in a pressurized can (Product#1) were applied to the test surface placed both vertically and horizontally. The spraying was carried out as described for the low dose. Each experiment was repeated 2–6 times. For the horizontal scenarios, the test board was placed at a height of 90 cm (Fig. 1).

The difference in particle concentrations by spraying on a cloth compared to spraying directly on a hard surface (the whiteboard) was also investigated. However, these tests were made solely for Product#14.

In Denmark, if a spray product cannot be avoided, it is recommended by cleaning companies to spray into a cloth and then use the cloth to wipe the surface to be cleaned. Thus, additional experiments were performed to measure the effect of the distance between the nozzle and the cloth. Product#14 and Nozzle#F were chosen for these experiments since it was one of three nozzles (#B, #E, and #F) that released the highest *mass* concentration of aerosols. Spraying was carried out at two distances to the cloth: 1 cm and 10 cm, respectively. The standard test setup with spraying on the vertical whiteboard at 20 cm was used as a reference. The experiments were carried out in triplicate.

2.10. First order decay model

A 1st order decay model is described by the following equation:

$$C = C_0 \cdot \exp(-k \cdot t),$$

where C = concentration at time t ($\mu g/m^3$), C_0 = initial concentration ($\mu g/m^3$), k = 1st order decay constant (h^{-1}) and t = time (h).

To obtain a 1st order decay equation where C is a function of the *half-life time* ($t_{½}$) of the particle *mass* concentrations, the following equations were used and rearranged:

$$t_{½} = \ln(2)/k <=> k = \ln(2)/t_{½}$$

$$C = C_0 \cdot \exp(-(\ln(2)/t_{½}) \cdot t)$$

To obtain $t_{½}$ this expression was fitted to the total particle *mass* concentration (TPMC) versus time data using the non-linear regression function in Minitab® 20.

2.11. ConsExpo modeling

ConsExpo Web (modification date 2021/10/26) was used to model the experimental spray scenarios, i.e. instant release of the aerosol from the spray can and decay of the aerosol concentration. The input parameters were based on the experimental data and using the recorded spray time and calculated MGR. The airborne fraction was based on the measured particle peak *mass* concentration of the individual particle monitors. The geometric mean diameter (GMD) of the particles and the arithmetic coefficient of variation was based on fitting a lognormal distribution to the measured particle size distributions. It should be noted that particle size distributions in ConsExpo are 'initial' distributions, see Discussion.

2.12. Data treatment and statistics

The programing language for statistical computing, R, was used to treat data from the aerosol particle measurements to visualize the particle size distributions $dN/d\log D_p$ over time. $dN/d\log D_p$ data was extracted directly from each instrument and plotted using the computer program packages tidyr, dplyr, and plotly. From the measurements, particle *mass* distributions, based on the measured *number* concentrations, were calculated according to the formula:

$$dM/d\log D_{p,i}\ (\mu g/m^3) = (\pi/6)\rho D_{p,i}^3 (10^6 dN/d\log D_{p,i})$$

Where ρ (= 1 g/cm^3) is the assumed density of the particles and $D_{p,i}$ (m) is the geometric mean diameter of particle size bin *i*. Finally, $dN/d\log D_{p,i}$ was measured by the instruments ($\#/cm^3$).

Total particle *number* and *mass* concentrations were found by multiplying each particle size bin with the corresponding $d\log D_p$ for both $dN/d\log D_p$ and $dM/d\log D_p$ and then summing across size bins.

The measured particle concentrations were corrected by subtracting background concentrations, which in some cases resulted in negative concentrations since the background concentration was high. These data were omitted from statistical analysis. The 10-min background was measured immediately prior to spraying.

Average and peak total *mass* concentrations and average and peak total *number* concentrations were used to compare experiments. For OPS and APS the 10-min averages were calculated from t = 0 min (start of spraying) to t = 10 min. OPS and APS measured aerosol concentration peaks typically about 1–2 min after the start of spraying. For NS the aerosol concentration typically peaked later than for OPS and APS; however, we have no explanation for this observation. In case of a late peak, the 10-min average was calculated at ± 5 min around the peak. All concentrations were normalized to the amount of sprayed product before statistical analysis.

Heatmaps with a wide size-range of particles were formed by combining mobility, optical, and aerodynamic particle size distributions measured by NS (10–300 nm), OPS (30–10000 nm), and APS (10–20 μm), respectively. For comparison, it was assumed that particle mobility, optical, and aerodynamic diameters were equivalent even though the optical diameter may differ from mobility and aerodynamic diameters depending on the particle shape, refractive index, and size.

Excel 2016 was used for further data calculations including the calculation of MGRs and airborne fractions, and further statistical tests were performed using Minitab®20.

3. Results and discussion

3.1. Mass generation rates

MGRs were within 0.5–3.1 g/s (Fig. 2). The mean of all MGRs of the 19 products are 1.6 g/s for low dose and 1.7 g/s for high dose and the values are not significantly different (p = 0.40). There was less variation in the MGRs for high doses, especially for pressurized spray cans, and this might be due to a relatively more accurate timing when spray time is longer. The average spray time for low doses was 8.7 s and 17.2 s for high doses. Typically, the MGRs decrease as a function of the number of times the spray can has been used.

Comparison of MGRs of pressurized spray cans and trigger sprays at low and high doses with a one-way ANOVA shows no significant difference (p = 0.73). The mean MGR values of low dose from pressurized spray cans and trigger sprays were 1.56 g/s and 1.63 g/s, respectively, and for high dose, it was 1.81 g/s and 1.67 g/s, respectively. Two-sample t-tests showed no significant difference between pressurized spray cans and trigger sprays. Despite the large uncertainty in our mean values of MGRs, they agree well with the default value of 1.6 g/s set for all-purpose and glass cleaner trigger sprays in the ConsExpo exposure model (Meesters et al., 2018).

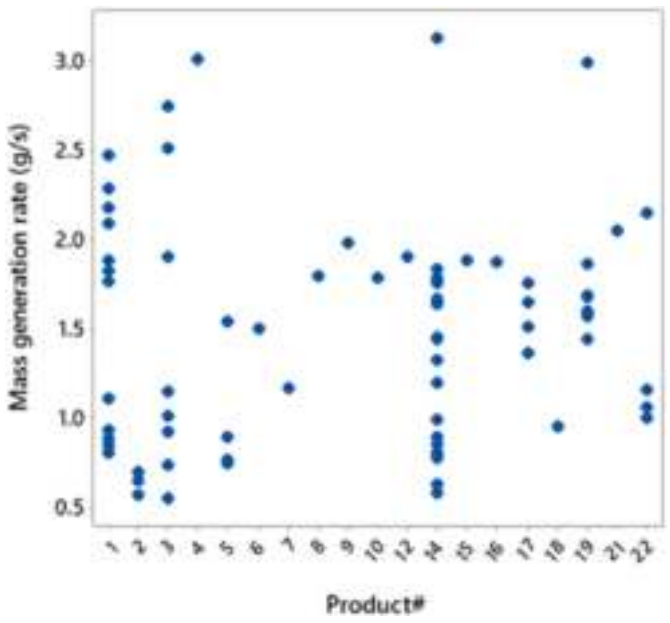

Fig. 2. Individual value plot of all measured mass generation rates (MGRs) at low doses of 19 spray cleaning and disinfection products.

For a variety of 23 different pressurized cans and trigger spray products, Delmaar and Bremmer (2009) found an average MGR of 1.25 g/s for full spray cans and 0.83 g/s for nearly empty spray cans within the ranges of 0.48–4.30 g/s and 0.29–1.60 g/s, respectively. This is similar to the variation observed in the present study as shown in Fig. 2. Furthermore, the spray cans were only used a few times so they were never empty.

3.2. First order decay modeling of the released aerosols from 19 products

The *half-life times* of all 19 products were estimated to be between 0.07 and 0.7 h (Fig. 3). The first order decay model fits well with most of the experimental data. An example of a fitted curve is shown in Fig. S1.

The average TPMC *half-life time* of the 6 pressurized spray cans was twice that of the 13 trigger sprays, 0.41 h versus 0.20 h for OPS data and 0.59 h versus 0.27 for APS data. Two-sample t-tests showed a significant difference between *half-life times* of pressurized spray cans and trigger sprays for OPS ($p = 0.002$) and APS ($p = 0.004$). NS data have insufficient number of values for a *t*-test.

The average total particle *number* concentration *half-life time* of the 6 pressurized spray cans was three times that of the 13 trigger sprays, 1.1 h versus 0.37 h for OPS, 1.2 h versus 0.42 for APS, and 1.0 versus 0.74 for NS. Two-sample t-tests showed significant difference between *half-life times* of pressurized spray cans and trigger sprays for OPS ($p = 0.019$) but not for APS ($p = 0.088$) and NS ($p = 0.50$). The larger half-life time of the *number* concentration was expected since the terminal settling velocity is proportional to the square of the particle diameter (Hinds, 1999). This causes faster removal of the large particles, which means a faster decrease of the TPMC because the large particles carry the largest mass. The particle size distribution over time of the mass concentrations was calculated for all spray products and these show consistently that the mass concentration of large particles decreased significantly after 1 h. This is illustrated by the example in Fig. 4. Particle size distributions over time for all products are shown in Fig. S2.

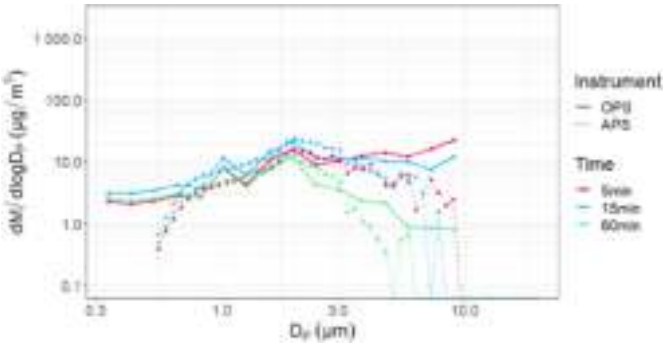

Fig. 4. Particle size distribution over time of the mass concentration of Product#5. The particle size distribution at 5 min, 15 min, and 60 min is plotted for both OPS and APS.

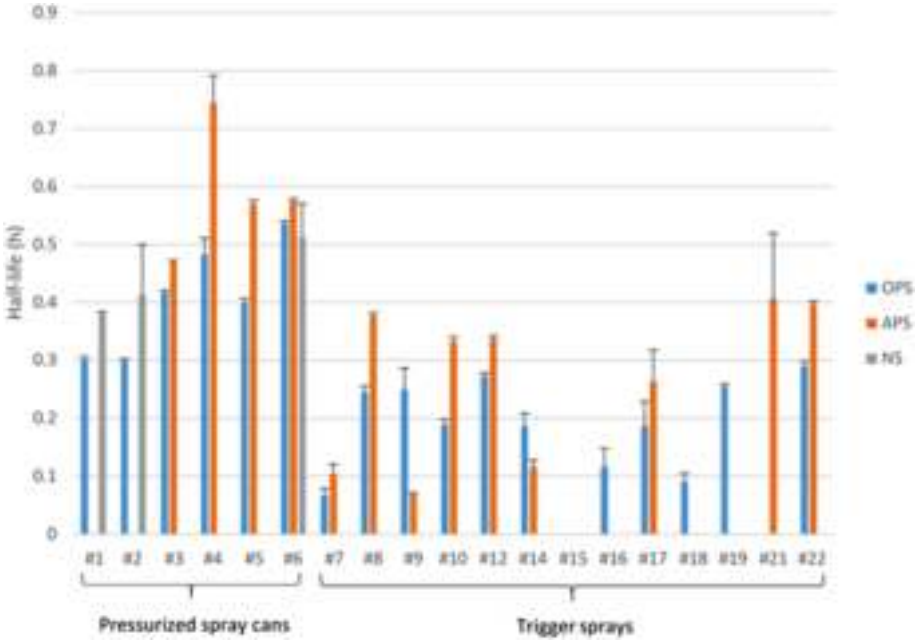

Fig. 3. *Half-life times* of the decay of the total particle *mass* concentrations released by 19 spray cleaning products, six pressurized spray cans (Product#1–6), and 13 trigger sprays (Product#7–22). Product# is shown on the x-axis. Error bars are the standard error of mean (SEM) of the *half-life times*.

The doubled *half-life time* for pressurized spray cans compared to trigger sprays is probably due to a larger fraction of small particles. The heatmap for particle *number* concentration shown in Fig. S4 shows that all pressurized spray cans showed a tendency to a larger fraction of small particles. An assumed higher pressure in pressurized spray cans than in trigger sprays is believed to result in a larger fraction of small particles from the pressurized cans since droplet size decreases with higher spraying pressure (Wolf and Bretthauer, 2009). Comparison of normalized average and peak total *number* concentrations from the pre-tests showed that only for OPS average and peak *number* concentrations, pressurized spray cans released significantly more particles than trigger sprays ($p = 0.024$ and $p = 0.026$, respectively). APS data showed the same tendency but not NS data. It is a reasonable assumption that these excess particles are smaller since the MGRs of pressurized spray cans and trigger sprays do not differ significantly (see section 3.1). It has previously been observed that trigger sprays tend to release larger particles than pressurized spray cans (Delmaar and Bremmer, 2009) and that the median aerosol diameters ranged from about 70 μm up to well over 100 μm for trigger sprays. A large fraction of larger particles in the aerosols would be expected to result in a larger 1st order rate constant (k), but a fit of k versus the geometric mean diameter of the particles (Table S11) provided meaningless results (see discussion in Section 3.6).

3.3. Airborne fraction

The gravimetrically measured airborne fractions showed large variation as also observed for MGRs (see Fig. S5). The data showed that pressurized spray cans tend to release higher airborne fractions. A comparison of the airborne fractions showed that the pressurized spray cans on average released 15.6% versus 6.5% for the trigger sprays; however, the difference was not significant ($p = 0.092$). This is in line with previous observations that airborne fractions are largest for sprays that produce small particles (Delmaar and Bremmer, 2009). Higher airborne fractions (4–20%) for trigger sprays measured with a similar gravimetric method have previously been published (Olsen, 2017).

The tendency to generally higher airborne fractions from the pressurized spray cans may be explained by finer aerosols, which are slower to deposit on the aluminum foil that was used to collect the fraction that was not airborne, than larger droplet aerosols formed by trigger sprays. A larger fraction of small particles in aerosols from pressurized spray cans is also supported by their longer *half-life times*, see Section 3.2. Further, very large particles were visually observed from trigger sprays, and they may be gravitationally settled before they reach the aluminum foil; thus, they are counted as airborne fraction though they are settled fast. Consequently, the measured airborne fraction will be larger than it actually was since the very large particles are counted as airborne though they are settled fast. Furthermore, large particles/droplets (>20 μm) cannot be measured by APS.

When the aerosol is sprayed out of the spray can, fast evaporation from the aerosol particles may be expected (Losert et al., 2014; Xie et al., 2007). If all liquids are evaporated from the aerosol before it reaches the aerosol monitors, only the non-volatile constituents of the product remain as aerosols. This remaining aerosol of non-volatile constituents may constitute the smallest peak *mass* concentration of particles expected to be measured if no particles escape the aerosol monitors. An equation for calculating this minimum concentration (C_{min}) has been derived and presented in Supplementary Material. C_{min} was based on content information obtained from the Danish Product Registry (Clausen et al., 2020a). We found that C_{min} for the 19 pre-tested products was approximately 40 times higher than the measured particle peak *mass* concentration for pressurized spray cans and around 100 times for trigger sprays. Thus, the aerosol monitors did not measure the major fraction of the sprayed products. Probably, the evaporation is effectively instantaneous from the particles which have a huge surface area (Xie et al., 2007). Based on calculations, it has previously been assumed that this is the case for compounds as volatile as water (Delmaar and Bremmer, 2009). Most of the investigated products in the present study contain more than 99% water. The particles that survive in the air are probably in equilibrium with the gas-phase and constitute the measured particles. Large droplets (>20 μm) may partly explain the higher factor for trigger sprays. Despite a generally higher airborne fraction for pressurized spray cans, the calculated minimum particle concentrations are closer to the measured particle peak *mass* concentrations than it is for trigger sprays. Based on these observations, it is obvious that the gravimetrically determined airborne fractions (Fig. S5) are overestimated in comparison to the measured particle peak *mass* concentrations. This is partly because the gravimetrically measured airborne fraction constitutes both fast-settled particles, airborne particles, and gasses.

Airborne fractions based on measured peak *mass* concentrations (Σ(OPS + NS) 0.01–10 μm) were on average 0.012% for pressurized spray cans and 0.0054% for trigger sprays, though not significantly different ($p = 0.31$). It would have been better to use the APS data since they cover particles up to 20 μm, but analysis was not possible due to missing values.

Since C_{min} is much higher than the actual measured particle peak *mass* concentrations it must be due to either large particles >20 μm or very small particles <0.01 μm, which are not measured by the particle monitors, and evaporation of liquids from the particles, mainly water. However, it is highly unlikely that the very small particles contribute significantly to the 'missing' mass because of their extremely small mass even if they are numerous. Since C_{min} is much higher than the measured particle peak *mass* concentrations, the non-volatile constituents simply 'disappear' because it is unlikely that large droplets would account for up to 99% of the 'missing' mass.

3.4. The effect on the aerosol formation of different nozzles with the same product

ANOVA of both the mean MGRs and masses sprayed for all six nozzles (N#A – N#F) showed a large within-nozzle variation, but not a significant between-nozzle variation (Tables S2 and S3). Thus, a direct comparison of the nozzle variations was possible. However, all following data analysis was carried out using normalized concentration data. There was a large variation in the measured MGRs. One reason can be that the first use of the spray product has a higher MGR than in the following uses. We propose this is caused by a partial blocking/coating of the nozzle by residues of the product, which we observed for some of the nozzles.

The original nozzle (N#A) and the five other nozzles have qualitatively comparable size distributions based on the 10-min average *mass* concentrations (Fig. 5A). As expected the large particles dominate the mass distributions because most of the mass remains in the large particles. However, N#B, N#E, and N#F have the highest and the original nozzle (N#A1), N#C, and N#D the lowest concentrations. This is consistent with N#A and N#C being foam sprays, which are expected to release low concentrations of aerosols. In the size distribution of the 10-min average *number* concentrations, small particles dominate, as expected (Fig. 5B). It shows that the two entries for N#A have very different concentrations, that N#B has the lowest and N#C – N#F have the highest concentrations. The first entry N#A1, N#C, and N#E have the highest concentration of small particles. N#A1 in Fig. 5A shows release of the lowest particle mass, which was, however, not reflected by the MGRs (Table S2). It might be the case that the original nozzle releases a smaller measurable airborne fraction than the other nozzles, e.g., due to the generation of large particles >20 μm that are not measured by APS.

One-way ANOVA of OPS, APS, and NS 10-min-average and peak *mass* and *number* concentrations (Table S4) was also used for quantitative evaluation of the nozzle experiments. One-way ANOVA of OPS and APS average and peak *mass* concentrations shows significant difference between the six different nozzles. The omission of the N#A in the one-

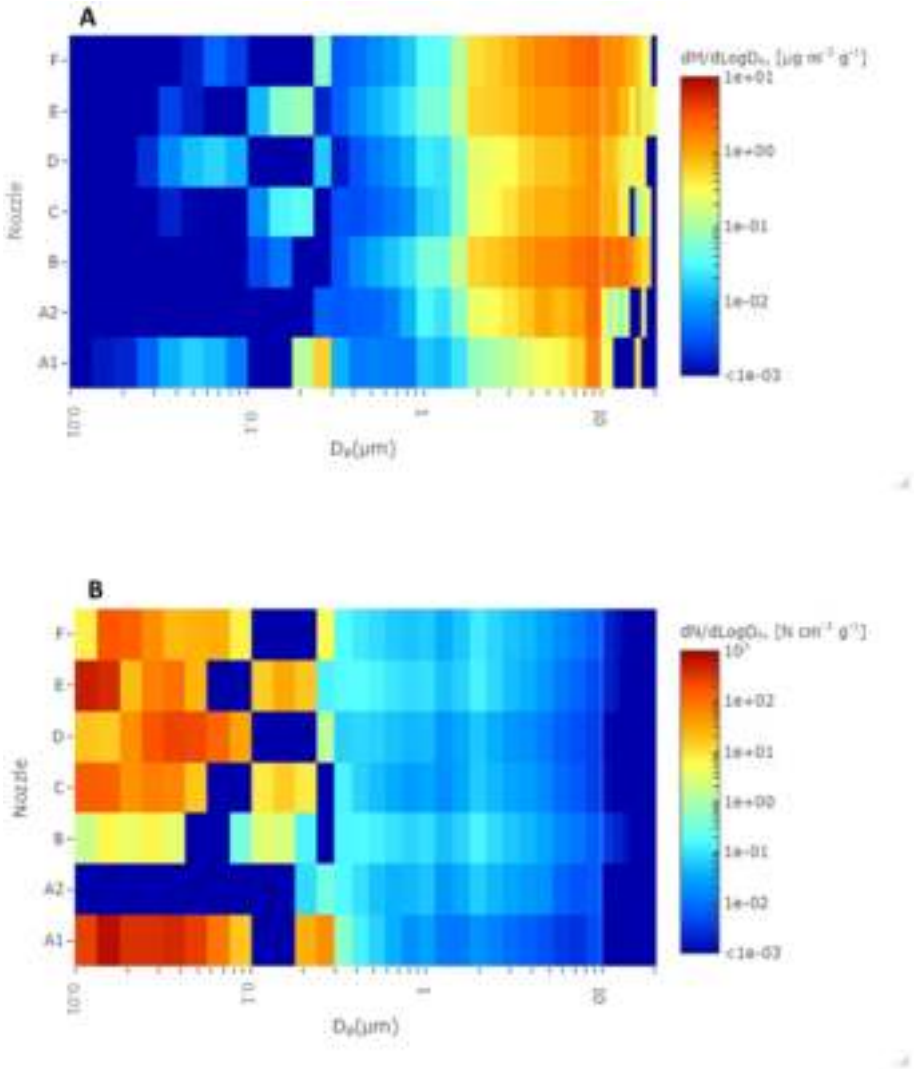

Fig. 5. Heatmap of 10-min average of NS, OPS, and APS normalized *mass* (A) and *number* (B) concentration particle size distributions of the 6 different nozzles sprayed with the same product (Product#14). The two entries for the original nozzle are for two different bottles (showing differences in particle distributions for the same product and nozzle).

way ANOVA still shows significant differences between the five other nozzles. OPS data showed that N#A released a peak *mass* concentration of 0.4 times and N#B 1.5 times the average *mass* concentration of the remaining four nozzles. For APS data, it was 0.1 and 1.8 times, respectively.

These results imply that a change of nozzle can reduce exposure to aerosols. Thus, new nozzles should be developed not only to optimize for the specific cleaning task but also for the reduction of generated aerosols.

3.5. The effect on aerosol formation when spraying on a vertical versus horizontal surface and spraying on a cloth

One-way ANOVA of MGRs and absolute amounts sprayed for Product#14 showed significant differences (Tables S5 and S6). For Product#1 two-sample *t*-test showed that they did not differ significantly ($p = 0.12$ and 0.10, respectively). However, further data analysis was carried out using concentration data normalized to the sprayed mass of the product.

The individual results of Product#14 and Product#1 for average and peak *mass* and *number* concentrations showed large variations. One-way ANOVA showed no significant differences between vertical, horizontal, and cloth spraying for Product#14 (Table S7). Two-sample *t*-test showed no significant difference between vertical and horizontal spraying for Product#1 (Table S8).

Additional experiments were carried out since the originally designed experiments did not show any significant differences. Since cleaning companies in Denmark recommend spraying in a cloth if a spray product cannot be avoided the additional experiments were designed to answer the question: 'How close should the nozzle be to the cloth to significantly reduce the release of the aerosols compared to spraying on a vertical surface?' Nozzle#F was chosen for the experiments since it was one of three nozzles (N#B, N#E, and N#F), which released the highest *mass* concentration of aerosols. Product#14 was filled in the bottle with N#F. Spraying on a cloth was performed at two distances, 10 cm and 1 cm, respectively, and compared to the standard spray scenario i.e., spraying on a vertical surface at a distance of 20 cm.

The aerosol release at the 10 cm and 1 cm distance from a cloth compared to spraying at a vertical surface at a distance of 20 cm were significantly different by ANOVA (Table S9); the 1-cm distance released the lowest amount of aerosols. The aerosol release was also in some cases at the 10 cm distance less than spraying on a vertical surface at 20 cm. Spraying at 10 cm and 1 cm distance to a cloth resulted on average in a total peak *mass* concentration of 0.8 and 0.1 times, respectively, that

of the normal scenario of spraying on a vertical surface at 20 cm.

3.6. ConsExpo modeling

The applied input parameters are shown in Tables S10 and S11. The model only predicts well with airborne fractions based on measured particle peak *mass* concentrations. The experimental peak concentrations of the total particle *mass* concentrations were well predicted by ConsExpo (Fig. 6). This is probably not surprising since a simple equation, which is presented in the Supplementary Material, can be used to calculate the particle peak *mass* concentration.

Although the peak *mass* concentrations were well predicted by ConsExpo, this was not the case for the decay of the aerosol concentrations. Roughly, half of the predictions of the OPS decays and 1/7th of the APS decays were well predicted by ConsExpo. NS decays were not predicted due to insufficient data. A well-predicted decay is illustrated in Fig. 7. The reason why only few predictions describe the aerosol decay adequately could be a poor estimation of the geometric mean diameter (GMD) of the particles and the arithmetic coefficient of variation of the particle size distributions. GMD of the measured aerosols probably differ from the 'initial' particles right outside the spray nozzle, because the aerosol monitor inlets were placed 140 cm from the spray nozzle (Fig. 1), cf. Losert et al. (2014). Since ConsExpo requires the 'initial' particle diameter this may explain why only few ConsExpo modelings were successful.

It has previously been found that the use of adequate input parameters is a prerequisite to obtaining good model results with ConsExpo (Clausen et al., 2020b); however, the measured air concentrations of the active substances in biocide sprays were underestimated (Clausen et al., 2020b; Delmaar and Meesters, 2020). Delmaar and Meesters (2020) concluded that the discrepancy between ConsExpo and measured concentrations generally is within a factor of two and that this is due to both simplifications of the model and poor input parameters such as the particle size distribution.

4. Conclusions

The average mass generation rates of six pressurized spray cans and 13 trigger sprays were not significantly different and were about 1.7 ± 0.3 g/s (std.dev.)

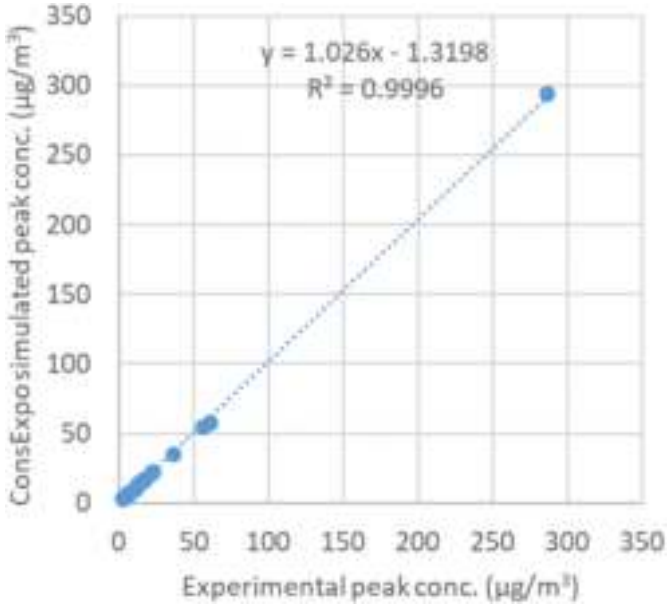

Fig. 6. Comparison of ConsExpo simulated particle peak concentrations with measured OPS particle peak concentrations.

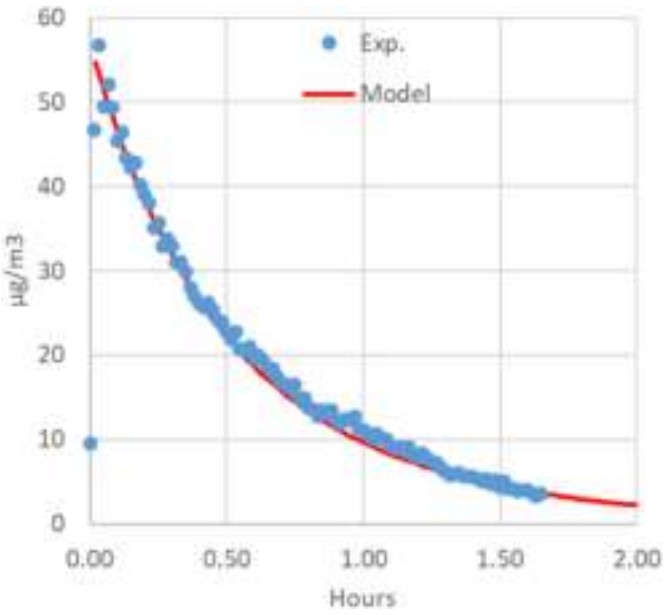

Fig. 7. Comparison of experimental OPS data for total particle *mass* concentrations with ConsExpo predicted decay. The data is from the pre-test of Product#3.

The average *half-life time* of the total particle *mass* concentration (TPMC) of the six pressurized spray cans was twice that of the 13 trigger sprays, approximately 0.5 h versus 0.25 h. This was most likely due to a larger fraction of fine particles from the pressurized spray cans. The implication of the doubled particle mass-based *half-life time* for pressurized spray cans is that it requires twice as long time to ventilate a room after spraying compared to trigger sprays.

The gravimetrically determined airborne fractions of pressurized spray cans showed a tendency to be higher than for trigger sprays. On average, the airborne fractions of the pressurized spray cans were 15.6% versus 6.5% for the trigger sprays, but they do not differ significantly. However, it turned out that they were greatly overestimated and that airborne fractions based on TPMC were a better estimate and were on average only 0.012% for pressurized spray cans and 0.0054% for trigger sprays, though not significantly different.

A comparison of six different trigger spray nozzles with the same product showed that the original nozzle (N#A) released a lower and another nozzle (N#B) a higher TPMC than the remaining four nozzles (which also showed significant but smaller differences). OPS data showed that N#A released a peak TPMC of 0.4 times and N#B 1.5 times the average TPMC of the remaining four nozzles. For APS data it was 0.1 and 1.8 times, respectively. The implication is that the nozzle geometry is critical in decreasing the exposure.

Spraying at a 10-cm and 1-cm distance on a cloth resulted in a peak TPMC of 0.8 times and 0.1 times, respectively, that of the normal scenario of spraying on a vertical surface at a distance of 20 cm. This information is relevant since cleaning companies in Denmark recommend, spraying into a cloth followed by wiping the surface to be cleaned, if a spray product cannot be avoided.

Modeling of the particle concentrations using ConsExpo showed that the measured peak *mass* concentrations were well predicted with good input parameters, but the decay was less well. This may be due to poor estimation of the (time-dependent) particle size distribution.

Author responsibilities

The manuscript was written through contributions from all authors. All authors have given approval to the final version of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2023.114220.

References

Bello, A., Quinn, M.M., Milton, D.K., Perry, M.J., 2013. Determinants of exposure to 2-butoxyethanol from cleaning tasks: a Quasi-experimental study. Ann. Occup. Hyg. 57, 125–135.

Chen, B.T., Afshari, A., Stone, S., Jackson, M., Schwegler-Berry, D., Frazer, D.G., Castranova, V., Thomas, T.A., 2010. Nanoparticles-containing spray can aerosol: characterization, exposure assessment, and generator design. Inhal. Toxicol. 22, 1072–1082.

Clausen, P.A., Frederiksen, M., Sejbæk, C.S., Sørli, J.B., Hougaard, K.S., Frydendall, K.B., Carøe, T.K., Flachs, E.M., Meyer, H.W., Schlünssen, V., Wolkoff, P., 2020a. Chemicals inhaled from spray cleaning and disinfection products and their respiratory effects. A comprehensive review. Int. J. Hyg Environ. Health 229, 113592.

Clausen, P.A., Mørck, T.A., Jensen, A.C.Ø., Schou, T.W., Kofoed-Sørensen, V., Koponen, I. K., Frederiksen, M., Detmar, A., Fink, M., Nørgaard, A.W., Wolkoff, P., 2020b. Biocidal spray product exposure: measured gas, particle and surface concentrations compared with spray model simulations. J. Occup. Environ. Hyg. 17, 15–29.

Delmaar, C., Meesters, J., 2020. Modeling consumer exposure to spray products: an evaluation of the ConsExpo Web and ConsExpo nano models with experimental data. J. Expo. Sci. Environ. Epidemiol.

Delmaar, J.E., Bremmer, H.J., 2009. The ConsExpo Spray Model - Modelling and Experimental Validation of the Inhalation Exposure of Consumers to Aerosols from Spray Cans and Trigger Sprays, pp. 1–70. RIVM Report 320104005/2009.

Dimitroulopoulou, C., 2012. Ventilation in European dwellings: a review. Build. Environ. 47, 109–125.

Fedoruk, M.J., Bronstein, R., Kerger, B.D., 2005. Ammonia exposure and hazard assessment for selected household cleaning product uses. J. Expo. Anal. Environ. Epidemiol. 15, 534.

Folletti, I., Zock, J.P., Moscato, G., Siracusa, A., 2014. Asthma and rhinitis in cleaning workers: a systematic review of epidemiological studies. J. Asthma 51, 18–28.

Garrod, A.N.I., Rimmer, D.A., Robertshaw, L., Jones, T., 1998. Occupational exposure through spraying remedial pesticides. Ann. Occup. Hyg. 42, 159–165.

Gerster, F.M., Hopf, N.B., Wild, P.P., Vernez, D., 2014. Airborne exposures to monoethanolamine, glycol ethers, and benzyl alcohol during professional cleaning: a pilot study. Ann. Occup. Hyg. 58, 846–859.

Gonzalez, M., Jégu, J., Kopferschmitt, M.C., Donnay, C., Hedelin, G., Matzinger, F., Velten, M., Guilloux, L., Cantineau, A., de Blay, F., 2013. Asthma among workers in healthcare settings: role of disinfection with quaternary ammonium compounds. Clin. Exp. Allergy 44, 393–406.

Hadrup, N., Frederiksen, M., Wedeby, E.B., Nikolov, N.G., Carøe, T.K., Sørli, J.B., Frydendall, K.B., Liguori, B., Sajbaek, C.S., Wolkoff, P., Flachs, E.M., Schlûnssen, V., Meyer, H., Clausen, P.A., Hougaard, K.S., 2022. Asthma-inducing potential of 28 substances in spray cleaning products – assessed by quantitative structure activity relationship (QSAR) testing and literature review. J. Appl. Toxicol. 42, 130–153.

Hinds, W.C., 1999. Aerosol Technology: Properties, Behavior, and Measurements of Airborne Particles. John Wiley & Sons, INC., New York.

Lorenz, C., Hagendorfer, H., von Goetz, N., Kaegi, R., Gehrig, R., Ulrich, A., Scheringer, M., Hungerbühler, K., 2011. Nanosized aerosols from consumer sprays: experimental analysis and exposure modeling for four commercial products. J. Nanoparticle Res. 13, 3377–3391.

Losert, S., von Goetz, N., Bekker, C., Fransman, W., Wijnhoven, S.W.P., Delmaar, C., Hungerbuhler, K., Ulrich, A., 2014. Human exposure to conventional and nanoparticle-containing sprays – critical review. Environ. Sci. Technol. 48, 5366–5378.

Meesters, J., Nijkamp, M., Schuur, A., te Biesebeek, J., 2018. Cleaning Products Fact Sheet: Default Parameters for Estimating Consumer Exposure-Updated Version 2018, pp. 1–248.

Olsen, R., 2017. Rengjøringsmidler i sprayform. STAMI-rapport Årgang 18, 1–34.

Park, J., Ham, S., Jang, M., Lee, J., Kim, S., Kim, S., Lee, K., Park, D., Kwon, J., Kim, H., 2017. Spatial–Temporal dispersion of aerosolized nanoparticles during the use of consumer spray products and estimates of inhalation exposure. Environ. Sci. Technol. 51, 7624–7638.

Quirce, S., Barranco, P., 2010. Cleaning agents and asthma. J. Invest. Allergol. Clin. Immunol. 20, 542–550.

Rogers, R.E., Isola, D.A., Jeng, C.J., Lefebvre, A., Smith, L.W., 2005. Simulated inhalation levels of fragrance materials in a surrogate air freshener formulation. Environ. Sci. Technol. 39, 7810–7816.

Shin, H., Jang, Y., Lim, M., Park, J.Y., Yang, W., Lee, K., 2020. Characteristics of exposure factors and inhalation exposure to selected spray consumer products in Korean population. Regul. Toxicol. Pharmacol. 110, 104513.

Singer, B.C., Destaillats, H., Hodgson, A.T., Nazaroff, W.W., 2006. Cleaning products and air fresheners: emissions and resulting concentrations of glycol ethers and terpenoids. Indoor Air 16, 179–191.

Vandenplas, O., Wiszniewska, M., Raulf, M., de Blay, F., Gerth van Wijk, R., Moscato, G., Nemery, B., Pala, G., Quirce, S., Sastre, J., Schlünssen, V., Sigsgaard, T., Siracusa, A., Tarlo, S.M., van Kampen, V., Zock, J.P., Walusiak-Skorupa, J., 2014. EAACI position paper: irritant-induced asthma. Allergy 69, 1141–1153.

Vincent, G., Kopferschmitt-Kubler, M., Mirabel, P., Pauli, G., Millet, M., 2007. Sampling and analysis of quaternary ammonium compounds (QACs) traces in indoor atmosphere. Environ. Monit. Assess. 133, 25–30.

Vincent, R., Cicolella, A., Subra, I., Rieger, B., Poirot, P., Pierre, F., 1993. Occupational exposure to 2-butoxyethanol for workers using window cleaning agents. Appl. Occup. Environ. Hyg 8, 580–586.

Wolf, R.E., Bretthauer, S., 2009. Droplet Size Calibration: A New Approach to Effective Spraying. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, pp. 1–4.

Xie, X., Li, Y., Chwang, A., Ho, P., Seto, W.J.I.a., 2007. How Far Droplets Can Move in Indoor Environments-Rrevisiting the Wells Evaporation-Falling Curve, vol. 17, pp. 211–225.

Zock, J.-P., Plana, E., Jarvis, D., Antó, J.M., Kromhout, H., Kennedy, S.M., Künzli, N., Villani, S., Olivieri, M., Torén, K., Radon, K., Sunyer, J., Dahlman-Hoglund, A., Norbäck, D., Kogevinas, M., 2007. The Use of Household Cleaning Sprays and Adult Asthma, vol. 176, pp. 735–741.

Zock, J.P., Vizcaya, D., Le Moual, N., 2010. Update on asthma and cleaners. Curr. Opin. Allergy Clin. Immunol. 10, 114–120.

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Exposure to perfluoroalkyl substances (PFAS) and association with thyroid hormones in adolescent males

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ABSTRACT

Background: Perfluoroalkyl substances (PFAS) are found in a wide range of consumer products. Exposure to PFAS in children and adolescents may be associated with alterations in thyroid hormones, which have critical roles in brain function.

Objective: This study investigated the association between plasma concentrations of PFAS and serum levels of total triiodothyronine (T3), free thyroxine (T4), and thyroid-stimulating hormone (TSH) in adolescent males.

Methods: In 2017–2019, 151 boys from the Environment and Childhood (INMA)-Granada birth cohort, Spain, participated in a clinical follow up visit at the age of 15–17 years. Plasma concentrations of ten PFAS (PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFOS, and PFHxS) and serum thyroid hormones were measured in 129 of these boys. Linear regression analysis was performed to determine associations of individual PFAS with total T3, free T4, TSH, and free T4/TSH ratio, and quantile g-computation models were performed to assess the mixture effect. Additional models considered iodine status as effect modifier.

Results: PFOS was the most abundant PFAS in plasma (median = 2.22 μg/L), followed by PFOA (median = 1.00 μg/L), PFNA (median = 0.41 μg/L), and PFHxS (median = 0.40 μg/L). When adjusted by confounders (including age, maternal schooling, and fish intake), PFOA and PFUnDA were associated with an increase in free T4 (β [95% CI] = 0.72 [0.06; 1.38] and 0.36 [0.04; 0.68] pmol/L, respectively, per two-fold increase in plasma concentrations), with no change in TSH. PFOS, the sum of PFOA, PFNA, PFOS, and PFHxS, and the sum of long-chain PFAS were marginally associated with increases in free T4. Associations with higher free T4 and/or total T3 were seen for several PFAS in boys with lower iodine intake (<108 μ/day) alone. Moreover, the PFAS mixture was association with an increase in free T4 levels in boys with lower iodine intake (% change [95% CI] = 6.47 [–0.69; 14.11] per each quartile increase in the mixture concentration).

Conclusions: Exposure to PFAS, considered individually or as a mixture, was associated with an increase in free T4 levels in boys with lower iodine intake. However, given the small sample size, the extent of these alterations remains uncertain.

1. Introduction

Perfluoroalkyl substances (PFAS) are man-made chemicals used in a wide range of commercial and industrial products, including fire-fighting foams, semiconductors, water- and oil-repellent textiles, leather, food contact materials, cosmetics, medical devices, biocides, pharmaceuticals, and paints (Glüge et al., 2020). Food, contaminated drinking water, and indoor air and dust are major routes of exposure to

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PFAS in the general population (Cornelis et al., 2012; EFSA, 2020; Haug et al., 2011). Factors associated with internal levels of PFAS such as perfluorooctane sulfonate (PFOS) and perfluorononanoic acid (PFNA) in children include the intake of fast foods and snacks (Halldorsson et al., 2008; Wu et al., 2015) and seafood (Dassuncao et al., 2018), the frequency of wearing waterproof clothing (Wu et al., 2015), and the presence of PFAS in residential dust (Wu et al., 2015). PFAS are highly persistent in the environment and have been detected in adults and children from various countries (Bartolomé et al., 2017; Calafat et al., 2007; Kannan et al., 2004; Lewis al., 2015). The half-life of PFAS in human serum can vary widely, being 3.5 and 4.8 years, respectively, for perfluorooctanoic acid (PFOA) and PFOS, and up to 7.3 years for perfluorohexane sulfonate (PFHxS) (Olsen and Zobel, 2007). In 2009, PFOS was added to the Stockholm Convention, a global treaty to eliminate or restrict the use of several persistent organic pollutants (Stockholm Convention, 2017), PFOA was added to the Convention in 2019 (Regulation (EU) 2019/1021) but had been phased out in the European Union (EU) since 2008 (Commission Regulation (EU) 2017/1000). Thus, the use of PFOA is banned in the EU and the use of PFOS is only allowed for a few applications. Other PFAS such as PFHxS remain under review by the European Chemicals Agency (ECHA, 2019).

Both long (≥8 carbon compounds) and short (<8 carbon compounds) chain PFAS may act as endocrine disrupting chemicals (Diamanti-Kandarakis et al., 2009; Gore, 2016), and some are also suspected of disrupting thyroid hormone homeostasis (Coperchini et al., 2017, 2021). Thyroid homeostasis is controlled by the hypothalamus-pituitary-thyroid axis feedback mechanism, which depends on interactions among thyroid stimulating hormone (TSH), thyroxin (T4), and triiodothyronine (T3). These hormones play critical roles in regulating different physiological functions, including metabolism, circadian rhythm, reproductive function, and fetal and child nervous system development (Zoeller, 2007). Various epidemiological studies have assessed the association between PFAS exposure and thyroid hormone levels in adults, reporting varied results and suggesting that associations are dependent on the compound, dose, age, and sex (Blake et al., 2018; Bloom et al., 2010; Byrne et al., 2018; Ji et al., 2012; Kim et al., 2018; Knox et al., 2011; Lewis et al., 2015; Melzer et al., 2010). However, there has been only limited research in children and adolescents, who are more sensitive than adults to thyroid-disrupting chemicals because their bodies and brains are still developing (Lanphear, 2015). In this way, Lin et al. (2013) found a positive association of serum PFNA concentrations with free T4 levels but not with TSH in adolescents and young Taiwanese adults. Likewise, Lopez-Espinosa et al. (2012) described slight positive associations of serum PFNA, PFOA, and PFOS with total T4 in children living near a petrochemical plant in the USA. In the same line, Caron-Beaudoin et al. (2019) observed that high serum PFNA concentrations were positively associated with free T4 levels but not TSH in children and adolescents from a Native Community in Quebec. Finally, analysis of data from the U.S. National Health and Nutrition Examination Survey (NHANES) revealed positive associations of serum PFNA and PFOS with TSH in male adolescents and a negative association between PFOA and TSH in female adolescents (Lewis et al., 2015). Overall, the role of PFAS as thyroid disrupting chemicals remains controversial, and there has been no consistent evidence of an association between a given PFAS and human thyroid function (Coperchini et al., 2021).

As part of the European Human Biomonitoring Initiative (HBM4EU), serum concentrations of 12 PFAS were measured in adolescents (12–18 years) from nine countries, including Spain, in 2014–2021 (Richterová et al., 2023). Around 14% of the adolescents exceeded the EFSA health-based guidance value of 6.9 μg/L for the total serum concentration of PFOS, PFOA, PFNA, and PFHxS with significantly higher PFAS concentrations in boys *versus* girls and in subjects from Northern and Western Europe (Richterová et al., 2023). The aim of the present study was to assess the relationship of plasma concentrations of ten PFAS, including long and short chain compounds, with serum levels of total T3, free T4, and TSH in adolescent Spanish males aged 15–17 years.

2. Material and methods

2.1. Study population

The Environment and Childhood (INMA) Project is a multicenter population-based birth cohort study designed to investigate the effect of environmental exposures and diet during pregnancy on fetal and child development in different parts of Spain (Guxens et al., 2012). The INMA-Granada birth cohort was established between 2000 and 2002 by recruiting 668 mother-son pairs at delivery in Granada province, Spain (Fernandez et al., 2007). Randomly selected pairs from the baseline cohort were contacted to request their participation in different clinical follow-ups at 4–5 (N = 220, 32.9%) and 9–11 years (N = 300, 44.9%). Those who attended both follow-up sessions (N = 269) were re-contacted and asked to participate in the most recent follow-up at the age of 15–17 years (2017–2019), from which 151 agreed to participate and underwent physical examination (Castiello et al., 2020). The follow-up visit included anthropometric and pubertal measures, questionnaire completion, and the collection of a non-fasting blood sample, which was obtained from 135 (89%) of the boys. Whole venous blood was collected from participants between 17:00 and 20:00 h and processed to obtain plasma and serum samples, which were stored at −80 °C until delivery to the *Instituto de Investigación Biosanitaria de Granada* (ibs.Granada), Granada, Spain, for analyses of PFAS exposure and thyroid hormones. The present study was conducted in 129 of the boys with available data on plasma PFAS and thyroid hormone levels. Further details on study participation were previously described (Castiello et al., 2020; Suárez et al., 2021). The parents of all participants signed informed consent, and the study protocol was approved by the Biomedical Research Ethics Committee of Granada. No significant differences in general characteristics were observed between boys included (N = 129) and not included (N = 26) in the present study (Supplementary material, Table S1).

2.2. Laboratory analysis

A previously described methodology based on salt-assisted liquid-liquid extraction (SALLE) and dispersive liquid-liquid microextraction (DLLME) (Vela-Soria et al., 2020) was used to measure plasma levels of three short chain PFAS (perfluorohexanoic acid [PFHxA], perfluoroheptanoic acid [PFHpA], and PFHxS) and seven long chain PFAS (PFOA, PFOS, PFNA, perfluorodecanoic acid [(PFDA], perfluoroundecanoic acid [PFUnDA], perfluorododecanoic acid [PFDoDA], and perfluorotridecanoic acid [PFTrDA]). After placing 1 mL of plasma sample in a polypropylene centrifuge tube, 1 mL Milli-Q water and 5.0 mL of acetonitrile were added, and the mixture was vortexed for 30 s. SALLE was then performed by adding a salt mixture of 600 mg NaCl, 200 mg disodium hydrogen citrate, and 200 mg trisodium citrate, followed by manual agitation for 60 s and centrifugation at 4000 rpm for 10 min. The supernatant was transferred into a glass 7-mL vial, concentrated to 1 mL under nitrogen stream, and poured into a 15-mL screw cap glass test tube. The sample was then prepared for DLLME by adding 10 mL of 10% NaCl aqueous solution (w/v) at pH 2. Next, 1500 μL of trichloromethane was rapidly injected by syringe, and the mixture was gently shaken for 40 s and then centrifuged for 5 min at 4000 rpm. The entire sedimented phase volume was transferred into a clean glass vial, the organic phase was evaporated under a nitrogen stream, and the residue was dissolved with 100 μL of a mixture of 5 mM ammonium acetate (pH 4.5) and acetonitrile 30:70 (v/v) and then vortexed for 30 s, thereby preparing the sample for injection into the high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) system. HPLC-MS/MS analysis was performed with a NexeraXR LC-20A liquid chromatography system (Shimadztu, Japan) and 4500 QTRAP MS/MS4500 mass spectrometer (ABSciex, USA). A

Gemini C18 column (100 mm × 2 mm i.d., 3-μm particle) from Phenomenex (Torrance, CA, USA) and a gradient mobile phase consisting of 5 mM ammonium acetate aqueous solution with pH of 4.5 (solvent A) and acetonitrile (solvent B) were used for the chromatography. Compounds were determined in negative ion mode. The tandem mass spectrometer was operated in selected reaction monitoring mode. The limits of detection (LOD) and quantification (LOQ) were 0.02 μg/L and 0.05 μg/L, respectively, for all PFAS.

Serum thyroid hormone measurements were performed by electrochemiluminescence immunoassay using a Roche® kit (Elecsys System, Roche Diagnostics). The ratio of FT4 to TSH was calculated by dividing FT4 (pmol/L) by TSH (mU/mL) as a marker of the negative feedback control mechanism of the hypothalamus-pituitary-thyroid axis. Limits of detection were 0.005 μIU/mL for TSH, 0.5 pmol/L for free T4, and 0.300 nmol/L for total T3. Age-specific laboratory reference ranges (11–18 years old) were 10.29–24.45 pmol/L for free T4; 1.23–3.23 nmol/L for total T3; and 0.32–3.0 μIU/mL for TSH (Iwaku et al., 2013).

2.3. Covariate data

The weight, height, and waist circumference of the boys were measured following standardized procedures (Castiello et al., 2020). Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2) and converted to age-specific z-scores based on World Health Organization growth reference standards for children aged 5–19 years (de Onis et al., 2007), classifying the boys as underweight (<-1 standard deviation [SD]), normal weight (±1 SD), overweight (>+1 SD, equivalent to BMI≥25 kg/m^2 at 19 years), or obese (>+2 SD; equivalent to BMI≥30 kg/m^2 at 19 years). Weight status was finally categorized into underweight/normal weight or overweight/-obese. The waist-to-height ratio was also calculated by dividing waist circumference (cm) by height (cm), and abdominal obesity was defined as waist-to-height ratio≥0.5 (Browning et al., 2010). Tanner stages of genital development (G) and pubic hair growth (PH) were assessed by a pediatric endocrinologist and categorized as reaching sexual maturity (Tanner G = 5, Tanner PH = 5) or no (Castiello et al., 2023). Information was also obtained from questionnaire responses on the following covariates: maternal schooling (primary [from age of 6–12 years], secondary, or university), and child passive smoking (living with a smoker, yes or no), alcohol intake (never; 1 beverage per month; 2 beverages per month; and ≥3 beverages per month), total fish intake (g/day), iodine intake (μg/day), history of physician-diagnosed thyroid disease (yes or no), and current or recent (<12 months) medication use. Information on fish intake was gathered from a validated semiquantitative food frequency questionnaire (FFQ) completed by the adolescents, who were asked to report the average frequency of consumption for the specified serving or portion size for each food item of the FFQ, based on the previous year (Notario-Barandiaran et al., 2020, 2021). To estimate iodine intake, the published food composition tables of the US Department of Agriculture (USDA) and other published sources for specific Spanish food and portion sizes were used. Average daily iodine intake was estimated by multiplying the frequency of consumption of each food item by iodine composition of the serving size specified in the FFQ and summed the results for all foods (Notario-Barandiaran et al., 2020).

2.4. Statistical analysis

Concentrations of PFAS below the LOD were assigned a value of LD/$\sqrt{2}$ (Croghan and Egeghy, 2003). The total concentration of all PFAS analyzed (∑PFAS), the most abundant PFAS commonly found in human blood samples (∑4 PFAS=PFOA + PFOS + PFNA + PFHxS) (EFSA, 2020), long chain PFAS (∑LC PFAS), and short chain PFAS (∑SC PFAS) were calculated as the sum of molar concentrations of the compounds based on molecular weight and were expressed as PFOA (or PFHxS in the case of ∑SC PFAS).

Data were missing on passive smoking for two participants, on alcohol, fish, and iodine intake for four, and on maternal schooling for eight. Missing data were imputed by using the mode for categorical covariates (passive smoking, alcohol intake, and maternal schooling) and the median for continuous variables (fish and iodine intake). The association between the concentrations of each PFAS and each thyroid parameter (total T3, free T4, TSH, and free T4/TSH) was assessed using linear regression analysis. Concentrations of PFAS, TSH, and free T4/TSH ratio showed a non-normal distribution and were natural log-transformed (ln) before inclusion in models. Total T3 and free T4 were normally distributed and modeled untransformed.

Confounders were selected a priori among the above-reported covariates using a directed acyclic graph (DAG) (Textor et al., 2011) (Figure S1) that included adolescent age (in years, continuous), maternal schooling (primary, secondary, or university), and fish intake (ln-transformed). Models were adjusted for fish intake as a relevant source of both PFAS exposure and iodine (Carlsen et al., 2018; Menzel et al., 2021), which is in turn essential for thyroid hormone biosynthesis. Further regression analyses were conducted by categorizing PFAS exposure in tertiles. All linear regression models were checked for normality and homoscedasticity of residuals, and outliers. Regression coefficients of models of ln(PFAS) on total T3 or free T4 were transformed to represent the average change in total T3 or free T4 associated with a two-fold increase in the concentration of each PFAS compound or group. For TSH and free T4/TSH, regression coefficients were transformed to represent the percentage change (%) in TSH or free T4/TSH associated with a two-fold increase in the concentration of each PFAS compound or group, calculated as the complement of the exponentiated regression coefficient [(expβ-1) x 100]. Results of models based on PFAS tertiles are expressed as the difference in hormone level or the percentage change relative to the first tertile of PFAS exposure.

Quantile g-computation was used to assess the combined effect of PFAS on thyroid hormones. Quantile g-computation estimates the joint effect using a parametric generalized linear model that simultaneously increases all exposures by one tertile (Keil et al., 2020). Advantages of this method include the possibility of assessing individual exposure-effect relationships within the mixture in opposite directions, producing an unbiased estimate of the overall joint effect, and its usefulness for small sample sizes (Eick et al., 2021; Keil et al., 2020). The functioning of quantile g-computation is based on the categorization of urinary biomarkers of exposure to phenols, metals, and pesticides into quartiles. Each biomarker is given a negative or positive weight. If the individual compound shows a different direction of the effect, the weight is interpreted as the proportion of the partial effect in a negative or positive direction.

We examined the potential effect modification by iodine intake through stratification of single-exposure and mixture effect models by using the median of iodine intake (*i.e.*, 108 μg/day). Finally, associations were examined after excluding boys who had ever been diagnosed with thyroid disease (N = 3 hyperthyroidism; N = 2 hypothyroidism) and/or received thyroid medication (N = 1), finding no major differences (results not shown). The significance level was established at 0.05. SPSS version 28 (IBM, Chicago, IL) and R version 4.1.2, package "qgcomp" (https://cran.r-project.org/web/packages/qgcomp/index.html) were used for statistical analyses.

3. Results

Participants had a mean age of 16.2 years; 38% had mothers with primary schooling, 70% resided in an urban area, 42% were passive smokers, 12% consumed at least 2 alcoholic beverages per month; 28% had overweight or obesity, and 23% had a weight-to-height ratio ≥0.50. All boys were in pubertal stage 4 or 5, 45% were in stage G5 and 61% in stage PH5. Average fish and iodine intakes were 79.8 g/day and 104.6 μg/day, respectively (Table 1).

All plasma samples had detectable concentrations of PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFOS, and PFHxS, while PFDoDA was below the

Table 1
Characteristics of 129 adolescents from the INMA-Granada cohort.

Variables	n (%) or mean ± SD
Adolescent age (years)	16.2 ± 0.42
Maternal schooling	
Primary	49 (38.0)
Secondary	48 (37.2)
University	32 (24.8)
Passive smoking	54 (41.9)
Alcohol intake	50 (36.8)
Never	79 (61.2)
1 beverage/month	35 (27.1)
2 beverage/month	7 (5.4)
≥3 beverage/month	8 (6.2)
Weight status[a]	
Underweight/normal weight	93 (72.1)
Overweight/obese	36 (27.9)
Waist-to-height ratio≥0.50	30 (23.3)
Puberty development	
Stage G5	58 (45.0)
Stage PH5	79 (61.2)
Total fish intake (g/day)	79.8 ± 83.7
Iodine intake (μg/day)	140.6 ± 102.9

[a] Based on age-specific z-scores based on World Health Organization growth reference standards.

LOD in 10.7% of samples, and PFHxA and PFTrDA was below in 13.8% of samples (Table 2). PFOS was the PFAS with the highest concentration (median = 2.224 μg/L), which was more than two-fold higher than the concentration of PFOA (median = 0.997 μg/L) and five-fold higher than the concentrations of PFNA and PFHxS (median = 0.408 and 0.398 μg/L, respectively). There was a higher total concentration of LC PFAS than of SC PFAS (median = 3.93 *vs.* 1.04 μg/L). In general, positive correlations were observed between PFAS; however, PFHxA was only weakly correlated with PFOA and PFUnDA and was inversely correlated with PFTrDA (Fig. 1). Median total T3 and free T4 values were 2.15 nmol/L and 16.20 pmol/L, respectively, and median TSH and free T4/TSH ratio values were 1.84 mU/mL and 9.06, respectively. None of the participants had total T3, free T4, or TSH levels outside the age-specific pediatric range (Table 3).

Table 2
Plasma concentrations of PFAS (μg/L) in 129 adolescent males.

PFAS		%> LD	25th percentile	Median	75th percentile
PFHxA	Perfluorohexanoic acid	86.2	0.046	0.110	0.230
PFHpA	Perfluroheptanoic acid	100	0.084	0.139	0.236
PFOA	Perfluorooctanoic acid	100	0.805	0.997	1.338
PFNA	Perfluorononanoic acid	100	0.271	0.408	0.715
PFDA	Perfluorodecanoic acid	100	0.170	0.218	0.267
PFUnDA	Perfluoroundecanoic acid	100	0.134	0.250	0.382
PFDoDA	Perfluorododecanoic acid	89.3	0.043	0.061	0.089
PFTrDA	Perfluorotridecanoic acid	86.3	0.040	0.069	0.119
PFOS	Perfluorohexane sulfonic acid	100	1.626	2.224	3.079
PFHxS	Perfluorooctane sulfonic acid	100	0.244	0.398	0.819
Sum of PFAS[a]					
∑4 PFAS[b]	PFOA + PFOS + PFNA + PFHxS	–	3.238	4.316	5.765
∑SC PFAS[c]	PFHxA + PFHpA + PFHxS	–	0.603	1.040	1.782
∑LC PFAS[d]	PFOA + PFOS + PFNA + PFDA + PFUnA + PFDoA + PFTrDA	–	3.145	3.926	5.224
∑PFAS	Sum of all 10 PFAS	–	4.028	5.263	7.171

LD: Limit of detection.
[a] Sum of the molar concentrations of PFAS based on molecular weight and expressed as PFOA (or PFHxS in the case of ∑SC PFAS).
[b] Most abundant PFAS in human serum (EFSA, 2020).
[c] Long-chain PFAS.
[d] Short-chain PFAS.

In single-exposure models, PFOA and PFUnDA were associated with slight increases in free T4 (β [95% CI] = 0.72 [0.06; 1.38] and 0.36 [0.04; 0.68] pmol/L, respectively, per two-fold increase in plasma concentrations). In addition, PFOS, the sum of PFOA, PFNA, PFOS, and PFHxS (∑4 PFAS), and the sum of LC PFAS were marginally associated with increases in free T4 (β [95% CI] = 0.42 [–0.08; 0.93], 0.54 [–0.03; 1.13], and 0.57 [–0.09; 1.23] pmol/L, respectively) (Table 4). No association was observed for total T3 or TSH (Table 4). Stratification by iodine status revealed that associations were mostly significant in boys with iodine intake <108 μ/day (Table S2). Thus, PFOA and ∑4 PFAS were associated with a slight increase in total T3, and PFNA, PFUnDA, ∑4 PFAS, and ∑LC PFAS with an increase in free T4 in boys with lower iodine intake alone (*e.g.*, free T4 increased by 1.09 and 0.95 pmol/L, respectively, per two-fold increase in ∑4 PFAS and ∑LC PFAS) (Table S2).

Analysis based on PFAS tertiles did not show a clear trend towards higher thyroid hormone levels with increasing PFAS exposure. Nonetheless, second-tertile concentrations of PFOA and ∑LC PFAS were associated with a slight increase in total T3 (β [95% CI] = 0.17 [0.03; 0.32] and 0.16 [0.02; 0.29], respectively); second tertile of ∑4 PFAS with an increase in free T4 (β [95% CI] = 1.07 [0.13; 2.01]); third tertiles of PFOA and PFHxS with an increase in free T4; and third tertile of PFHxS with a decrease in TSH (Table S3). Analysis of residuals and outliers indicated that linear regression assumptions are met (data not shown).

In the quantile g-computation model, the mixture was not associated with thyroid parameters when considering the total sample of boys (Table 5 and Fig. 1). However, after stratification by iodine intake, there was a suggestive positive association between the PFAS mixture and free T4 levels in boys with lower iodine intake (% change [95% CI] = 6.47 [–0.69; 14.11] per each quartile increase in the mixture concentration) (Table 5), with PFTrDA, PFNA, PFOS, and PFHxS contributing most to this effect (Fig. 2).

4. Discussion

This cross-sectional study on the association of plasma PFAS concentrations with thyroid hormone parameters in adolescent males found that higher PFOA, PFUnDA, PFOS, ∑4 PFAS, and ∑LC PFAS concentrations were associated with a slight increase in free T4 that ranged from 0.36 to 0.72 pmol/L per two-fold increase in plasma concentrations. In addition, it was observed that these and other PFAS biomarkers, including PFNA and PFHxS, and the PFAS mixture were positively associated with free T4 and/or total T3 in boys with lower iodine intake alone. These results suggest that exposure to environmentally relevant concentrations of PFAS may be associated with subtle alterations in the thyroid hormone levels of adolescents, notably an increase in free T4, and that this association is modified by their iodine status. Thyroid hormone levels were within normal reference ranges and may not be of clinical significance. Nevertheless, the thyroid-disrupting effects of PFAS in adolescents may have a significant impact on their health, given the major role played by thyroid hormones in growth, metabolism, and brain maturation and in behavior and mood (Anderson, 2001; Bauer et al., 2003).

PFOS was the most abundant PFAS in the present plasma samples, and concentrations were in the range recently reported in sera from European teenagers (median = 2.22 μg/L in plasma *vs.* geometric mean [GM] = 2.13 μg/L in serum) (Richterová et al., 2023). Plasma PFOA, PFNA, and PFHxS concentrations were also similar to those observed in

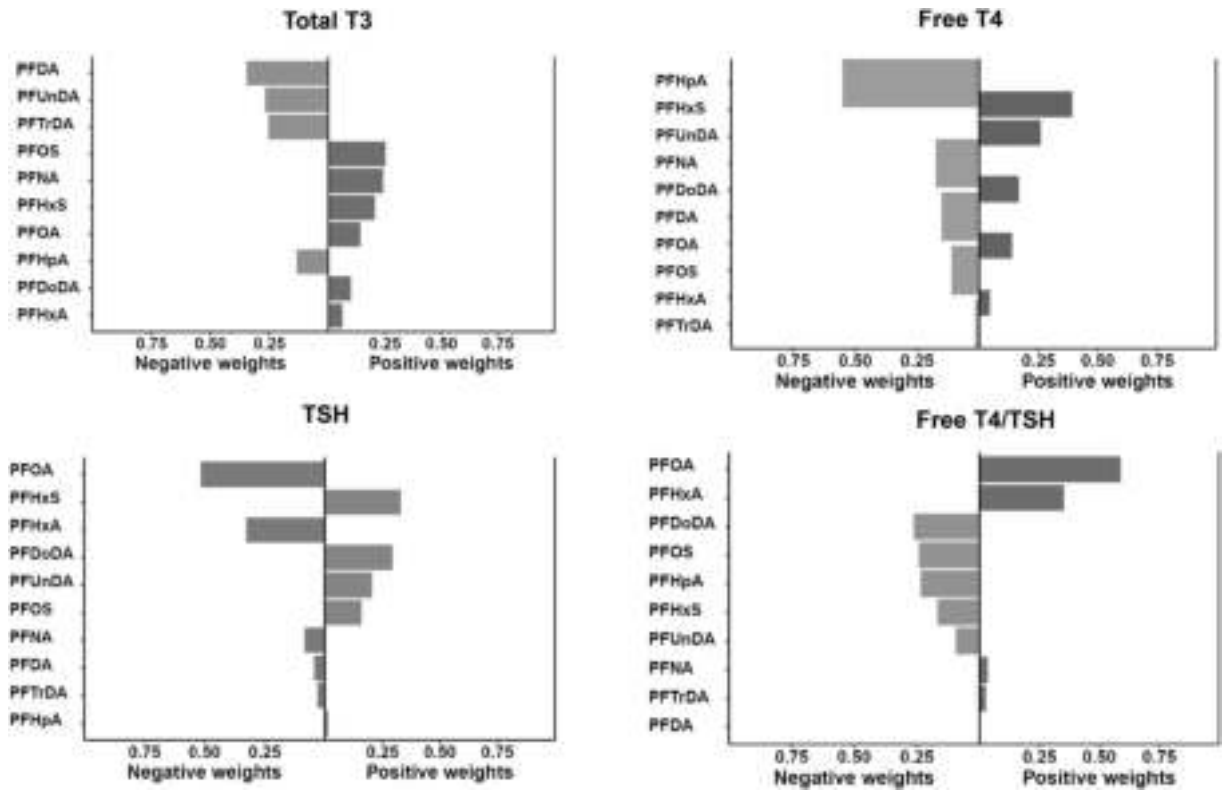


Fig. 1. G-computation models for the mixture effect of PFAS on thyroid parameters among adolescent males (N = 129). Dark-colored bars refer to chemicals with an effect in the same direction to the overall effect. Grey-colored bars refer to chemicals with an effect in the opposite direction to the overall effect.

Table 3
Serum thyroid parameters.

Thyroid hormones	Median	5th-95th percentile	Range
Total T3 (nmol/L)	2.15	1.61–2.73	1.48–3.00
Free T4 (pmol/L)	16.20	13.10–19.62	7.8–22.11
TSH (μIU/mL)	1.84	0.81–3.89	0.63–4.69
Free T4/TSH	9.06	4.23–21.15	3.51–32.6

Reference values (11–18 years): Total T3: 1.23–3.23 nmol/L; free T4: 10.29–24.45 pmol/L; TSH: 0.6–5.8 μIU/mL.

the teenagers (GM = 0.97, 0.30, and 0.41 μg/L in serum, respectively) (Richterová et al., 2023). However, plasma concentrations of PFOA, PFNA, PFDA, PFOS, and PFHxS were around two-fold lower than observed in serum samples collected from Spanish adults in 2009–2010 (Bartolomé et al., 2017), suggesting a decline in PFAS exposure over the past decade due to their regulation in the EU and other countries. Overall, the present data corroborate recent findings from the HBM4EU Project indicating generalized exposure to legacy and newer PFAS among European teenagers.

Epidemiological studies have shown that exposure to individual PFAS has varied effects on thyroid hormones, with differences in their magnitude and direction. However, studies in children and adolescents provide some evidence of a positive association between serum PFNA and higher total or free T4 levels (Caron-Beaudoin et al., 2019; Lin et al., 2013; Lopez-Espinosa et al., 2012), and this association was stronger in males in two of these studies (Caron-Beaudoin et al., 2019; Lin et al., 2013). In the present sample of boys, PFNA was not associated with thyroid hormones in the main analysis, but stratification by iodine intake revealed a significant positive association between PFNA and free T4 and a suggestive positive association with total T3 in subjects with lower iodine intake. Despite the between-study variations in the age of participants, which was 12–30 years in Lin et al. (2013), 1–17 years in Lopez-Espinosa et al. (2012), and 6–19 years in Caron-Beaudoin et al. (2019), our findings are in partial agreement with these results. The associations of PFOS and PFOA with higher free T4 (and higher total T3 for second-tertile PFOA) observed in the main analysis are in line with the positive association of serum PFOA and PFOS with total T4 reported by Lopez-Espinosa et al. (2012) in a large sample of children living near a Teflon manufacturing facility exposed to high PFOA levels. Strikingly, PFOA and PFOS levels in North American children were nearly 30- and 10-fold higher, respectively, than in the present boys. However, no association was found between PFOA or PFOS and T4 or T3 levels in other studies of children or adolescents (Caron-Beaudoin et al., 2019; Lewis et al., 2015; Lin et al., 2013). To our knowledge, this is the first report that links PFUnDA, a 11-carbon compound that has been used as an alternative to the 8-carbon compounds PFOS and PFOA in the fluoropolymer industry, to thyroid function in children or adolescents. Several studies in pregnant women reported inverse association between maternal PFUnDA and thyroid hormones (Boesen et al., 2020), but findings in children are not comparable to those in pregnant women. In the present study, the increase in free T4 or total T3 was not accompanied by a decrease in TSH, and previous studies in children have also failed to find an association between PFAS exposure and TSH levels (Caron-Beaudoin et al., 2019; Lopez-Espinosa et al., 2012; Lewis et al., 2015; Lin et al., 2013). There was a suggestive inverse association of TSH with PFHxA, and suggestive positive associations with PFHxS and $\sum$4 PFAS in boys with higher iodine intake, but these results may be spurious and should be taken with caution.

Altered thyroid hormone levels following prenatal and postnatal exposure to PFAS have been found in experimental animal studies (Coperchini et al., 2021). For instance, increased T4 and T3 levels were observed in zebrafish larvae after exposure to PFDoDA (Zhang et al., 2018), while serum levels of PFAS, particularly PFOS, were significantly higher in hyperthyroid *versus* non-hyperthyroid cats (D Wang et al., 2018a,b). *In vitro* models reported several effects of legacy and newer PFAS on thyroid function, including cytotoxicity and genotoxicity in thyroid cells and interference with thyroid hormone synthesis, thyroid

Table 4
Single-exposure models[a] for the association between plasma concentrations of PFAS and thyroid parameters.

PFAS	Total T3	Free T4	TSH	Free T4/TSH
	β (95% CI)	β (95% CI)	% change (95% CI)	% change (95% CI)
PFHxA	−0.00 (−0.03; 0.03)	0.06 (−0.15; 0.27)	−2.72 (−7.31; 1.39)	3.51 (−0.69; 7.89)
PFHpA	−0.01 (−0.06; 0.03)	−0.14 (−0.46; 0.17)	0.00 (−6.67; 6.41)	−0.69 (−7.31; 6.41)
PFOA	0.06 (−0.03; 0.15)	**0.72 (0.06; 1.38)****	−5.37 (−17.57; 7.89)	10.90 (−2.72; 27.32)
PFNA	0.02 (−0.03; 0.08)	0.18 (−0.21; 0.57)	2.80 (−4.72; 11.67)	−1.37 (−9.21; 6.41)
PFDA	0.04 (−0.04; 0.12)	−0.08 (−0.66; 0.50)	3.51 (−7.95; 16.39)	−3.39 (−14.67; 8.63)
PFUnDA	0.00 (−0.04; 0.05)	**0.36 (0.04; 0.68)****	2.09 (−4.72; 9.38)	0.00 (−6.02; 7.14)
PFDoDA	0.00 (−0.05; 0.05)	0.01 (−0.28; 0.40)	4.95 (−2.05; 12.45)	−4.06 (−10.45; 2.80)
PFTrDA	−0.01 (−0.05; 0.03)	−0.08 (−0.37; 0.20)	2.09 (−3.39; 8.63)	−2.72 (−8.58; 3.51)
PFOS	0.03 (−0.04; 0.10)	**0.42 (-0.08; 0.93)***	3.51 (−6.67; 14.80)	−0.69 (−11.07; 10.14)
PFHxS	0.03 (−0.01; 0.07)	0.13 (−0.14; 0.41)	4.23 (−1.37; 10.90)	−3.39 (−9.21; 2.09)
∑4 PFAS	0.06 (−0.02; 0.14)	**0.54 (-0.03; 1.13)***	4.23 (−7.31; 18.01)	−0.69 (−12.29; 11.67)
∑SC PFAS	0.02 (−0.03; 0.07)	0.01 (−0.35; 0.36)	0.69 (−6.02; 8.63)	−0.69 (−7.95; 6.41)
∑LC PFAS	0.04 (−0.05; 0.14)	**0.57 (-0.09; 1.23)***	2.09 (−11.07; 17.20)	2.09 (−11.68; 17.20)
∑PFAS	0.05 (−0.04; 0.14)	0.46 (−0.17; 1.08)	2.09 (−10.45; 16.39)	0.69 (−11.68; 15.59)

Models are adjusted by adolescent age (years), maternal schooling (primary, secondary, or university), and (log) total fish intake.
Regression estimates were transformed to represent the average change (total T3, free T4) or percentage change (TSH, free T4/TSH) in thyroid parameter associated with a two-fold increase in PFAS concentration.
**p < 0.05; *p < 0.10.
[a] Each PFAS compound or group was separately modeled with each thyroid parameter.

Table 5
Plasma PFAS mixture effect on thyroid parameters.

Thyroid parameters	% change	95% CI
Total sample (N = 129)		
Total T3	2.02	−3.93; 7.25
Free T4	2.02	−2.96; 7.25
TSH	−0.99	−15.64; 16.18
Free T4/TSH	3.05	−12.19; 22.14
Iodine intake <108 μg/L (N = 64)		
Total T3	4.29	−3.62; 12.91
Free T4	**6.47**	**−0.69; 14.11***
TSH	2.78	−19.21; 30.73
Free T4/TSH	3.50	−18.12; 31.21
Iodine intake ≥108 μg/L (N = 65)		
Total T3	−0.17	−7.38; 7.25
Free T4	−0.99	−8.37; 6.60
TSH	−2.09	−21.53; 22.14
Free T4/TSH	1.10	−19.01; 26.24

Mixture components: PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA. PFTrDA, PFOS, and PFHxS.
All models are adjusted by adolescent age, maternal schooling, and total fish intake.
Estimates are expressed as percentage of change in thyroid hormone parameter. Pear each quartile increase in the mixture concentration.
*p < 0.10.

peroxidase (TPO) function, iodine uptake, and thyroid hormone transport and clearance (Coperchini et al., 2021; Weiss et al., 2009; Yu et al., 2009, 2011). Thus, Kim et al. (2021) suggested that PFUnDA and PFTrDA cause transcriptional changes of thyroid regulating genes that may increase thyroid hormone synthesis (Kim et al., 2021), which could explain the positive association between PFUnDA and free T4.

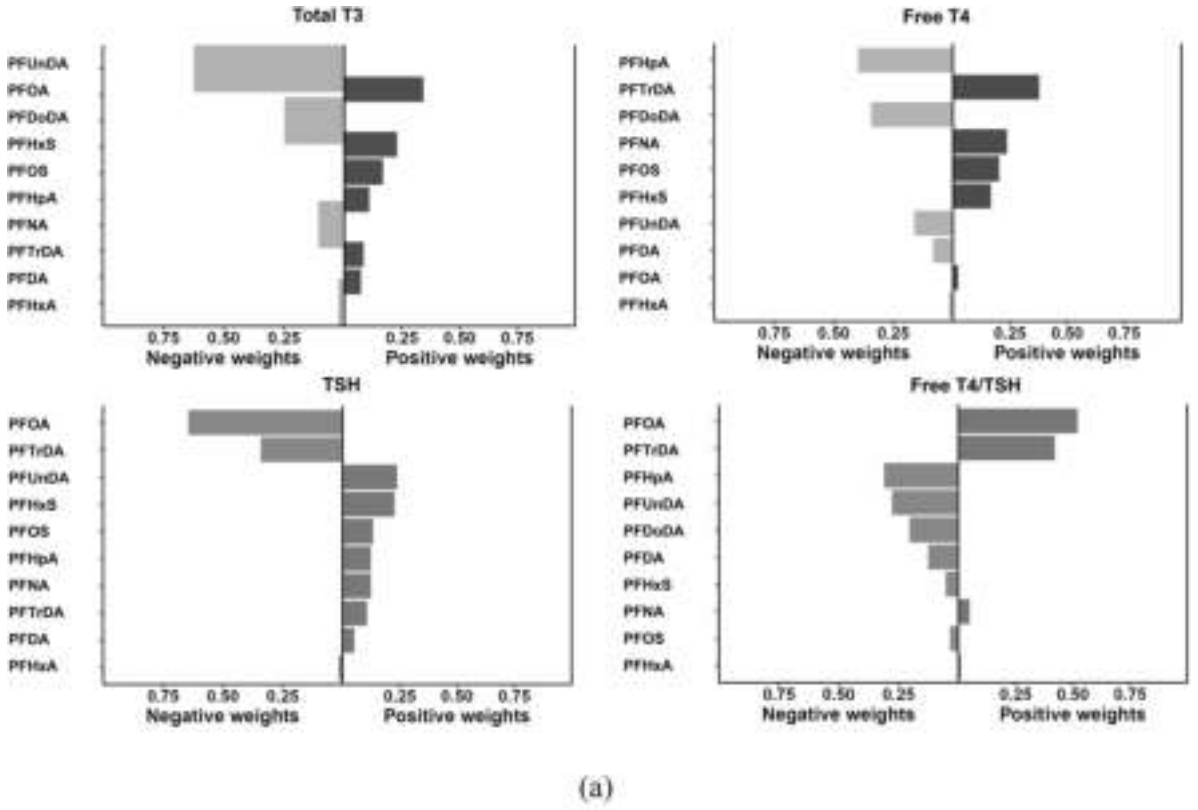
Overall, direct comparisons with previous findings on the effects of PFAS on thyroid hormones in children and adolescents are hampered by differences in the age of participants, their PFAS concentrations, and/or the types of thyroid hormones measured. For instance, regarding age, levels of free T4, free T3, and TSH are generally higher in younger children than in older children, with a gradual decline in concentrations as adult age is approached (Taylor et al., 2023). In addition, complex mechanisms are involved in thyroid homeostasis, and PFAS has been reported to interfere with this endocrine system at several levels (Coperchini et al., 2021).

When stratified by iodine intake, single-exposure and g-computation models suggested that exposure to individual PFAS and their mixture may increase levels of free T4 only in boys with lower iodine intake. In a mother-child cohort study, iodine deficiency did not modify the association between maternal serum PFAS and thyroid hormones (Lebeaux et al., 2020), while serum PFOA, PFOS, PFNA, and PFHxS were positively associated with free T3, total T3, and TSH in adults from the NHANES with high TPO antibody and low urinary iodine levels (Webster et al., 2016). According to Webster et al. (2014), effect modification by iodine status contribute to the "multiple hit hypothesis", a theory that thyroid function may be more susceptible to disruption by chemicals such as PFAS if the system is already impacted by multiple stressors. Although iodine deficiency is still present in some parts of Europe, recent data suggest that the iodine status of the population is optimal in the Southern region of Spain (Andalusia) where the INMA-Granada cohort was established, with higher urinary iodine concentrations in schoolchildren than in adults (Ittermann et al., 2020). Nevertheless, the median iodine intake of the present sample of adolescents was below the recommended level of 120 μg/day, possibly due to low fish intake in the study sample (mean = 80 g/day) while underestimation of iodine intake cannot be ruled out. Further studies using robust mixture models in larger populations are required to corroborate these findings.

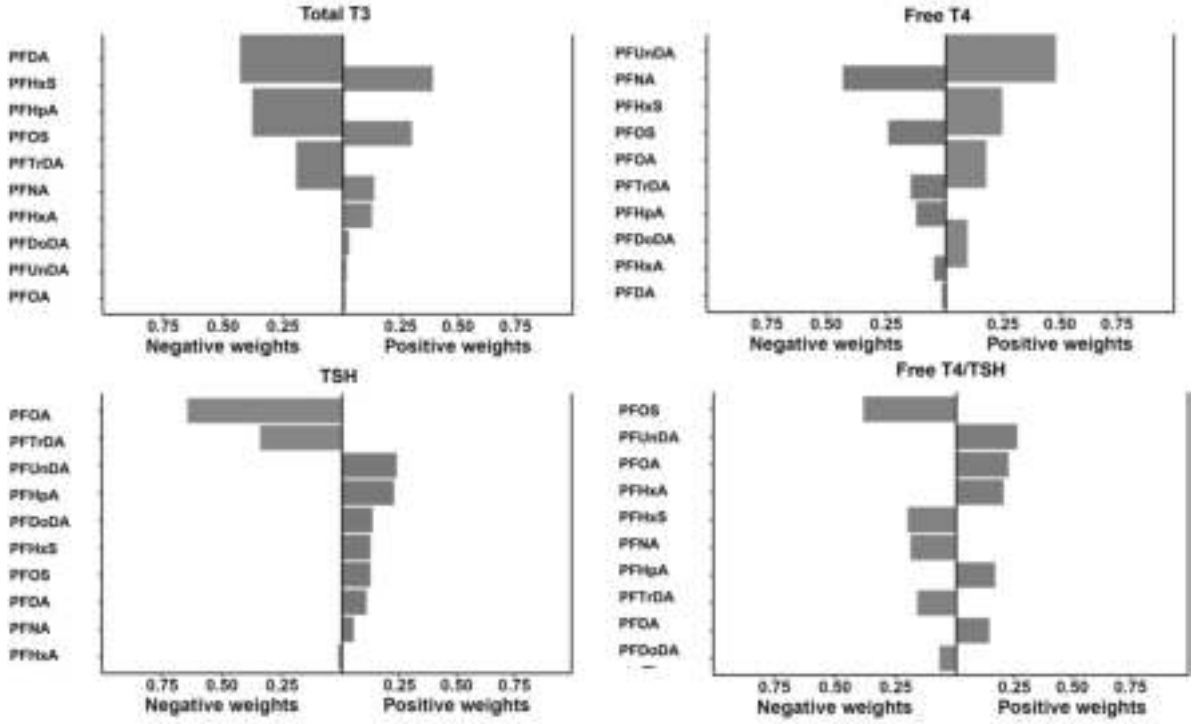
The main strengths of this study include the evaluation of ten different PFAS with high detection frequencies, the performance of mixture effect analysis, examination of the association between PFAS and thyroid hormones in a novel population, and assessment of effect modification by iodine status. The cross-sectional design of the study is a major limitation, preventing evaluation of the potential causality of the association between PFAS exposure and thyroid hormone levels. The small sample size may have resulted in imprecise estimates of the effect size, thus preventing the ascertainment of the extent to which PFAS exposure altered thyroid hormone levels. Additionally, it is unclear whether the associations observed in single-exposure models represent cause-effect relationships or result from the performance of multiple comparisons (*i.e.*, 10 PFAS x 3 thyroid hormones = 30 effective comparisons). However, the mixture analysis corroborated the effect observed in single-exposure models. Furthermore, the sex-specific influence of PFAS on thyroid hormones could not be assessed in the INMA-Granada cohort, which contains only boys.

5. Conclusions

This study found a wide presence of legacy and newer PFAS in plasma samples from Spanish adolescent males and observed that higher concentrations of some PFAS (including PFOA PFOS, and PFUnDA) and the PFAS mixture were associated with a mild increase in thyroid hormone levels, particularly higher free T4 levels in boys with lower iodine intake. Larger longitudinal studies are needed to confirm the associations observed and to improve understanding of the effects of PFAS on thyroid function in young populations.



(a)



(b)

Fig. 2. G-computation models for the mixture effect of PFAS on thyroid parameters by iodine intake: a) lower iodine intake (<108 μg/day, N = 64); b) higher iodine intake (≥108 μg/day, N = 65). Dark-colored bars refer to chemicals with an effect in the same direction to the overall effect. Grey-colored bars refer to chemicals with an effect in the opposite direction to the overall effect.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijheh.2023.114219.

References

Anderson, G.W., 2001. Thyroid hormones and the brain. Front. Neuroendocrinol. 22 (1), 1–17. https://doi.org/10.1006/frne.2000.0208.

Bartolomé, M., Gallego-Picó, A., Cutanda, F., Huetos, O., Esteban, M., Pérez-Gómez, B., Bioambient, es, Castaño, A., 2017. Perfluorinated alkyl substances in Spanish adults: geographical distribution and determinants of exposure. Sci. Total Environ. 603–604, 352–360. https://doi.org/10.1016/j.scitotenv.2017.06.031.

Bauer, M., London, E.D., Silverman, D.H., Rasgon, N., Kirchheiner, J., Whybrow, P.C., 2003. Thyroid, brain and mood modulation in affective disorder: insights from molecular research and functional brain imaging. Pharmacopsychiatry 36 (Suppl. 3), S215–S221. https://doi.org/10.1055/s-2003-45133.

Blake, B.E., Pinney, S.M., Hines, E.P., Fenton, S.E., Ferguson, K.K., 2018. Associations between longitudinal serum perfluoroalkyl substance (PFAS) levels and measures of thyroid hormone, kidney function, and body mass index in the Fernald Community Cohort. Environ. Pollut. 242 (Pt A), 894–904. https://doi.org/10.1016/j.envpol.2018.07.042.

Bloom, M.S., Kannan, K., Spliethoff, H.M., Tao, L., Aldous, K.M., Vena, J.E., 2010. Exploratory assessment of perfluorinated compounds and human thyroid function. Physiol. Behav. 99 (2), 240–245. https://doi.org/10.1016/j.physbeh.2009.02.005.

Boesen, S.A.H., Long, M., Wielsøe, M., Mustieles, V., Fernandez, M.F., Bonefeld-Jørgensen, E.C., 2020. Exposure to perflouroalkyl acids and foetal and maternal thyroid status: a review. Environ. Health 19 (1), 107. https://doi.org/10.1186/s12940-020-00647-1.

Browning, L.M., Hsieh, S.D., Ashwell, M., 2010. A systematic review of waist-to-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0·5 could be a suitable global boundary value. Nutr. Res. Rev. 23 (2) https://doi.org/10.1017/S0954422410000144, 247–69.

Byrne, S.C., Miller, P., Seguinot-Medina, S., Waghiyi, V., Buck, C.L., von Hippel, F.A., Carpenter, D.O., 2018. Exposure to perfluoroalkyl substances and associations with serum thyroid hormones in a remote population of Alaska Natives. Environ. Res. 166, 537–543. https://doi.org/10.1016/j.envres.2018.06.014.

Calafat, A.M., Wong, L.Y., Kuklenyik, Z., Reidy, J.A., Needham, L.L., 2007. Polyfluoroalkyl chemicals in the U.S. Population: data from the national health and nutrition examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. Environ. Health Perspect. 115 (11), 1596–1602. https://doi.org/10.1289/ehp.10598.

Carlsen, M.H., Andersen, L.F., Dahl, L., Norberg, N., Hjartåker, A., 2018. New iodine food composition database and updated calculations of iodine intake among Norwegians. Nutrients 10, 930. https://doi.org/10.3390/nu10070930.

Caron-Beaudoin, É., Ayotte, P., Laouan Sidi, E.A., 2019. Community of lac simon; community of winneway – long point first nation; CSSS tshukuminu kanani of nutashkuan; community of unamen shipu, gros-louis McHugh N, lemire M. Exposure to perfluoroalkyl substances (PFAS) and associations with thyroid parameters in first nation children and youth from Quebec. Environ. Int. 128, 13–23. https://doi.org/10.1016/j.envint.2019.04.029.

Castiello, F., Olmedo, P., Gil, F., Molina, M., Mundo, A., Romero, R.R., Ruíz, C., Gómez-Vida, J., Vela-Soria, F., Freire, C., 2020. Association of urinary metal concentrations with blood pressure and serum hormones in Spanish male adolescents. Environ. Res. 182, 108958 https://doi.org/10.1016/j.envres.2019.108958.

Castiello, F., Suárez, B., Gómez-Vida, J., Torrent, M., Fernández, M.F., Olea, N., Freire, C., 2023. Exposure to non-persistent pesticides and sexual maturation of Spanish adolescent males. Chemosphere 324, 138350. https://doi.org/10.1016/j.chemosphere.2023.138350.

Commission Regulation (EU). 2017/1000 of 13 june 2017 amending annex XVII to regulation (EC) No 1907/2006 of the European parliament and of the council concerning the registration, evaluation, authorisation and restriction of chemicals (REACH) as regards perfluorooctanoic acid (PFOA), its salts and PFOA-related substances. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R1000&from=EN. (Accessed 18 October 2022).

Coperchini, F., Awwad, O., Rotondi, M., Santini, F., Imbriani, M., Chiovato, L., 2017. Thyroid disruption by perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). J. Endocrinol. Invest. 40 (2), 105–121. https://doi.org/10.1007/s40618-016-0572-z.

Coperchini, F., Croce, L., Ricci, G., Magri, F., Rotondi, M., Imbriani, M., Chiovato, L., 2021. Thyroid disrupting effects of old and new generation PFAS. Front. Endocrinol. 11, 612320 https://doi.org/10.3389/fendo.2020.612320.

Cornelis, C., D'Hollander, W., Roosens, L., Covaci, A., Smolders, R., Van Den Heuvel, R., Govarts, E., Van Campenhout, K., Reynders, H., Bervoets, L., 2012. First assessment of population exposure to perfluorinated compounds in Flanders, Belgium. Chemosphere 86 (3), 308–314. https://doi.org/10.1016/j.chemosphere.2011.10.034.

Croghan, C., Egeghy, P.P., 2003. Methods of Dealing with Values below the Limit of Detection Using SAS. Presented at Southern SAS User Group, St. Petersburg, FL. September 22-24.

Dassuncao, C., Hu, X.C., Nielsen, F., Weihe, P., Grandjean, P., Sunderland, E.M., 2018. Shifting Global Exposures to poly- and perfluoroalkyl substances (PFASs) evident in longitudinal birth cohorts from a seafood-consuming population. Environ. Sci. Technol. 52 (6), 3738–3747. https://doi.org/10.1021/acs.est.7b06044.

de Onis, M., Onyango, A.W., Borghi, E., Siyam, A., Nishida, C., Siekmann, J., 2007. Development of a WHO growth reference for school-aged children and adolescents. Bull. World Health Organ. 85 (9), 660–667. https://doi.org/10.2471/blt.07.043497.

Diamanti-Kandarakis, E., Bourguignon, J.P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., et al., 2009. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr. Rev. 30 (4), 293–342. https://doi.org/10.1210/er.2009-0002.

ECHA (European Chemicals Agency), 2019. Candidate list of substances of very high concern for authorisation. Available online: https://echa.europa.eu/candidate-list-table. (Accessed 18 October 2022).

EFSA (European Food Safety Authority), 2020. Risk to human health related to the presence of perfluoroalkyl substances in food. Scientific opinion of the panel on contaminants in the food chain. EFSA J. 18 (9), 6223.

Eick, S.M., Goin, D.E., Cushing, L., DeMicco, E., Park, J.S., Wang, Y., Smith, S., Padula, A. M., Woodruff, T.J., Morello-Frosch, R., 2021. Mixture effects of prenatal exposure to per- and polyfluoroalkyl substances and polybrominated diphenyl ethers on maternal and newborn telomere length. Environ. Health 20 (1), 76. https://doi.org/10.1186/s12940-021-00765-4.

Glüge, J., Scheringer, M., Cousins, I.T., DeWitt, J.C., Goldenman, G., Herzke, D., Lohmann, R., Ng, C.A., Trier, X., Wang, Z., 2020. An overview of the uses of per- and polyfluoroalkyl substances (PFAS). Environ Sci Process Impacts 22 (12), 2345–2373. https://doi.org/10.1039/d0em00291g.

Gore, A.C., 2016. Endocrine-disrupting chemicals. JAMA Intern. Med. 176 (11), 1705–1706. https://doi.org/10.1001/jamainternmed.2016.5766.

Guxens, M., Ballester, F., Espada, M., Fernández, M.F., Grimalt, J.O., Ibarluzea, J., Olea, N., Rebagliato, M., Tardón, A., Torrent, M., Vioque, J., Vrijheid, M., Sunyer, J., INMA Project, 2012. Cohort profile: the INMA–INfancia y medio ambiente–(environment and childhood) Project. Int. J. Epidemiol. 41 (4), 930–940. https://doi.org/10.1093/ije/dyr054.

Halldorsson, T.I., Fei, C., Olsen, J., Lipworth, L., McLaughlin, J.K., Olsen, S.F., 2008. Dietary predictors of perfluorinated chemicals: a study from the Danish National Birth Cohort. Environ. Sci. Technol. 42 (23), 8971–8977. https://doi.org/10.1021/es801907r.

Haug, L.S., Huber, S., Becher, G., Thomsen, C., 2011. Characterisation of human exposure pathways to perfluorinated compounds–comparing exposure estimates with biomarkers of exposure. Environ. Int. 37 (4), 687–693. https://doi.org/10.1016/j.envint.2011.01.011.

Ittermann, T., Albrecht, D., Arohonka, P., Bilek, R., de Castro, J.J., Dahl, L., Filipsson Nystrom, H., Gaberscek, S., Garcia-Fuentes, E., Gheorghiu, M.L., Hubalewska-Dydejczyk, A., Hunziker, S., Jukic, T., Karanfilski, B., Koskinen, S., Kusic, Z., Majstorov, V., Makris, K.C., Markou, K.B., Meisinger, C., Milevska Kostova, N., Mullen, K.R., Nagy, E.V., Pirags, V., Rojo-Martinez, G., Samardzic, M., Saranac, L., Strele, I., Thamm, M., Top, I., Trofimiuk-Müldner, M., Ünal, B., Koskinen, S., Vila, L., Vitti, P., Winter, B., Woodside, J.V., Zaletel, K., Zamrazil, V., Zimmermann, M., Erlund, I., Völzke, H., 2020. Standardized map of iodine status in Europe. Thyroid 30 (9), 1346–1354. https://doi.org/10.1089/thy.2019.0353. Erratum in: Thyroid. 2022;32(5):603.

Iwaku, K., Noh, J.Y., Minagawa, A., Kosuga, Y., Suzuki, M., Sekiya, K., Matsumoto, M., Ohye, H., Kunii, Y., Yoshihara, A., Watanabe, N., Mukasa, K., Ito, K., Ito, K., 2013. Determination of pediatric reference levels of FT3, FT4 and TSH measured with ECLusys kits. Endocr. J. 60 (6), 799–804. https://doi.org/10.1507/endocrj.ej12-0390.

Ji, K., Kim, S., Kho, Y., Paek, D., Sakong, J., Ha, J., Kim, S., Choi, K., 2012. Serum concentrations of major perfluorinated compounds among the general population in Korea: dietary sources and potential impact on thyroid hormones. Environ. Int. 45, 78–85. https://doi.org/10.1016/j.envint.2012.03.007.

Kannan, K., Corsolini, S., Falandysz, J., Fillmann, G., Kumar, K.S., Loganathan, B.G., Mohd, M.A., Olivero, J., Van Wouwe, N., Yang, J.H., Aldoust, K.M., 2004. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. Environ. Sci. Technol. 38 (17), 4489–4495. https://doi.org/10.1021/es0493446.

Keil, A.P., Buckley, J.P., O'Brien, K.M., Ferguson, K.K., Zhao, S., White, A.J., 2020. A quantile-based g-computation approach to addressing the effects of exposure mixtures. Environ. Health Perspect. 128 (4), 47004. https://doi.org/10.1289/EHP5838.

Kim, J., Lee, G., Lee, Y.M., Zoh, K.D., Choi, K., 2021. Thyroid disrupting effects of perfluoroundecanoic acid and perfluorotridecanoic acid in zebrafish (Danio rerio) and rat pituitary (GH3) cell line. Chemosphere 262, 128012. https://doi.org/10.1016/j.chemosphere.2020.128012.

Kim, M.J., Moon, S., Oh, B.C., Jung, D., Ji, K., Choi, K., Park, Y.J., 2018. Association between perfluoroalkyl substances exposure and thyroid function in adults: a meta-analysis. PLoS One 13 (5), e0197244. https://doi.org/10.1371/journal.pone.0197244.

Knox, S.S., Jackson, T., Frisbee, S.J., Javins, B., Ducatman, A.M., 2011. Perfluorocarbon exposure, gender, and thyroid function in the C8 Health Project. J. Toxicol. Sci. 36 (4), 403–410. https://doi.org/10.2131/jts.36.403.

Lanphear, B.P., 2015. The impact of toxins on the developing brain. Annu. Rev. Publ. Health 36, 211–230. https://doi.org/10.1146/annurev-publhealth-031912-114413.

Lebeaux, R.M., Doherty, B.T., Gallagher, L.G., Zoeller, R.T., Hoofnagle, A.N., Calafat, A. M., Karagas, M.R., Yolton, K., Chen, A., Lanphear, B.P., Braun, J.M., Romano, M.E., 2020. Maternal serum perfluoroalkyl substance mixtures and thyroid hormone concentrations in maternal and cord sera: the HOME Study. Environ. Res. 185, 109395 https://doi.org/10.1016/j.envres.2020.109395.

Lewis, R.C., Johns, L.E., Meeker, J.D., 2015. Serum biomarkers of exposure to perfluoroalkyl substances in relation to serum testosterone and measures of thyroid function among adults and adolescents from NHANES 2011-2012. Int. J. Environ. Res. Publ. Health 12 (6), 6098–6114. https://doi.org/10.3390/ijerph120606098.

Lin, C.Y., Wen, L.L., Lin, L.Y., Wen, T.W., Lien, G.W., Hsu, S.H., Chien, K.L., Liao, C.C., Sung, F.C., Chen, P.C., Su, T.C., 2013. The associations between serum perfluorinated chemicals and thyroid function in adolescents and young adults. J. Hazard Mater. 244–245, 637–644. https://doi.org/10.1016/j.jhazmat.2012.10.049.

Lopez-Espinosa, M.J., Mondal, D., Armstrong, B., Bloom, M.S., Fletcher, T., 2012. Thyroid function and perfluoroalkyl acids in children living near a chemical plant. Environ. Health Perspect. 120 (7), 1036–1041. https://doi.org/10.1289/ehp.1104370.

Melzer, D., Rice, N., Depledge, M.H., Henley, W.E., Galloway, T.S., 2010. Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. National Health and Nutrition Examination Survey. Environ. Health Perspect. 118 (5), 686–692. https://doi.org/10.1289/ehp.0901584.

Menzel, J., Abraham, K., Dietrich, S., Fromme, H., Völkel, W., Schwerdtle, T., Weikert, C., 2021. Internal exposure to perfluoroalkyl substances (PFAS) in vegans and omnivores. Int. J. Hyg Environ. Health 237, 113808. https://doi.org/10.1016/j.ijheh.2021.113808.

Notario-Barandiaran, L., Freire, C., García-de-la-Hera, M., Compañ-Gabucio, L.M., Torres-Collado, L., González-Palacios, S., Mundo, A., Molina, M., Fernández, M.F., Vioque, J., 2020. Reproducibility and validity of a food frequency questionnaire for dietary assessment in adolescents in a self-reported way. Nutrients 12 (7), 2081. https://doi.org/10.3390/nu12072081.

Notario-Barandiaran, L., Navarrete-Muñoz, E.M., Valera-Gran, D., Hernández-Álvarez, E., Donoso-Navarro, E., González-Palacios, S., García-de-la-Hera, M., Fernández, M.F., Freire, C., Vioque, J., 2021. Biochemical validation of a self-administered food frequency questionnaire to assess diet using carotenoids and vitamins e and d in male adolescents in Spain. Antioxidants 10 (5), 750. https://doi.org/10.3390/antiox10050750.

Olsen, G.W., Zobel, L.R., 2007. Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers. Int. Arch. Occup. Environ. Health 81 (2), 231–246. https://doi.org/10.1007/s00420-007-0213-0.

Regulation (EU). 2019/1021 of the European Parliament and of the Council of 20 June 2019 on persistent organic pollutants. Available online: http://data.europa.eu/eli/reg/2019/1021/oj. (Accessed 18 October 2022).

Richterová, D., Govarts, E., Fábelová, L., Rausová, K., Rodriguez Martin, L., Gilles, L., Remy, S., Colles, A., Rambaud, L., Riou, M., Gabriel, C., Sarigiannis, D., Pedraza-Diaz, S., Ramos, J.J., Kosjek, T., Snoj Tratnik, J., Lignell, S., Gyllenhammar, I., Thomsen, C., Palkovičová Murínová, Ľ., 2023. PFAS levels and determinants of variability in exposure in European teenagers – Results from the HBM4EU aligned studies (2014–2021). Int. J. Hyg. Environ. Health 247, 114057. https://doi.org/10.1016/j.ijheh.2022.114057.

Stockholm Convention, 2017. Available online: http://www.pops.int/Portals/0/download.aspx?d=UNEP-POPS-COP-CONVTEXT-2017.English.pdf (accessed on 18 October 2022).

Suárez, B., Vela-Soria, F., Castiello, F., Olivas-Martinez, A., Acuña-Castroviejo, D., Gómez-Vida, J., Olea, N., Fernández, M.F., Freire, C., 2021. Organophosphate pesticide exposure, hormone levels, and interaction with PON1 polymorphisms in male adolescents. Sci. Total Environ. 769, 144563 https://doi.org/10.1016/j.scitotenv.2020.144563.

Taylor, P.N., Lansdown, A., Witczak, J., Khan, R., Rees, A., Dayan, C.M., Okosieme, O., 2023. Age-related variation in thyroid function - a narrative review highlighting important implications for research and clinical practice. Thyroid Res. 16 (1), 7. https://doi.org/10.1186/s13044-023-00149-5. Erratum in: Thyroid Res. 2023;16 (1):20.

Textor, J., Hardt, J., Knuppel, S., 2011. Dagitty: a graphical tool for analyzing causal diagrams. Epidemiology 22 (5), 745. https://doi.org/10.1097/EDE.0b013e318225c2be.

Vela-Soria, F., Serrano-López, L., García-Villanova, J., de Haro, T., Olea, N., Freire, C., 2020. HPLC-MS/MS method for the determination of perfluoroalkyl substances in breast milk by combining salt-assisted and dispersive liquid-liquid microextraction. Anal. Bioanal. Chem. 412 (28), 7913–7923. https://doi.org/10.1007/s00216-020-02924-x.

Wang, D., Cheng, X., Yu, S., Qiu, L., Lian, X., Guo, X., Hu, Y., Lu, S., Yang, G., Liu, H., 2018a. Data mining: seasonal and temperature fluctuations in thyroid-stimulating hormone. Clin. Biochem. 60, 59–63. https://doi.org/10.1016/j.clinbiochem.2018.08.008.

Wang, M., Guo, W., Gardner, S., Petreas, M., Park, J.S., 2018b. Per- and polyfluoroalkyl substances in Northern California cats: temporal comparison and a possible link to cat hyperthyroidism. Environ. Toxicol. Chem. 37 (10), 2523–2529. https://doi.org/10.1002/etc.4239.

Webster, G.M., Venners, S.A., Mattman, A., Martin, J.W., 2014. Associations between perfluoroalkyl acids (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study. Environ. Res. 133, 338–347. https://doi.org/10.1016/j.envres.2014.06.012.

Webster, G.M., Rauch, S.A., Marie, N.S., Mattman, A., Lanphear, B.P., Venners, S.A., 2016. Cross-sectional associations of serum perfluoroalkyl acids and thyroid hormones in U.S. adults: variation according to TPOAb and iodine status (NHANES 2007-2008). Environ. Health Perspect. 124 (7), 935–942. https://doi.org/10.1289/ehp.1409589.

Weiss, J.M., Andersson, P.L., Lamoree, M.H., Leonards, P.E., van Leeuwen, S.P., Hamers, T., 2009. Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin. Toxicol. Sci. 109 (2), 206–216. https://doi.org/10.1093/toxsci/kfp055.

Wu, X.M., Bennett, D.H., Calafat, A.M., Kato, K., Strynar, M., Andersen, E., Moran, R.E., Tancredi, D.J., Tulve, N.S., Hertz-Picciotto, I., 2015. Serum concentrations of perfluorinated compounds (PFC) among selected populations of children and adults in California. Environ. Res. 136, 264–273. https://doi.org/10.1016/j.envres.2014.09.026.

Yu, W.G., Liu, W., Jin, Y.H., Liu, X.H., Wang, F.Q., Liu, L., Nakayama, S.F., 2009. Prenatal and postnatal impact of perfluorooctane sulfonate (PFOS) on rat development: a cross-foster study on chemical burden and thyroid hormone system. Environ. Sci. Technol. 43 (21), 8416–8422. https://doi.org/10.1021/es901602d.

Yu, W.G., Liu, W., Liu, L., Jin, Y.H., 2011. Perfluorooctane sulfonate increased hepatic expression of OAPT2 and MRP2 in rats. Arch. Toxicol. 85 (6), 613–621. https://doi.org/10.1007/s00204-010-0613-x.

Zhang, S., Guo, X., Lu, S., Sang, N., Li, G., Xie, P., et al., 2018. Exposure to PFDoA causes disruption of the hypothalamus-pituitary-thyroid axis in zebrafish larvae. Environ. Pollut. 235, 974–982. https://doi.org/10.1016/j.envpol.2018.01.015.

Zoeller, R.T., 2007. Environmental chemicals impacting the thyroid: targets and consequences. Thyroid 17 (9), 811–817. https://doi.org/10.1089/thy.2007.0107.

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Firefighters and the liver: Exposure to PFAS and PAHs in relation to liver function and serum lipids (CELSPAC-FIREexpo study)

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ABSTRACT

Introduction: Firefighting is one of the most hazardous occupations due to exposure to per- and polyfluoroalkyl substances (PFAS) and polycyclic aromatic hydrocarbons (PAHs). Such exposure is suspected to affect the cardiometabolic profile, e.g., liver function and serum lipids. However, only a few studies have investigated the impact of this specific exposure among firefighters.

Methods: Men included in the CELSPAC-FIREexpo study were professional firefighters (n = 52), newly recruited firefighters in training (n = 58), and controls (n = 54). They completed exposure questionnaires and provided 1–3 samples of urine and blood during the 11-week study period to allow assessment of their exposure to PFAS (6 compounds) and PAHs (6 compounds), and to determine biomarkers of liver function (alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin (BIL)) and levels of serum lipids (total cholesterol (CHOL), low-density lipoprotein cholesterol (LDL) and triglycerides (TG)). The associations between biomarkers were investigated both cross-sectionally using multiple linear regression (MLR) and Bayesian weighted quantile sum (BWQS) regression and prospectively using MLR. The models were adjusted for potential confounders and false discovery rate correction was applied to account for multiplicity.

Results: A positive association between exposure to PFAS and PAH mixture and BIL (β = 28.6%, 95% CrI = 14.6–45.7%) was observed by the BWQS model. When the study population was stratified, in professional firefighters and controls the mixture showed a positive association with CHOL (β = 29.5%, CrI = 10.3–53.6%) and LDL (β = 26.7%, CrI = 8.3–48.5%). No statistically significant associations with individual compounds were detected using MLR.

Conclusions: This study investigated the associations between exposure to PFAS and PAHs and biomarkers of cardiometabolic health in the Czech men, including firefighters. The results suggest that higher exposure to a mixture of these compounds is associated with an increase in BIL and the alteration of serum lipids, which can result in an unfavourable cardiometabolic profile.

1. Introduction

Firefighting, as one of the most hazardous occupations, combines extreme physical and psychological demands, themselves potential risk factors, with the former including risk of acute trauma and exposure to both high temperatures and a complex mixture of hazardous pollutants released during fire suppression activities as well as from contaminated equipment and protective gear (Barros et al., 2021; Trowbridge et al., 2020). Previous studies have reviewed the increased incidence of cardiovascular disease (CVD) among firefighters (Soteriades et al., 2011,

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2019). The exposure of firefighters to per- and polyfluoroalkyl substances (PFAS) and polycyclic aromatic hydrocarbons (PAHs) may be of particular relevance due to recently reported associations between exposure to these compounds and adverse health outcomes in humans, many of which are related to CVD (Alshaarawy et al., 2016; Attanasio, 2019; Gallo et al., 2012a; Gleason et al., 2015a; Li et al., 2020a; Moorthy et al., 2015; Sakr et al., 2007a; Sakr et al., 2007b; Stanifer et al., 2018; Wagner et al., 2015; Xu et al., 2021; Yamaguchi et al., 2013).

PFAS are omnipresent and highly persistent synthetic chemicals extensively used for a variety of commercial and industrial applications due to their grease-, stain-, and water-repellent properties (Ho et al., 2022). Drinking water and diet have been identified as major sources of exposure in humans (Fenton et al., 2021). Firefighters are additionally exposed due to the application of PFAS in class B firefighting aqueous film-forming foams (AFFFs, used for extinguishing hydrocarbon-fuel and chemical solvent fires) and the use of PFAS-coated firefighting equipment (Khalil et al., 2022; Laitinen et al., 2014a; Pitter et al., 2020). Elevated levels of PFAS in firefighters' blood serum, especially perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), and perfluorohexane sulfonic acid (PFHxS), have been reported in several studies (Dobraca et al., 2015; Jin et al., 2011; Laitinen et al., 2014a; Rotander et al., 2015a; Trowbridge et al., 2020).

PAHs consist of two or more fused benzene rings and are generated by the incomplete combustion of organic matter (Kim et al., 2013). The main exposure routes are the inhalation of polluted air or cigarette smoke, the ingestion of contaminated food, or dermal absorption (Li et al., 2020b). Several biomonitoring studies reported increased internal exposure among firefighters after firefighting activity compared to controls, even when using self-contained breathing apparatuses (SCBAs) for protection against the inhalation of airborne contaminants (Banks et al., 2021; Ekpe et al., 2021; Fent et al., 2020; Rossbach et al., 2020), suggesting dermal absorption as the most relevant route for PAH uptake (Andersen et al., 2018; Fent et al., 2019; Rossbach et al., 2020; Wingfors et al., 2018) as well as inhalation uptake due to SCBAs removal during the overhaul stage at the fire incident site (Banks et al., 2021; Baxter et al., 2014).

The liver, with its central role in the metabolism of xenobiotics, is considered to be the main target organ of both PFAS and PAHs. Epidemiological studies suggest associations between PFAS exposure and altered levels of biomarkers of liver function (e.g., liver enzymes, bilirubin, and serum lipids). However, reported associations are often inconsistent and cause-and-effect relationships have not yet been established (Costello et al., 2022; Darrow et al., 2016; Gallo et al., 2012a; Gleason et al., 2015a; Lin et al., 2010; Omoike et al., 2021; Salihovic et al., 2018; Stratakis et al., 2020). A high abundance of cytochrome P450 in the liver is responsible for the oxidation of PAHs, resulting in a complex mixture of hydroxylated metabolites (OH-PAHs), which are excreted predominantly through urine (Oliveira et al., 2020; Weyand and Bevan, 1986) and used as biomarkers of PAH exposure. Exposure to PAHs was also associated with altered liver biomarkers (Alshaarawy et al., 2016; Brucker et al., 2014; Wang et al., 2019). Abnormal liver function (altered levels of liver enzymes and bilirubin) and dyslipidaemia (altered levels of serum cholesterol, low-density lipoprotein, or triglycerides) are considered risk factors for developing CVD (Choi et al., 2018; Ekstedt et al., 2015; Ismaiel and Dumitraşcu, 2019; Soderberg et al., 2010).

Monitoring exposure among firefighters is challenging due to the unpredictability and specificity of particular firefighting activities, resulting in exposure to many chemicals in multiple exposure pathways. Many studies monitoring exposure both on- and off-duty are available (Banks et al., 2021; Ekpe et al., 2021; Rossbach et al., 2020; Rotander et al., 2015b; Trowbridge et al., 2020; Wingfors et al., 2018), though only a minority of these consider multiple exposures (Bessonneau et al., 2021; Fent et al., 2020; Laitinen et al., 2012; Park et al., 2015). A more consistent assessment of the simultaneous exposure to a wide range of chemicals is essential for the evaluation of potential health effects. Only a few studies have considered the simultaneous assessment of both exposure and effect biomarkers in firefighters (Andersen et al., 2018; Bessonneau et al., 2021; Oliveira et al., 2020) and, to the best of our knowledge, none of them have focused on the liver and lipidic health or simultaneous exposure to PAHs and PFAS. Therefore, this study aimed to assess the effects of exposure to PAHs and PFAS on liver function and serum lipid profile with a special focus on firefighters at different professional stages.

2. Materials and methods

2.1. Study population

The study population is described in detail in Říháčková et al. (2023). Briefly, between 2019 and 2020, a total of 166 participants were recruited for the CELSPAC-FIREexpo study, a collaborative research project with the aim of assessing firefighters' exposure to PAHs and PFAS while firefighting and training, and determining chemical and biochemical biomarkers of exposure and its effects. Participants were divided into 3 sub-cohorts: newly recruited firefighters before professional training for active participation in responses to incidents ("NEW FF"; n = 58), professional firefighters actively participating in responses to incidents ("PROF"; n = 52), and a control group of non-firefighters ("CTRL"; n = 54). PROF and NEW FF were recruited by the chief accredited project deputy in the Training Center of the Fire Rescue Service in Brno (Czech Republic). Controls were recruited at the Faculty of Sport, Masaryk University, Brno (Czech Republic). The participants were men (until August 2022 no female professional firefighters were actively participating in responses to incidents), either firefighters (active or enrolled in training) or physically active men (for the control group), who were 18–35 years old and non-smokers with no chronic diseases. Two participants withdrew from the study before completion. Therefore, a total of 164 participants, who had answered questionnaires and for whom information with respect to exposure and biochemical analyses was complete, were included in the present study. The study was approved by the ELSPAC Ethics Committee in 2019, and all participants gave their written informed consent.

2.2. Study design

The complete design of the CELSPAC-FIREexpo study based on 3 subcohorts (NEW FF, PROF, and CTRL) is described in detail in Říháčková et al. (2023). In this study, a reduced dataset was used for statistical analyses due to the specificity of the biomarkers included (Fig. 1). Upon inclusion in the study, all participants filled out exposure questionnaires about lifestyle and dietary factors possibly contributing to PFAS exposure (wearing GoreTex or eVent clothing, work in the ski service sector, skiing activities, source of drinking water, use of dental floss, and blood donation), PAH exposure (former smoking habit, years since quitting smoking, exposure to fire, type of heating at home), or exposure to both PFAS and PAH (length of firefighting career if relevant, diet and frequency of consumption of relevant foods and supplements). The questionnaires also included information on the presence of acute or chronic infectious disease, the participant's subjective health assessment, and employment (Table S1).

Participants from NEW FF were completing a 15-week initial professional training programme prior to becoming active firefighters; hence, they followed a specific experimental design. In phase 1 (weeks 1–5), NEW FF provided a morning void urine sample and fasting blood sample for the further analysis of exposure biomarkers (PFAS, OH-PAHs). Phase 2 (week 6) corresponded with the time 4 h after the firefight training in an indoor environment. In this phase, NEW FF again provided urine and non-fasting blood samples for the analysis of exposure biomarkers (PFAS, OH-PAHs). In phase 3 (week 10), which corresponded with the period 1 week after training with AFFFs (considered as PFAS exposure) and 4 weeks after phase 2, NEW FF once more provided

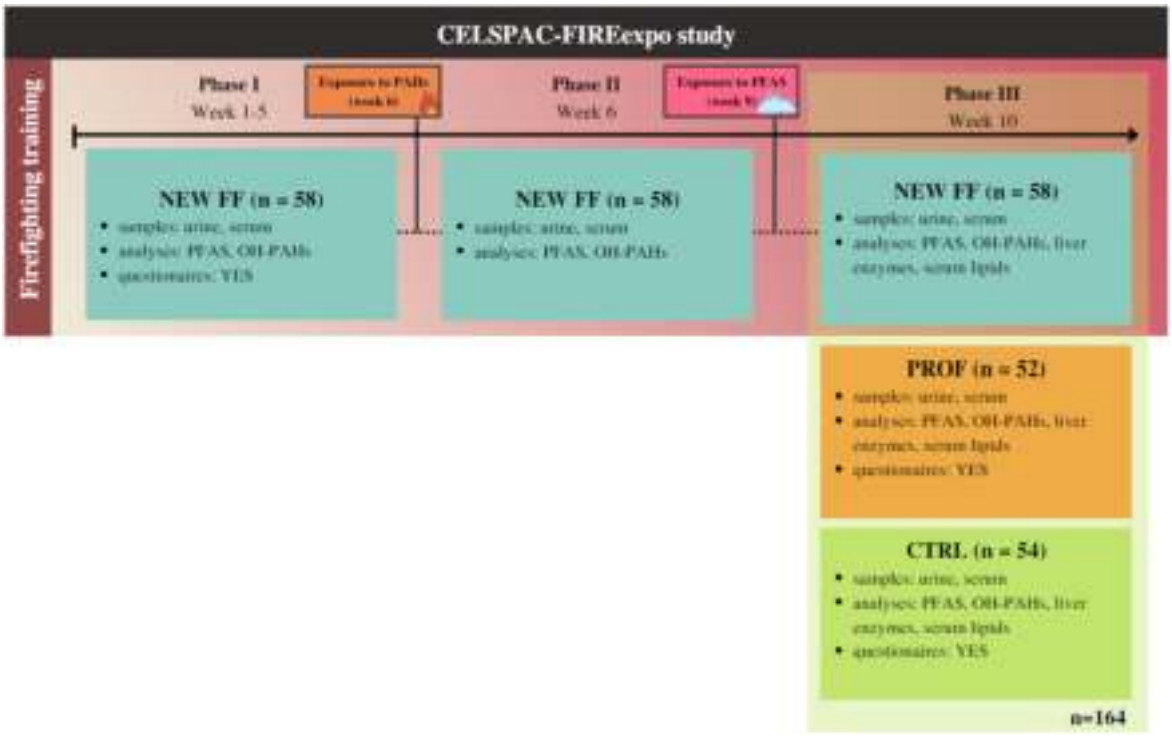

Fig. 1. Design of CELSPAC-FIREexpo study consisting of 3 sub-cohorts: new firefighters in training ("NEW FF"), professional firefighters ("PROF"), and controls ("CTRL"). In this study, NEW FF were monitored 3 times (correspondingly with phases 1, 2, and 3) throughout their firefighting training, while PFOF and CTRL provided just one set of samples.

fasting blood samples and morning void urine for the analysis of biomarkers of exposure (OH-PAHs, PFAS) and also biomarkers of liver function and serum lipids (biochemical analyses). A detailed description of the training activities and the equipment of trainees is provided in the supplementary material. Participants from the PROF- and CTRL-subcohorts provided a single sample of morning void urine and fasting blood, which was used for the analyses of both exposure biomarkers and biomarkers of liver function and serum lipids.

2.3. Blood and urine collection

Blood samples were collected by medical personnel in an operational ambulance. Urine samples were collected at the workplace by own urine collection following the instruction of medical personnel. In phases 1 and 3, morning void midstream urine was sampled, along with venous blood on an empty stomach. In phase 2, the sampling of morning void urine and venous blood on an empty stomach was not possible due to training schedule.

Venous blood for serum isolation was sampled in 7.5 mL S-Monovette® tube containing the Z-gel clotting activator. Each participant provided approximately 40 mL of midstream urine, which was collected in a 50 mL centrifuge tube. Both the venous blood and urine samples were immediately transported to laboratories in a cooling box set at 8 °C.

Once the clot had formed in the venous blood tube, it was centrifuged at 2500×*g* and 20 °C for 10 min. Subsequently, 0.5 mL aliquots were separated and placed into 1.2 mL cryotubes, which were then gradually frozen and stored in a biobank facility at −80 °C for further analyses of the biomarkers and biochemical analysis. Similarly, the urine samples in 50 mL centrifuge tubes were divided into 1 mL aliquots in 1.2 mL cryotubes, frozen gradually, and stored in a biobank facility at −80 °C until further analyses.

2.4. Determination of OH-PAHs and PFAS

A total of 6 PFAS in blood serum (perfluorohexane sulfonate [PFHxS], perfluorooctanoate [PFOA], perfluorooctane sulfonate [PFOS], perfluorononanoate [PFNA], perfluorodecanoate [PFDA] and perfluoroundecanoate [PFUnDA]) and 6 hydroxylated PAH metabolites (OH-PAHs) (1-hydroxynaphtalene [1-OH-NAP], 2-hydroxynaphtalene [2-OH-NAP], 2-hydroxyfluorene [2-OH-FLU], 3-hydroxyfluorene [3-OH-FLU], 1-hydroxypyrene [1-OH-PYR] and 2/3-hydroxyphenanthrene [2/3-OH-PHEN]) in urine were measured. Samples were analysed at RECETOX (Brno, Czech Republic) following the methods described in detail elsewhere (Říhačková et al., 2023).

OH-PAHs were analysed using the modified CDC method 6705.02 (CDC's National Center for Environmental Health (NCEH), n.d.). Briefly, 500 μL of each urine sample were transferred to a 96-well plate and β-glucuronidase solution and internal standards in hydrolysing buffer were added into each well. All components were then mixed and incubated at 55 °C for 2 h. Samples were extracted using SPE plate Oasis HLB (60 mg) and analysed using an Agilent 1200 series liquid chromatography (HPLC) system with analyte detection performed on AB Sciex Qtrap 5500 tandem mass spectrometer (MS) operating in negative electrospray ionization (ESI) mode. Regrading PFAS analysis, modified CDC method 6304.04 was used (CDC's National Center for Environmental Health (NCEH), 2013). Each serum sample was transferred to a 96-well plate (Phenomenex, USA) and internal standards and acetonitrile with an addition of 1% formic acid were added to each sample. Samples were then mixed, filtered, transferred to glass vials, and evaporated to the last drop, after which methanol and ammonium acetate (1:1) were added. Then, the samples were analysed using Qtrap 5500 LC-MS/MS system (ABSciex, CA, USA) with ESI. The mobile phases were methanol with 5 mM ammonium acetate in water (55:45, component A) and methanol (component B). Gradient elution was used. Laboratory and method performances were successfully verified by participation in third-party proficiency testing (ICI-EQUAS, OSEQA). List of chemicals and information regarding QA/QC is available in supplementary

material.

2.5. Biochemical measurements and specific gravity

The levels of alanine aminotransferase (ALT, in μkat/L), gamma-glutamyl transferase (GGT, in μkat/L), aspartate aminotransferase (AST, in μkat/L), alkaline phosphatase (ALP, in μkat/L), and total bilirubin (BIL, in μmol/L) in blood serum were considered as markers of liver function. Indicators of blood lipids included total cholesterol (CHOL, mmol/L), low-density lipoprotein (LDL, mmol/L), and triglycerides (TG, mmol/L). All markers were measured spectrophotometrically with an Alinity c instrument (©Abbott, Illinois, U.S.A). The specific gravity of urine samples (SG) was measured by a handheld refractometer (Atago PAL-10S).

2.6. Statistical analysis

The concentrations of PFAS and OH-PAHs below LOQ were imputed using maximum likelihood multiple estimation based on the observed values and an expected log-normal distribution (Lubin et al., 2004). SG-standardized concentrations of OH-PAHs in urine (based on Eq. S1 in SI), serum concentrations of PFAS, and all biochemical measurements were $\log_2$ transformed and IQR standardized to approach normality and reduce the influence of outliers. Spearman correlation coefficients were calculated to estimate correlations between the concentrations of PFAS and OH-PAHs as well as between the biochemical measurements. Differences in internal exposure and biochemical parameters between the study sub-cohorts were investigated using ANOVA/Kruskal-Wallis ANOVA with Tukey/Wilcox post hoc tests and $\chi 2$ test with post hoc tests.

Firstly, using individual multiple linear regression models (MLR), associations between each biomarker of exposure (considered individually) and each biomarker of liver function or serum lipids were examined cross-sectionally including all participants (PROF, CTRL, and NEW FF from phase 3). For each chemical compound, results are expressed as the relative change in the median of liver or serum lipids biomarker for a doubled concentration of OH-PAHs or PFAS in urine or serum, respectively. Secondly, associations between the OH-PAHs and PFAS mixture and biomarkers of liver function and serum lipids were assessed on the same dataset using Bayesian weighted quantile sum (BWQS) regression. BWQS is a novel approach that extends original weighted quantile sum (WQS) regression, which is designed to estimate the effect of a mixture of correlated chemicals by creating a single score summarizing overall exposure while accounting for the individual contributions of mixture components using weights. BWQS overcomes certain limitations of WQS, especially the requirement of the *a priori* selection of the directionality of the coefficients associated with the mixture, which improves the statistical power and stability of the estimates (Colicino et al., 2020; Maitre et al., 2022; Pedretti and Colicino, 2021). In addition, prospective associations were examined in the NEW FF sub-cohort by studying the effect of exposure measured in the 1st and 2nd phases of firefighting training on liver and serum lipids biomarkers measured in the third phase using MLR. All models were adjusted for the same set of confounders, identified on the basis of *a priori* knowledge and a directed acyclic graph (DAG) approach (Fig. S1) (Shrier and Platt, 2008): age (in years), body mass index (BMI, in [kg/m^2]), previous smoking (yes/no), length of firefighting career (in years) and study sub-cohorts (NEW FF/PROF/CTRL; except the prospective model for NEW FF).

The CELSPAC-FIREexpo study population was already quite specific and homogenous – it comprised men of similar ages from the same geographical region; hence, in this study, just a few sensitivity analyses were performed. To explore associations in the subpopulations with potentially different vulnerabilities, the dataset was stratified into 3 study sub-cohorts (NEW FF, PROF, and CTRL) and MLRs were performed. To evaluate the robustness of the associations with the mixture, the BWQS regression model was run for 3 population subsets – each time excluding one sub-cohort (subset *A* – professional FF and new FF; subset *B* – new FF and controls; subset *C* – professional FF and controls). Moreover, BWQS regression models for separate mixtures of PFAS and OH-PAHs were additionally run. To account for multiple testing in the case of linear regression models, correction for multiple comparisons with the false discovery rate controlled at <5% was performed (Benjamini and Yekutieli, 2005). All statistical analyses were performed using Rstudio version 4.0.2 (Rstudio Team, 2020).

3. Results

3.1. Study population characteristics

The main characteristics of the participants are given in Table 1. The participants included in the study were 26.4 years old on average, with professional firefighters being the oldest and new firefighters the youngest, which in the case of professionals corresponds with the length of the firefighting career. Professional firefighters and new firefighters had higher BMI compared to the control group. The highest rate of former smoking was reported among professional firefighters (Table 1).

Regarding liver function and serum lipids biomarkers, the correlation matrix is presented in supplementary material (Fig. S2). The strongest positive correlation observed was between CHOL and LDL. Serum lipids (CHOL, LDL, TG) were correlated with each other and with BMI. Moreover, CHOL and LDL were correlated with age and length of FF career. Length of FF career was also significantly positively correlated with age and BMI. There was no clear correlation pattern among the liver enzymes. Statistically significant differences between the sub-cohorts were observed in ALT, GGT, CHOL, LDL (PROF had higher levels compared to CTRL and NEW FF) and ALP (PROF had lower levels compared to NEW FF) (Table 1) (Fig. S3). PROF had also significantly higher proportion of participants with levels above the physiological limits in case of CHOL (compared to NEW FF) and LDL (compared to NEW FF and CTRL) (Table 1).

1-OH-NAP, 2-OH-NAP, 2-OH-FLU, and 2/3-OH-PHEN were detected in all samples, with 2-OH-NAP having the highest median concentration. For PFAS, the highest detection frequency was observed for PFNA and PFOA, and PFOS had the highest median concentration (Table 2, Table S2). There were no or only weak inter-compound correlations between PFAS and OH-PAHs; however, intra-class correlations were stronger suggesting similar sources of exposure (Fig. S4). Firefighters (PROF and NEW FF) had higher total PFAS concentrations compared to controls. ΣOH-PAHs levels were not different among PROF, NEW FF (phases 1 and 3), and CTRL. ΣOH-PAHs in NEW FF from phase 2 was significantly higher compared to the median concentrations in other sub-cohorts (Table 2).

3.2. Cross-sectional associations

The results from MLR applied cross-sectionally to the overall study population ($n = 164$) suggest a negative association between the levels of PFOS and TAG ($\beta = -11.6\%$, $p = 0.0225$), and positive associations between all OH-PAHs and BIL ($p < 0.05$). However, after FDR correction for multiple testing, no associations remained statistically significant (Fig. 2, Table S3).

The BWQS regression model for the mixture indicates similar results – the only statistically significant association of the full mixture was with bilirubin ($\beta = 28.6\%$, CrI = 14.6–45.7%), with PFHxS, 1-OH-PYR, and 2/3-OH-PHEN as the most active compounds in the mixture (Fig. 3). Nearly significant positive tendencies were observed between the mixture and CHOL and LDL. When the PFAS mixture was considered separately (without OH-PAHs), a negative association between the mixture and TG became significant (Table 3).

Table 1
Characteristics of the study population. ×- statistically different from new firefighters in training; *- statistically different from new firefighters in training and controls.

Characteristics		Overall study population	New firefighters in training	Professional firefighters	Controls
		n = 164	n = 58	n = 52	n = 54
		Mean ± SD			
Age (years)		26.4 ± 4.3	25.0 ± 3.6	28.4 ± 3.6	26.0 ± 4.9
BMI		25.82 ± 2.70	26.31 ± 2.83	26.14 ± 2.35	24.99 ± 2.72
Former smoking					
yes		21 (13%)	7 (12%)	10 (19%)	4 (7.4%)
no		143 (87%)	51 (88%)	42 (81%)	50 (92.6%)
Length of FF career (years)		1.76 ± 2.77	0.88 ± 0.67	4.58 ± 3.43	0.00 ± 0.00
Biomarkers	**Normal range**	**Median ± IQR**			
ALP (μkat/L)	≤2.15	1.17 (1.0–1.3)	1.21 (1.1–1.4)	1.10 × (0.98–1.2)	1.19 (1.0–1.4)
	out of the range:	0.6%	0.0%	0.0%	1.9%
ALT (μkat/L)	≤0.68	0.42 (0.33–0.55)	0.40 (0.31–0.54)	0.48 * (0.38–0.64)	0.41 (0.32–0.49)
	out of the range:	13.4%	13.8%	21.2%	5.6%
AST (μkat/L)	≤0.62	0.47 (0.39–0.59)	0.46 (0.40–0.57)	0.45 (0.38–0.60)	0.50 (0.4–0.62)
	out of the range:	22.6%	20.7%	23.1%	24.1%
GGT (μkat/L)	≤1.00	0.34 (0.26–0.43)	0.33 (0.25–0.40)	0.39 * (0.29–0.53)	0.31 (0.25–0.36)
	out of the range:	1.2%	0.0%	3.8%	0.0%
BIL (μmol/L)	≤18.70	13 (9.0–17)	14 (9.0–17)	13 (9.0–17)	12 (10–16)
	out of the range:	17.7%	19.0%	15.4%	18.5%
CHOL (mmol/L)	≤5.00	4.50 (4.1–5.2)	4.20 (4.0–4.7)	4.90 * (4.4–5.4)	4.45 (3.7–5.2)
	out of the range:	29.3%	12.1%	48.1% ×	29.6%
LDL (mmol/L)	≤3.00	2.8 (2.4–3.3)	2.65 (2.4–3.1)	3.15 * (2.7–3.7)	2.75 (2.3–3.3)
	out of the range:	38.4%	29.3%	55.8% *	31.5%
TAG (mmol/L)	≤1.70	1.06 (0.83–1.4)	1.02 (0.83–1.3)	1.15 (0.87–1.6)	1.02 (0.79–1.4)
	out of the range:	18.3%	15.5%	23.1%	16.7%

Abbreviations: IQR – interquartile range; BMI – body-mass index; FF – firefighting; ALT – alanine aminotransferase; GGT – gamma-glutamyl transferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase, BIL – total bilirubin, CHOL – total cholesterol, LDL – low-density lipoprotein cholesterol, TG – triglycerides.

3.3. Prospective associations

To assess the prospective effects of internal exposure on liver and serum lipids in NEW FF, associations between the exposure from phases 1 and 2 and liver and serum lipids biomarkers from phase 3 were determined. Phase 1 levels of serum PFUnDA and PFDA showed a negative association with ALT. Phase 1 2-OH-FLU and 3-OH-FLU were positively associated with BIL from phase 3. After FDR correction, no associations remained statistically significant (Table S4). Internal exposure from phase 2 was measured 4 h after indoor training with fire; hence, increased concentrations of OH-PAHs in urine were observed in this phase (Table 2). Phase 2 2-OH-NAP and PFHxS showed significant positive and negative associations with ALP from phase 3, respectively. Negative associations were observed between phase 2 2-OH-FLU and 3-OH-FLU and phase 3 CHOL; and between 2/3-OH-PHEN, 2-OH-FLU and 3-OH-FLU and phase 3 LDL. Phase 2 PFNA showed a positive association with BIL. After FDR correction for multiple testing, all associations became insignificant (Table S4).

3.4. Sensitivity analyses

When we stratified our study population into sub-cohorts (NEW, PROF, and CTRL), the cross-sectional associations from MLR indicated similar trends (Table S5). In the NEW FF sub-cohort, we detected positive associations between PFNA, 2/3-OH-PHEN, 2-OH-FLU and 3-OH-FLU and bilirubin, negative associations between 2/3-OH-PHEN and CHOL and LDL, and negative associations between 1-OH-NAP, 2-OH-NAP, 2-OH-FLU, and 3-OH-FLU, and TG. In the case of PROF, positive associations between 1-OH-NAP and ALP, PFDA and LDL, and between 1-OH-NAP, 2/3-OH-PHEN, and 2-OH-FLU and TG were detected. A negative association between PFUnDA and TG was also observed. In the case of CTRL, positive associations between 4 OH-PAHs (1-OH-NAP, 2-OH-NAP, 1-OH-PYR, 2/3-OH-PHEN, 2-OH-FLU) and BIL and between 1-OH-PYR and CHOL were detected. However, after correction for multiplicity, all associations became insignificant (Table S5).

BWQS regressions were run for 3 population subsets, each time excluding one sub-cohort and including the other two (*A* – PROF and NEW FF; *B* –NEW FF and CTRL; *C* – PROF and CTRL). The results from the stratified BWQS models are summarized in Table 4. Regarding subsets A and B, the results are very similar to the main analysis – significant positive association with BIL (β_{A_BIL} = 23.4%, β_{B_BIL} = 38.8%). However, when excluding NEW FF (subset *C* – PROF and CTRL), besides the association with BIL (β_{C_BIL} = 25.8%), the mixture also showed significant positive associations with CHOL and LDL (β_{C_CHOL} = 29.5%, β_{C_LDL} = 26.7%).

4. Discussion

In this original cohort study including occupationally exposed firefighters at different professional stages, we found that exposure to the mixture of PFAS and PAHs is associated with an increase in bilirubin and changes in the lipid serum profile.

In general, lower bilirubin levels and dyslipidaemia are considered indicators of the risk of developing CVDs (Choi et al., 2018; Ekstedt

Table 2
Levels of PFAS in serum and SG-adjusted levels of OH-PAHs in the urine of participants. "*" – significantly different from other sub-cohorts; "(1)" – significantly different from new firefighters in training in phase 1; "(2)"– significantly different from new firefighters in training in phase 3.

	Overall study population	New firefighters in training			Professional firefighters	Controls
	n = 164	n = 58			n = 52	n = 54
	Phase III	Phase I	Phase II	Phase III	Phase III	Phase III
PFAS		**Median (25th – 75th percentile) [ng.mL^{-1}]**				
PFOA	1.03 (0.76–1.3)	1.18 (1.0–1.5)	1.22 (0.88–1.5)	1.12 (0.83–1.3)	1.21 (0.92–1.5)	0.82 * (0.54–1.1)
PFNA	0.36 (0.26–0.45)	0.41 (0.3–0.55)	0.41 (0.32–0.51)	0.39 (0.28–0.46)	0.4 (0.29–0.54)	0.29 * (0.24–0.36)
PFDA	0.16 (0.12–0.22)	0.19 (0.12–0.27)	0.21 (0.15–0.29)	0.18 (0.12–0.26)	0.19 (0.14–0.25)	0.13 * (0.1–0.16)
PFUnDA	0.06 (0.04–0.08)	0.05 (0.02–0.08)	0.07 (0.03–0.09)	0.07 (0.04–0.09)	0.05 (0.04–0.07)	0.06 (0.03–0.08)
PFHxS	0.45 (0.34–0.58)	0.46 (0.35–0.59)	0.45 (0.35–0.55)	0.44 (0.39–0.56)	0.49 (0.38–0.66)	0.43 (0.33–0.54)
PFOS	2.72 (1.9–3.8)	2.82 (2.1–4.0)	3.13 (2.0–4.2)	2.90 (2.0–3.9)	3.22 (2.3–4.8)	2.19 * (1.5–2.7)
∑PFAS	4.78 (3.9–6.4)	5.38 (4.2–6.4)	5.67 (4.1–6.7)	5.00 (4.1–6.5)	5.56 (4.6–7.6)	3.93 * (2.9–4.9)
OH-PAHs		**Median (25th – 75th percentile) [ng.mL^{-1}]**				
1-OH-NAP	2.09 (1.2–3.7)	2.94 (1.6–4.9)	13.3 * (8.6–20)	1.95 (1.2–3.7)	2.43 (1.6–3.9)	1.7 (1) (1.2–3.3)
2–OH–NAP	5.11 (3.3–8.3)	5.48 (3.6–10)	18.1 * (11–27)	5.37 (3.6–8.6)	6.37 (3.8–8.8)	4.81 (3.0–6.6)
2–OH–FLU	0.37 (0.26–0.52)	0.36 (0.30–0.52)	0.99 * (0.69–1.6)	0.38 (0.27–0.54)	0.39 (0.28–0.49)	0.36 (0.22–0.51)
3–OH–FLU	0.08 (0.05–0.15)	0.11 (0.07–0.16)	0.2 * (0.13–0.3)	0.07 (0.05–0.15)	0.11 (0.06–0.16)	0.08 (0.05–0.12)
1–OH–PYR	0.13 (0.08–0.22)	0.15* (0.1–0.24)	0.46 * (0.31–0.63)	0.22 * (0.13–0.33)	0.11 (0.07–0.15)	0.10 (0.06–0.14)
2,3–OH–PHEN	0.24 (0.16–0.36)	0.25 (0.16–0.37)	0.62 * (0.49–0.86)	0.26 (0.18–0.29)	0.20 (2) (0.15–0.3)	0.26 (0.18–0.4)
∑OH–PAHs	8.69 (5.4–13)	10.16 (6.2–18)	37.81 * (21–53)	9.07 (5.5–15)	9.70 (5.6–13)	6.57 (4.8–12)

Abbreviations: IQR – interquartile range; PFAS – perfluoroalkyl substances; PFOA – perfluorooctanoate; PFNA – perfluorononanoate; PFDA – perfluorodecanoate; PFUnDA – perfluoroundecanoate; PFHxS – perfluorohexane sulfonate; PFOS – perfluorooctane sulfonate; OH-PAHs – hydroxylated polycyclic aromatic hydrocarbons; 1-OH-NAP – 1-hydroxynaphtalene; 2-OH-NAP – 2-hydroxynaphtalene; 2-OH-FLU – 2-hydroxyfluorene; 3-OH-FLU – 3-hydroxyfluorene; 1-OH-PYR – 1-hydroxyprene; 2/3-OH-PHEN – 2/3-hydroxyphenanthrene.

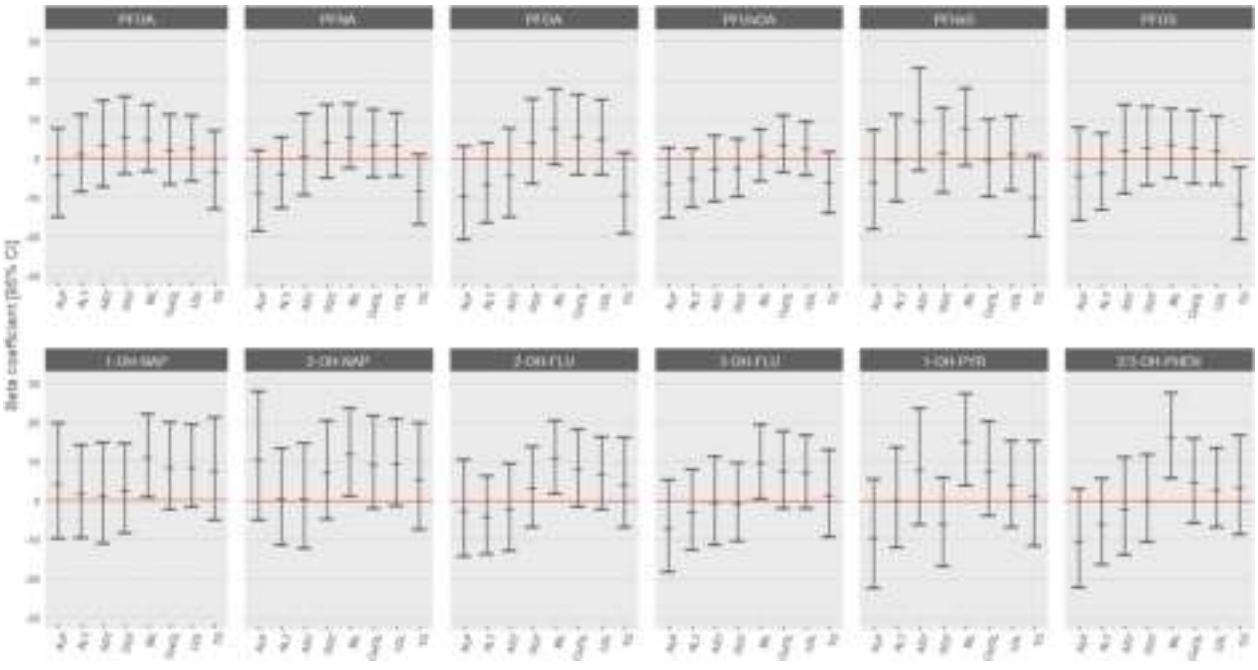

Fig. 2. Adjusted β-coefficients and 95% confidence intervals (CI) between the internal exposure (SG-adjusted urinary levels of OH-PAHs metabolites and serum PFAS levels) and biomarkers of liver function and serum lipids from cross-sectional multiple linear regression models (n = 164). Estimates are expressed as percent change in the median of biomarker upon doubling exposure levels. All models adjusted for age, BMI, former smoking, length of FF career, and study sub-cohort.

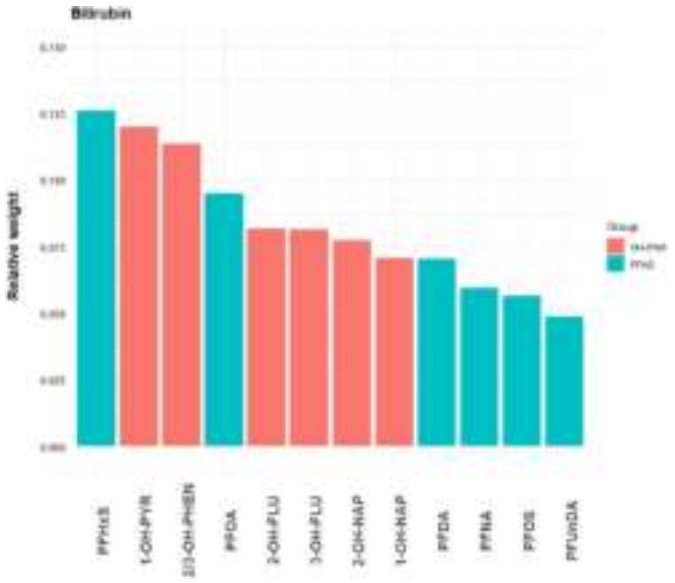

Fig. 3. Bayesian weighted quantile sum regression mixture composition estimates for total bilirubin (weights between 0 and 1).

et al., 2015; Ismaiel and Dumitraşcu, 2019; Méndez-Sánchez et al., 2017; Soderberg et al., 2010). Exposure to PFAS and PAHs, both common environmental pollutants, has been previously associated with the alteration of these biomarkers; however, the associations are not consistent. Firefighters are of particular interest due to their relevant occupational exposure and observed increased incidence of CVD (Soteriades et al., 2011, 2019). In spite of this, relatively few studies have focused on the associations between the exposure of firefighters and CVD biomarkers and risk factors; however, most of them suggest significant associations (Andersen et al., 2018; Oliveira et al., 2020; Semmens et al., 2016). The most robust association observed within this study was the positive association between exposure to the mixture of PFAS and PAHs and total serum bilirubin, tested by BWQS regression and supported by the results from MLR. Biliverdin, a precursor of bilirubin, is a product of the degradation of haemoproteins (e.g., haemoglobin, cytochrome P450) by haem oxygenase (HO), which is subsequently transformed into highly lipophilic bilirubin by biliverdin reductase. In blood, it is bound to the plasma protein albumin and then transported to the liver, where conjugates are formed, mostly bilirubin glucuronide by the action of UDP-glucuronosyltransferase 1, and then excreted via bile (Tomaro et al., 2002). The most active components of the mixture were PFHxS, 1-PYR, and 2/3-OH-PHEN (Fig. 3), which is partially in line with the results from MLR (Table S3). The association was significant also when PFAS and PAH mixtures were assessed individually (Table 3).

Studies focusing on the associations between liver functions and levels of urinary OH-PAHs are available (Alhamdow et al., 2017; Min et al., 2015; Xu et al., 2021); however, none of them focuses on bilirubin as a biomarker. Fortunately, similar trends have been observed in studies with rodents and hence support causality – after the administration of phenanthrene, pyrene, or their ozonized products, levels of bilirubin were increased compared to control treatments (Yoshikawa et al., 1985). Zhu et al. observed increased bilirubin levels after pyrene exposure in adult male rats compared to the control treatment (Zhu et al., 2018). Both studies used pyrene because, in general, pyrene is present in all PAH mixtures at relatively high concentrations, and, since its metabolite 1-OH-pyrene is stable and easy to measure, it is frequently used as a biomarker of exposure to PAHs (Kim et al., 2013).

Regarding PFAS, previous epidemiological studies focusing on PFAS exposure provide evidence suggestive of liver damage due to the alteration of serum enzymes and bilirubin levels associated with PFAS

Table 3
β-coefficients from BWQS mixture models with 95% credibility interval (CrI) for the full mixture (PFAS and OH-PAHs), PFAS-mixture, and OH-PAHs-mixture. All models were adjusted for age, BMI, former smoking, length of FF career, and study sub-cohort (n = 164). β represents a relative change in the median of the biomarker upon a doubling concentration of the mixture. **Bold** and "*" refer to statistically significant results.

	Full mixture				PFAS mixture				OH-PAH mixture			
	β	95% CrI			β	95% CrI			β	95% CrI		
ALP	−10.2	−24.4	7.5		−10.1	−21.0	3.2		−0.9	−12.1	12.0	
ALT	−7.5	−20.2	7.2		−6.5	−16.3	3.6		−1.3	−10.8	8.5	
AST	−3.2	−17.7	14.3		−1.4	−13.1	11.3		−1.8	−11.6	9.4	
GGT	6.4	−7.2	22.4		4.0	−6.9	16.5		3.2	−6.6	13.0	
BIL	**28.6**	**14.6**	**45.7**	*	**11.7**	**1.3**	**23.1**	*	**16.0**	**6.9**	**26.5**	*
CHOL	10.8	−3.0	27.8		2.6	−8.0	14.0		7.5	−1.7	17.0	
LDL	9.9	−4.0	24.2		1.2	−8.2	11.3		7.7	−0.6	16.7	
TG	−11.2	−24.9	5.8		**−14.3**	**−24.4**	**−3.3**	*	3.5	−7.1	15.7	

Abbreviations: PFAS – perfluoroalkyl substances, OH-PAH – hydroxylated polycyclic aromatic hydrocarbons, ALT – alanine aminotransferase, GGT – gamma-glutamyl transferase, AST – aspartate aminotransferase, ALP – alkaline phosphatase, BIL – total bilirubin, CHOL – total cholesterol, LDL – low-density lipoprotein, TG – triglycerides.

Table 4
β-values from BWQSR mixture models with 95% credibility interval (CrI) for the full mixture (PFAS and OH-PAHs) for specific subsets. **A** – new firefighting trainees (NEW FF) and firefighters professionals (PROF), n = 110; **B** – NEW FF and control sub-cohort (CTRL), n = 112; and **C** – PROF and CTRL, n = 106. All models were adjusted for age, BMI, former smoking, length of FF career, and study sub-cohort.

	A				B				C			
	β	95% CrI			β	95% CrI			β	95% CrI		
ALP	−0.5	−18.3	21.1		−18.9	−35.0	1.3		−10.8	−32.4	17.1	
ALT	−11.0	−25.9	6.9		−10.1	−24.4	7.7		−0.5	−18.0	19.7	
AST	−13.4	−29.8	6.9		−5.1	−20.8	13.9		4.7	−15.2	27.3	
GGT	4.1	−13.5	25.8		4.6	−10.0	22.3		15.5	−5.0	40.5	
BIL	**23.4**	**7.5**	**42.2**	*	**38.8**	**21.9**	**57.9**	*	**25.8**	**7.8**	**48.2**	*
CHOL	−1.1	−15.0	14.5		4.1	−11.1	22.0		**29.5**	**10.3**	**53.6**	*
LDL	0.2	−13.3	14.6		3.1	−11.5	20.1		**26.7**	**8.3**	**48.5**	*
TG	−12.4	−27.0	5.5		−13.3	−26.5	3.0		−1.8	−27.9	30.8	

exposure (Costello et al., 2022; Darrow et al., 2016; Gallo et al., 2012a; Gleason et al., 2015a; Lin et al., 2010; Omoike et al., 2021; Salihovic et al., 2018). Our results are in line with observations from the NHANES study, which suggested positive associations of total serum bilirubin with PFOS and PFOA, and an inconsistent positive association with PFHxS (Gleason et al., 2015a). In another study, BIL was positively associated with internal exposure to PFNA and PFHxS (Lin et al., 2010). Gallo et al. observed a clear positive association between bilirubin and serum PFOS levels (Gallo et al., 2012a). Positive associations between BIL and PFHxS, PFNA, PFOA, and PFOS were also observed in the recent NHANES study (Omoike et al., 2021). In contrast, many available studies report inverse associations of bilirubin with serum PFAS levels (Costa et al., 2009; Darrow et al., 2016; Olsen and Zobel, 2007; Sakr et al., 2007b) which is in line with the fact that decreased levels of BIL are associated with an increased risk of CVDs (Méndez-Sánchez et al., 2017).

Such inconsistency among available studies might be explained by the U-shaped relationship between bilirubin and serum PFAS levels, which was already proposed for PFOA (Gallo et al., 2012a). The authors suggest increasing levels of BIL per increasing levels of PFOA at low PFOA levels and decreasing BIL levels for concentrations of PFOA above about 40 ng/mL (Gallo et al., 2012a). This trend can be observed throughout the available studies – results from strongly/occupationally exposed populations with medians in the range of μg/mL show inverse associations (Costa et al., 2009; Darrow et al., 2016; Olsen and Zobel, 2007; Sakr et al., 2007b), while studies focusing on cohorts with milder exposure (in the range of ng/mL) report positive associations (Gallo et al., 2012a; Gleason et al., 2015a; Lin et al., 2010; Omoike et al., 2021).

Besides bilirubin being the end product of haem metabolism, it is also an endogenous antioxidant protectant. It is capable of scavenging hydroxyl (·OH), hydroperoxyl (HO_2·), and superoxide anion (O_2^-·) radicals while oxidising itself to biliverdin, which is thanks to a large excess of bilirubin reductase subsequently regenerated back into bilirubin. This cycle allows nanomolar concentrations of bilirubin (20–50 nM) (Sedlak et al., 2009) to effectively neutralize millimolar concentrations of toxic oxidant agents (Méndez-Sánchez et al., 2017). Bilirubin metabolism depends on several enzymes, such as haem-oxygenase (catalysing the cleavage of the tetrapyrrole ring of haem (Sedlak et al., 2009)), glutathione-S-transferase (binding lipophilic bilirubin in its non-substrate sites and providing the storage of bilirubin within cells (Fukai et al., 1989)) and UDP-glucuronosyltransferase 1 (catalysing the conjugation of bilirubin with glucuronic acid upon excretion via bile (Kapitulnik, 2004)). All three above-mentioned enzymes are of toxicological significance due to their irreplaceable functions in mitigating oxidative stress (defined as the increased production of reactive oxygen species (ROS)), and/or detoxifying xenobiotics in the human body (Dasari et al., 2018; Doré et al., 1999; Guillemette, 2003; Llesuy and Tomaro, 1994). Hence, increased oxidative stress can potentially affect the metabolism of bilirubin on several levels. Both PAHs and PFAS are known for their potential to increase levels of pro-oxidant moieties in the human body (Lin et al., 2020; Oliveira et al., 2020; Omoike et al., 2021; Wielsøe et al., 2015; Yang et al., 2015). Such exposure can trigger an increase in blood bilirubin, a potent antioxidant responding to exposure-related oxidative stress, probably via the induction of haem-oxygenase (Kapitulnik, 2004; Llesuy and Tomaro, 1994; Ryter and Tyrrell, 2000; Tomaro et al., 2002). When considering a complex mixture, our data suggest that already low levels of PFAS and OH-PAHs (in the range of ng/mL, Table 2, Říhačková et al., 2023) can probably initiate detoxifying activities in the human body. When the exposure is more severe (in the range of hundreds to thousands of ng/mL), it can be assumed that the capacity of antioxidant systems becomes exhausted (Bélanger et al., 1997; Niki, 2010; Sedlak and Snyder, 2004) which may lead to hepatotoxic effects, as observed in other studies. The lower exposure levels in participants from this study might also be the reason for there being no robust associations with liver enzymes detected by MLR or BWQS.

When the PFAS mixture was considered separately, a significant negative association with TG was observed (Table 2). Similar results were reported for the Swedish cohort (Donat-Vargas et al., 2019) as well as for prenatally exposed children (Papadopoulou et al., 2021). However, these hypolipidemic effects were not observed in the majority of previous studies, which reported either positive or non-significant associations with serum lipids (Canova et al., 2020; Ho et al., 2022; Sakr et al., 2007a, 2007b; Steenland et al., 2009). PFAS can affect the metabolism of lipids via several non-exclusive mechanisms, mainly in hepatocytes, including the activation of nuclear receptors such as peroxisome proliferator-activated receptor alpha (PPARα), PPARγ, constitutive androstane receptor (CAR), and pregnane X receptor (Andersen et al., 2021; Beggs et al., 2016; Behr et al., 2020; Canova et al., 2020; Fragki et al., 2021; Ho et al., 2022). The results from animal models (including primates) are in line with the findings from our study – inverse associations between the levels of PFAS and serum lipids, including TG (Guruge et al., 2006; Haughom and Spydevold, 1992; Martin et al., 2007; Seacat et al., 2002), suggesting PPARα is a key player in PFAS toxicity (Donat-Vargas et al., 2019). However, the relative importance of these mechanisms in humans is still debatable (Knutsen et al., 2018), mostly due to the differences between human and animal models as well as the potential non-causality of observations (Donat-Vargas et al., 2019; Ho et al., 2022). When PFAS and PAHs were considered together in one complex mixture, the association was not significant, suggesting different modes of action of PFAS and PAHs, a notion supported also by the opposite (positive) directionality of β-coefficients associated with OH-PAHs from BWQS as well as from cross-sectional MLR models (Fig. 2, Table 3). Although non-significant, these observations are in line with available epidemiological studies which suggest that exposure to PAHs in humans is positively associated with early markers of CVD and atherosclerosis, including levels of serum lipids (Alhamdow et al., 2017; Holme et al., 2019; Shahsavani et al., 2021).

In the case of CHOL and LDL, no significant associations resulted from MLR models. The results from the assessment of mixture effects (Table 3) are in line with observations from other studies (Alhamdow et al., 2017; Costa et al., 2009; Dong et al., 2019; Emmett et al., 2006; Holme et al., 2019; Olsen and Zobel, 2007; Sakr et al., 2007a, 2007b; Shahsavani et al., 2021) revealing the positive directionality of β-coefficients between exposure to the PFAS/PAHs mixture and serum CHOL (β = 10.8%) and LDL (β = 9.9%), although the associations were not statistically significant (95% CrI_{CHOL} = −3.0 – 27.8% and 95% CrI_{LDL} = −4.0 – 24.2%). When the NEW FF sub-cohort was excluded as part of the sensitivity analysis, the associations with CHOL and LDL became significant (Table 4). Interestingly, in the case of NEW FF, the prospective associations between phase 2 OH-PAHs and phase 3 CHOL and LDL were negative (Table S4), while mostly positive tendencies were observed in cross-sectional sensitivity analyses in the PROF and CTRL sub-cohorts (Table S5). In rodents, oral exposure to PAHs caused the dysregulation of lipid metabolism by altering the expression of genes responsible for *de novo* fatty acid synthesis and the accumulation of lipids in hepatocytes, suggesting the increased uptake of lipids from the blood by the liver (Jin et al., 2014; Li et al., 2019, 2020a). The alteration of serum lipids via this pathway due to the single-point exposure of NEW FF during indoor firefight training in the 6th week of the study (phase 2) might be an explanation for the observed trends that were inconsistent with other sub-cohorts. Hence, it can be hypothesised that results from the prospective analysis of NEW FF reflect a biological response to a short-term single exposure of higher magnitude (phase 2), and that this single exposure also influenced the results from the cross-sectional analysis of NEW FF in phase 3. In contrast, it can be assumed that the results from PROF and CTRL reflect a biological response to rather long-term stable exposure. However, in NEW FF, it cannot be ruled out that the observed results may have been affected by reduced sample size (n = 58) and by differences in the metabolization and excretion rate of PAHs due to the intensive physical training undertaken by participants before and during

the study period, which might have affected basal metabolic rates, since the confounding variable of physical activity was not included in the models (Durand et al., 2011; Speakman and Selman, 2003).

Levels of BIL, CHOL, and LDL are considered risk factors for CVDs; hence, the results from this study suggest that individuals exposed to higher levels of PFAS and PAHs are more prone to develop an unfavourable cardiometabolic profile in terms of the exhaustion of bilirubin antioxidant capacity and the alteration of serum lipid levels, both leading to an increased risk of developing CVDs in the future. Firefighters are among these individuals because firefighting as well as firefighting training increase internal exposure to PFAS and/or PAHs (Barros et al., 2021; Clarity et al., 2021; Durand et al., 2011; Fent et al., 2020; Jin et al., 2011; Laitinen et al., 2012; Laitinen et al., 2014a; Oliveira et al., 2020). There is a need to continuously monitor such exposure, identify the exposure sources (during both on- and off-duty periods), and minimise it. It is essential to communicate this information about potential health risks and how to reduce them through safety training with both firefighters and policymakers. Since PAHs and PFAS are omnipresent environmental pollutants (Giesy and Kannan, 2002; Kim et al., 2013), not only firefighters, but also other occupationally and non-occupationally exposed individuals (e.g., people living in contamination hot-spots (McMahon et al., 2022), production workers (Sakr et al., 2007a), coke-oven workers, and chimney sweeps (Wagner et al., 2015)) might face increased health risks.

The major strengths of this study can be summarized in 3 points:

a) the use of the cohort from central Europe with a special focus on the occupational exposure of firefighters including in total 110 firefighters at various professional stages, which is rare due to challenges arising from collaboration with fire rescue teams;
b) the collection of a rich dataset of both exposure biomarkers (6 PFAS in blood and 6 OH-PAHs in urine) and biomarkers of liver function and serum lipids accompanied with data from questionnaires;
c) the use of a complex statistical approach, including the assessment of associations of liver function and serum lipids biomarkers with both individual compounds by means of linear regression with adjustments for multiple comparisons, and their mixtures by means of Bayesian weighted quantile sum regression.

In terms of limitations of the study, despite the high participation of firefighters, the sample size was from the statistical point of view relatively small, which limited drawing firm conclusions, particularly for the stratified analyses. Small sample size along with homogeneity of the study population also limited generalization to the whole Czech male population. Although the models presented in this paper were controlled for factors such as age, BMI, former smoking, and others, residual confounding by unmeasured factors, such as physical activity and fitness, cannot be excluded. Despite the proposed molecular mechanisms, the causality of the observed associations cannot be clearly confirmed due to cross-sectional character of analyses or limited sample size in case of prospective analyses. Lastly, until recently women did not participate in firefighting in the Czech Republic, hence, only men were included in this study. However, in August 2022, the first woman qualified as a professional firefighter and began to participate in incident responses, hence, more women are expected to follow. Due to sex-related effects of PFAS (Sen et al., 2022), specific monitoring of female firefighters in Czech Republic will soon be required.

5. Conclusion

There are emerging gaps in the occupational epidemiology of firefighting, one of the most hazardous occupations. This study investigated the effects of PAH and PFAS exposure on liver function and serum lipids, with a special focus on firefighters at different professional stages. The findings from studying both the effects of individual compounds (by means of linear regression) as well as the effects of complex mixtures (by means of Bayesian weighted quantile sum regression) suggest that increased exposure to these compounds, typical in firefighters, is associated with increased levels of bilirubin (a potent antioxidant with a proposed U-shaped dose-response curve) and increased levels of total cholesterol and low-density lipoprotein (risk factors for developing cardiovascular diseases). Due to the low sample size and the cross-sectional design of the study, further research is required to confirm the associations observed.

Ethics approval

The study was approved by the ELSPAC Ethics Committee, ethical approval number No: ELSPAC/EK/1/2019. All participants received an information brochure and participated in personal interviews in order to be fully informed about the study and their participation. In addition, informed consent was obtained from each participant before participation began. All data were pseudonymized to protect the identity of the participants.

CRediT author contributions

The authors made substantial contributions to the acquisition, analysis, and interpretation of the data and the drafting and revision of the manuscript. All authors also approved the final version of the paper and agreed to be accountable for all aspects of the work.

N.P.: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing – Original Draft, Visualisation; **L.M.**: Methodology, Investigation, Writing – Review & Editing; **N.S.**: Methodology, Investigation, Writing – Review & Editing, **K.Ř.**: Conceptualization, Investigation, Visualisation, Writing – Review & Editing; **A.P.**: Conceptualization, Investigation, Writing – Review & Editing; **J.K.**: Methodology, Validation, Writing – Review & Editing, **P.Š.**: Methodology, Validation, Writing – Review & Editing; **A. B. P.**: Methodology, Investigation, Writing – Review & Editing; **P.G.**: Methodology, Visualisation, Writing – Review & Editing; **M.V.**: Methodology, Resources, Investigation, Writing – Review & Editing, Supervision; **P.Č.**: Methodology, Resources, Investigation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

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Declaration of competing interest

The authors declare that they have no known competing financial

interests nor personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2023.114215.

References

Alhamdow, A., Lindh, C., Albin, M., Gustavsson, P., Tinnerberg, H., Broberg, K., 2017. Early markers of cardiovascular disease are associated with occupational exposure to polycyclic aromatic hydrocarbons. Sci. Rep. 7 (1), 1–12. https://doi.org/10.1038/s41598-017-09956-x.

Alshaarawy, O., Elbaz, H.A., Andrew, M.E., 2016. The association of urinary polycyclic aromatic hydrocarbon biomarkers and cardiovascular disease in the US population. Environ. Int. 89 (90), 174–178. https://doi.org/10.1016/j.envint.2016.02.006.

Andersen, M.E., Hagenbuch, B., Apte, U., Corton, J.C., Fletcher, T., Lau, C., Roth, W.L., Staels, B., Vega, G.L., Clewell, H.J., Longnecker, M.P., 2021. Why is elevation of serum cholesterol associated with exposure to perfluoroalkyl substances (PFAS) in humans? A workshop report on potential mechanisms. Toxicology 459. https://doi.org/10.1016/j.tox.2021.152845.

Andersen, M.H.G., Saber, A.T., Clausen, P.A., Pedersen, J.E., Løhr, M., Kermanizadeh, A., Loft, S., Ebbehøj, N., Hansen, Å.M., Pedersen, P.B., Koponen, I.K., Nørskov, E.C., Møller, P., Vogel, U., 2018. Association between polycyclic aromatic hydrocarbon exposure and peripheral blood mononuclear cell DNA damage in human volunteers during fire extinction exercises. Mutagenesis 33 (1), 105–115. https://doi.org/10.1093/mutage/gex021.

Attanasio, R., 2019. Sex differences in the association between perfluoroalkyl acids and liver function in US adolescents: analyses of NHANES 2013–2016. Environ. Pollut. 254, 113061 https://doi.org/10.1016/j.envpol.2019.113061.

Banks, A.P.W., Thai, P., Engelsman, M., Wang, X., Osorio, A.F., Mueller, J.F., 2021. Characterising the exposure of Australian firefighters to polycyclic aromatic hydrocarbons generated in simulated compartment fires. Int. J. Hyg Environ. Health 231 (August 2020), 113637. https://doi.org/10.1016/j.ijheh.2020.113637.

Barros, B., Oliveira, M., Morais, S., 2021. Firefighters' occupational exposure: contribution from biomarkers of effect to assess health risks. Environ. Int. 156 (June) https://doi.org/10.1016/j.envint.2021.106704.

Baxter, C.S., Hoffman, J.D., Knipp, M.J., Reponen, T., Haynes, E.N., 2014. Exposure of firefighters to particulates and polycyclic aromatic hydrocarbons. J. Occup. Environ. Hyg. 11 (7), 37–41. https://doi.org/10.1080/15459624.2014.890286.

Beggs, K.M., McGreal, S.R., McCarthy, A., Gunewardena, S., Lampe, J.N., Lau, C., Apte, U., 2016. The role of hepatocyte nuclear factor 4-alpha in perfluorooctanoic acid- and perfluorooctanesulfonic acid-induced hepatocellular dysfunction. Toxicol. Appl. Pharmacol. 304, 18–29. https://doi.org/10.1016/j.taap.2016.05.001.

Behr, A.C., Plinsch, C., Braeuning, A., Buhrke, T., 2020. Activation of human nuclear receptors by perfluoroalkylated substances (PFAS). Toxicol. Vitro 62. https://doi.org/10.1016/j.tiv.2019.104700.

Bélanger, S., Lavoie, J.-C., Chessex, P., 1997. Influence of bilirubin on the antioxidant capacity of plasma in newborn infants. Biol. Neonate 71, 233–238. https://doi.org/10.1159/000244422.

Benjamini, Y., Yekutieli, D., 2005. False discovery rate-adjusted multiple confidence intervals for selected parameters. J. Am. Stat. Assoc. 100 (Issue 469), 71–81. https://doi.org/10.1198/016214504000001907. American Statistical Association.

Bessonneau, V., Gerona, R.R., Trowbridge, J., Grashow, R., Lin, T., Buren, H., Morello-Frosch, R., Rudel, R.A., 2021. Gaussian graphical modeling of the serum exposome and metabolome reveals interactions between environmental chemicals and endogenous metabolites. Sci. Rep. 11 (1), 1–15. https://doi.org/10.1038/s41598-021-87070-9.

Brucker, N., Charão, M.F., Moro, A.M., Ferrari, P., Bubols, G., Sauer, E., Fracasso, R., Durgante, J., Thiesen, F.v., Duarte, M.M., Gioda, A., Castro, I., Saldiva, P.H., Garcia, S.C., 2014. Atherosclerotic process in taxi drivers occupationally exposed to air pollution and co-morbidities. Environ. Res. 131, 31–38. https://doi.org/10.1016/j.envres.2014.02.012.

Canova, C., Barbieri, G., Zare Jeddi, M., Gion, M., Fabricio, A., Daprà, F., Russo, F., Fletcher, T., Pitter, G., 2020. Associations between perfluoroalkyl substances and lipid profile in a highly exposed young adult population in the Veneto Region. Environ. Int. 145 https://doi.org/10.1016/j.envint.2020.106117.

CDC's National Center for Environmental Health (NCEH), 2013. Laboratory Procedure Man- ual. Analyte:Polyfluoroalkyl chemicals: perfluorooctane sulfonamide, 2-(Nmethyl- perfluorooctane sulfonamido) acetate, 2-(N-ethylperfluorooctane sulfonamido) acetate, perfluorobutane sulfonate, perfluorohexane sulfonate, perfluorooc [Internet]. Available from: https://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/pfc_g_met.pdf.

CDC's National Center for Environmental Health (NCEH). Laboratory procedure manual. Analyte: eight monohydroxy-polycyclic aromatic hydrocarbons: 1-hydroxynaphthalene, 2- hydroxynaphthalene, 2-hydroxyfluorene, 3- hydroxyfluorene, 1-hydroxyphenanthrene, 2- & 3-hydroxyphenanthrene, 1-hydroxypyrene. Matrix: urine [Internet]. Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/abmethods/PAH_H_MET_Aromatic_Hydrocarbons.pdf.

Choi, K.M., Han, K., Park, S., Chung, H.S., Kim, N.H., Yoo, H.J., Seo, J.A., Kim, S.G., Kim, N.H., Baik, S.H., Park, Y.G., Kim, S.M., 2018. Implication of liver enzymes on incident cardiovascular diseases and mortality: a nationwide population-based cohort study. Sci. Rep. 8 (1), 1–9. https://doi.org/10.1038/s41598-018-19700-8.

Clarity, C., Trowbridge, J., Gerona, R., Ona, K., McMaster, M., Bessonneau, V., Rudel, R., Buren, H., Morello-Frosch, R., 2021. Associations between polyfluoroalkyl substance and organophosphate flame retardant exposures and telomere length in a cohort of women firefighters and office workers in San Francisco. Environ. Health: A Global Access Science Source 20 (1), 1–14. https://doi.org/10.1186/s12940-021-00778-z.

Colicino, E., Pedretti, N.F., Busgang, S.A., Gennings, C., 2020. Per- and poly-fluoroalkyl substances and bone mineral density: results from the Bayesian weighted quantile sum regression. Environmental Epidemiology 4 (3). https://doi.org/10.1097/EE9.0000000000000092.

Costa, G., Sartori, S., Consonni, D., 2009. Thirty years of medical surveillance in perfluooctanoic acid production workers. J. Occup. Environ. Med. 51 (3), 364–372. https://doi.org/10.1097/JOM.0b013e3181965d80.

Costello, E., Rock, S., Stratakis, N., Eckel, S.P., Walker, D.I., Valvi, D., Cserbik, D., Jenkins, T., Xanthakos, S.A., Kohli, R., Sisley, S., Vasiliou, V., Merrill, M. A. la, Rosen, H., Conti, D. v, Mcconnell, R., Chatzi, L., 2022. Exposure to per- and polyfluoroalkyl substances and markers of liver injury. A Systematic Review and Meta-Analysis 130 (April). https://doi.org/10.1289/EHP10092.

Darrow, L.A., Groth, A.C., Winquist, A., Shin, H.M., Bartell, S.M., Steenland, K., 2016. Modeled perfluorooctanoic acid (PFOA) exposure and liver function in a Mid-Ohio Valley community. Environ. Health Perspect. 124 (8), 1227–1233. https://doi.org/10.1289/ehp.1510391.

Dasari, S., Ganjayi, M.S., Yellanurkonda, P., Basha, S., Meriga, B., 2018. Role of glutathione S-transferases in detoxification of a polycyclic aromatic hydrocarbon, methylcholanthrene. Chem. Biol. Interact. 294, 81–90. https://doi.org/10.1016/j.cbi.2018.08.023. Elsevier Ireland Ltd.

Dobraca, D., Israel, L., McNeel, S., Voss, R., Wang, M., Gajek, R., Park, J.S., Harwani, S., Barley, F., She, J., Das, R., 2015. Biomonitoring in California firefighters: metals and perfluorinated chemicals. J. Occup. Environ. Med. 57 (1), 88–97. https://doi.org/10.1097/JOM.0000000000000307.

Donat-Vargas, C., Bergdahl, I.A., Tornevi, A., Wennberg, M., Sommar, J., Koponen, J., Kiviranta, H., Åkesson, A., 2019. Associations between repeated measure of plasma perfluoroalkyl substances and cardiometabolic risk factors. Environ. Int. 124, 58–65. https://doi.org/10.1016/j.envint.2019.01.007.

Dong, Z., Wang, H., Yu, Y.Y., Li, Y.B., Naidu, R., Liu, Y., 2019. Using 2003–2014 U.S. NHANES data to determine the associations between per- and polyfluoroalkyl substances and cholesterol: trend and implications. Ecotoxicol. Environ. Saf. 173, 461–468. https://doi.org/10.1016/j.ecoenv.2019.02.061.

Doré, S., Takahashi, M., Ferris, C.D., Hester, L.D., Guastella, D., Snyder, S.H., 1999. Bilirubin, formed by activation of heme oxygenase-2, protects neurons against oxidative stress injury. Neurobiology 96. https://doi.org/10.1073/pnas.96.5.2445.

Durand, G., Tsismenakis, A.J., Jahnke, S.A., Baur, D.M., Christophi, C.A., Kales, S.N., 2011. Firefighters' physical activity: relation to fitness and cardiovascular disease risk. Med. Sci. Sports Exerc. 43 (9), 1752–1759. https://doi.org/10.1249/MSS.0b013e318215cf25.

Ekpe, O.D., Sim, W., Choi, S., Choo, G., Oh, J.E., 2021. Assessment of exposure of Korean firefighters to polybrominated diphenyl ethers and polycyclic aromatic hydrocarbons via their measurement in serum and polycyclic aromatic hydrocarbon metabolites in urine. Environ. Sci. Technol. 55 (20), 14015–14025. https://doi.org/10.1021/acs.est.1c02554.

Ekstedt, M., Hagström, H., Nasr, P., Fredrikson, M., Stål, P., Kechagias, S., Hultcrantz, R., 2015. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology 61 (5), 1547–1554. https://doi.org/10.1002/hep.27368.

Emmett, E.A., Zhang, H., Shofer, F.S., Freeman, D., Rodway, N.V., Desai, C., Shaw, L.M., 2006. Community exposure to perfluorooctanoate: relationships between serum

levels and certain health parameters. J. Occup. Environ. Med. 48 (8), 771–779. https://doi.org/10.1097/01.jom.0000233380.13087.37.
Fent, K.W., Toennis, C., Sammons, D., Robertson, S., Bertke, S., Calafat, A.M., Pleil, J.D., Geer Wallace, M.A., Kerber, S., Smith, D.L., Horn, G.P., 2019. Firefighters' and instructors' absorption of PAHs and benzene during training exercises. Int. J. Hyg Environ. Health 222 (7), 991–1000. https://doi.org/10.1016/j.ijheh.2019.06.006.
Fent, K.W., Toennis, C., Sammons, D., Robertson, S., Bertke, S., Calafat, A.M., Pleil, J.D., Wallace, M.A.G., Kerber, S., Smith, D., Horn, G.P., 2020. Firefighters' absorption of PAHs and VOCs during controlled residential fires by job assignment and fire attack tactic. J. Expo. Sci. Environ. Epidemiol. 30 (2), 338–349. https://doi.org/10.1038/s41370-019-0145-2.
Fenton, S.E., Ducatman, A., Boobis, A., DeWitt, J.C., Lau, C., Ng, C., Smith, J.S., Roberts, S.M., 2021. Per- and polyfluoroalkyl substance toxicity and human health review: current state of knowledge and strategies for informing future research. Environ. Toxicol. Chem. 40 (3), 606–630. https://doi.org/10.1002/etc.4890.
Fragki, S., Dirven, H., Fletcher, T., Grasl-Kraupp, B., Bjerve Gützkow, K., Hoogenboom, R., Kersten, S., Lindeman, B., Louisse, J., Peijnenburg, A., Piersma, A. H., Princen, H.M.G., Uhl, M., Westerhout, J., Zeilmaker, M.J., Luijten, M., 2021. Systemic PFOS and PFOA exposure and disturbed lipid homeostasis in humans: what do we know and what not? Crit. Rev. Toxicol. 51 (Issue 2), 141–164. https://doi.org/10.1080/10408444.2021.1888073. Taylor and Francis Ltd.
Fukai, F., Yatomi, S., Morita, T., Nishizawa, S., Nagai, T., Katayama, T., 1989. Protection of glutathione S-transferase from bilirubin inhibition. J. Biochem. 105 https://doi.org/10.1093/oxfordjournals.jbchem.a122789.
Gallo, V., Leonardi, G., Genser, B., Frisbee, S.J., 2012a. Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) concentrations and liver function biomarkers in a population with elevated PFOA exposure. Environ. Health Perspect. 120 (5), 655–660.
Giesy, J.P., Kannan, K., 2002. Perfluorochemical Surfactants in the Environment. Environmental Science & Technology. https://doi.org/10.1021/es022253t.
Gleason, J.A., Post, G.B., Fagliano, J.A., 2015a. Associations of perfluorinated chemical serum concentrations and biomarkers of liver function and uric acid in the US population (NHANES), 2007-2010. Environ. Res. 136, 8–14. https://doi.org/10.1016/j.envres.2014.10.004.
Guillemette, C., 2003. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. Pharmacogenomics J. 3 (3), 136–158. https://doi.org/10.1038/sj.tpj.6500171.
Guruge, K.S., Yeung, L.W.Y., Yamanaka, N., Miyazaki, S., Lam, P.K.S., Giesy, J.P., Jones, P.D., Yamashita, N., 2006. Gene expression profiles in rat liver treated with perfluorooctanoic acid (PFOA). Toxicol. Sci. 89 (1), 93–107. https://doi.org/10.1093/toxsci/kfj011.
Haughom, B., Spydevold, O., 1992. The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and ciofibric acid. Biochim. Biophys. Acta 1128. https://doi.org/10.1016/0005-2760(92)90258-W.
Ho, S.H., Soh, S.X.H., Wang, M.X., Ong, J., Seah, A., Wong, Y., Fang, Z., Sim, S., Lim, J.T., 2022. Perfluoroalkyl substances and lipid concentrations in the blood: a systematic review of epidemiological studies. Sci. Total Environ. 850, 158036 https://doi.org/10.1016/j.scitotenv.2022.158036. NLM (Medline).
Holme, J.A., Brinchmann, B.C., Refsnes, M., Låg, M., Øvrevik, J., 2019. Potential role of polycyclic aromatic hydrocarbons as mediators of cardiovascular effects from combustion particles. Environ. Health: A Global Access Science Source 18 (Issue 1). https://doi.org/10.1186/s12940-019-0514-2. BioMed Central Ltd.
Ismaiel, A., Dumitraşcu, D.L., 2019. Cardiovascular risk in fatty liver disease: the liver-heart Axis—literature review. Front. Med. 6 (September), 1–18. https://doi.org/10.3389/fmed.2019.00202.
Jin, C., Sun, Y., Islam, A., Qian, Y., Ducatman, A., 2011. Perfluoroalkyl Acids Including Perfluorooctane Sulfonate and Perfluorohexane Sulfonate in Firefighters 53 (3), 324–328.
Jin, Y., Miao, W., Lin, X., Wu, T., Shen, H., Chen, S., Li, Y., Pan, Q., Fu, Z., 2014. Sub-chronically exposing mice to a polycyclic aromatic hydrocarbon increases lipid accumulation in their livers. Environ. Toxicol. Pharmacol. 38 (2), 353–363. https://doi.org/10.1016/j.etap.2014.07.014.
Kapitulnik, J., 2004. Bilirubin: an endogenous product of heme degradation with both cytotoxic and cytoprotective properties. Mol. Pharmacol. 66 (4), 773–779. https://doi.org/10.1124/mol.104.002832.
Khalil, N., Ducatman, A.M., Sinari, S., Billheimer, D., Hu, C., Sally, L., Burgess, J.L., 2022. Per- and polyfluoroalkyl substance and cardio metabolic markers in firefighters 62 (12), 1076–1081. https://doi.org/10.1097/JOM.0000000000002062.
Kim, K.H., Jahan, S.A., Kabir, E., Brown, R.J.C., 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ. Int. 60, 71–80. https://doi.org/10.1016/j.envint.2013.07.019.
Knutsen, H.K., Alexander, J., Barregård, L., Bignami, M., Brüschweiler, B., Ceccatelli, S., Cottrill, B., Dinovi, M., Edler, L., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. (Ron), Nebbia, C.S., Oswald, I.P., Petersen, A., Rose, M., Roudot, A.C., Vleminckx, C., Vollmer, G., et al., 2018. Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA J. 16 (12) https://doi.org/10.2903/j.efsa.2018.5194.
Laitinen, J.A., Koponen, J., Koikkalainen, J., Kiviranta, H., 2014a. Firefighters' exposure to perfluoroalkyl acids and 2-butoxyethanol present in firefighting foams. Toxicol. Lett. 231 (2), 227–232. https://doi.org/10.1016/j.toxlet.2014.09.007.
Laitinen, J., Mäkelä, M., Mikkola, J., Huttu, I., 2012. Firefighters' multiple exposure assessments in practice. Toxicol. Lett. 213 (1), 129–133. https://doi.org/10.1016/j.toxlet.2012.06.005.
Li, F., Xiang, B., Jin, Y., Li, C., Li, J., Ren, S., Huang, H., Luo, Q., 2019. Dysregulation of lipid metabolism induced by airway exposure to polycyclic aromatic hydrocarbons in C57BL/6 mice. Environ. Pollut. 245, 986–993. https://doi.org/10.1016/j.envpol.2018.11.049.
Li, F., Xiang, B., Jin, Y., Li, C., Ren, S., Wu, Y., Li, J., Luo, Q., 2020b. Hepatotoxic effects of inhalation exposure to polycyclic aromatic hydrocarbons on lipid metabolism of C57BL/6 mice. Environ. Int. 134, 105000 https://doi.org/10.1016/j.envint.2019.105000. June 2019.
Li, Y., Barregard, L., Xu, Y., Scott, K., Pineda, D., Lindh, C.H., Jakobsson, K., Fletcher, T., 2020a. Associations between perfluoroalkyl substances and serum lipids in a Swedish adult population with contaminated drinking water. Environ. Health: A Global Access Science Source 19 (1), 1–11. https://doi.org/10.1186/s12940-020-00588-9.
Lin, C.Y., Lee, H.L., Hwang, Y.T., Su, T.C., 2020. The association between total serum isomers of per- and polyfluoroalkyl substances, lipid profiles, and the DNA oxidative/nitrative stress biomarkers in middle-aged Taiwanese adults. Environ. Res. 182 https://doi.org/10.1016/j.envres.2019.109064.
Lin, C.Y., Lin, L.Y., Chiang, C.K., Wang, W.J., Su, Y.N., Hung, K.Y., Chen, P.C., 2010. Investigation of the associations between low-dose serum perfluorinated chemicals and liver enzymes in US adults. Am. J. Gastroenterol. 105 (6), 1354–1363. https://doi.org/10.1038/ajg.2009.707.
Llesuy, S.F., Tomaro, M.L., 1994. Heme oxygenase and oxidative stress. Evidence of involvement of bilirubin as physiological protector against oxidative damage. Biochim. Biophys. Acta 1223, 9–14. https://doi.org/10.1016/0167-4889(94)90067-1.
Lubin, J.H., Colt, J.S., Camann, D., Davis, S., Cerhan, J.R., Severson, R.K., Bernstein, L., Hartge, P., 2004. Epidemiologic evaluation of measurement data in the presence of detection limits. Environ. Health Perspect. 112 (17), 1691–1696. https://doi.org/10.1289/ehp.7199.
Maitre, L., Guimbaud, J.B., Warembourg, C., Güil-Oumrait, N., Petrone, P.M., Chadeau-Hyam, M., Vrijheid, M., Basagaña, X., Gonzalez, J.R., The Esposome Data Challenge Participant Consortium, 2022. State-of-the-art methods for exposure-health studies: results from the exposome data challenge event. Environ. Int. 168 https://doi.org/10.1016/j.envint.2022.107422.
Martin, M.T., Brennan, R.J., Hu, W., Ayanoglu, E., Lau, C., Ren, H., Wood, C.R., Corton, J.C., Kavlock, R.J., Dix, D.J., 2007. Toxicogenomic study of triazole fungicides and perfluoroalkyl acids in rat livers predicts toxicity and categorizes chemicals based on mechanisms of toxicity. Toxicol. Sci. 97 (2), 595–613. https://doi.org/10.1093/toxsci/kfm065.
McMahon, P.B., Tokranov, A.K., Bexfield, L.M., Lindsey, B.D., Johnson, T.D., Lombard, M.A., Watson, E., 2022. Perfluoroalkyl and polyfluoroalkyl substances in groundwater used as a source of drinking water in the eastern United States. Environ. Sci. Technol. 56 (4), 2279–2288. https://doi.org/10.1021/acs.est.1c04795.
Méndez-Sánchez, N., Vítek, L., Aguilar-Olivos, N.E., Uribe, M., 2017. Bilirubin as a biomarker in liver disease. In: Patel, Vinood B., Preedy, Vicotr R. (Eds.), Biomarkers in Liver Disease. https://doi.org/10.1007/978-94-007-7675-3.
Min, Y.S., Lim, H.S., Kim, H., 2015. Biomarkers for polycyclic aromatic hydrocarbons and serum liver enzymes. Am. J. Ind. Med. 58 (7), 764–772. https://doi.org/10.1002/ajim.22463.
Moorthy, B., Chu, C., Carlin, D.J., 2015. Polycyclic aromatic hydrocarbons: from metabolism to lung cancer. Toxicol. Sci. 145 (1), 5–15. https://doi.org/10.1093/toxsci/kfv040.
Niki, E., 2010. Assessment of antioxidant capacity in vitro and in vivo. Free Radic. Biol. Med. 49 (4), 503–515. https://doi.org/10.1016/j.freeradbiomed.2010.04.016.
Oliveira, M., Costa, S., Vaz, J., Fernandes, A., Slezakova, K., Delerue-Matos, C., Teixeira, J.P., Carmo Pereira, M., Morais, S., 2020. Firefighters exposure to fire emissions: impact on levels of biomarkers of exposure to polycyclic aromatic hydrocarbons and genotoxic/oxidative-effects. J. Hazard Mater. 383 https://doi.org/10.1016/j.jhazmat.2019.121179. March 2019.
Olsen, G.W., Zobel, L.R., 2007. Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers. Int. Arch. Occup. Environ. Health 81 (2), 231–246. https://doi.org/10.1007/s00420-007-0213-0.
Omoike, O.E., Pack, R.P., Mamudu, H.M., Liu, Y., Strasser, S., Zheng, S., Okoro, J., Wang, L., 2021. Association between per and polyfluoroalkyl substances and markers of inflammation and oxidative stress. Environ. Res. 196 https://doi.org/10.1016/j.envres.2020.110361.
Papadopoulou, E., Stratakis, N., Basaga, X., Brantsæter, A.L., Casas, M., Fossati, S., Gražulevičiene, R., Haug, L.S., Heude, B., Maitre, L., McEachan, R.R.C., Robinson, O., Roumeliotaki, T., Sabidó, E., Borràs, E., Urquiza, J., Vafeiadi, M., Zhao, Y., Slama, R., Wright, J., Conti, D.V., Vrijheid, M., Chatzi, L., 2021. Prenatal and postnatal exposure to PFAS and cardiometabolic factors and inflammation status in children from six European cohorts cien e. Environ. Int. 157 (September) https://doi.org/10.1016/j.envint.2021.106853.
Park, J.S., Voss, R.W., McNeel, S., Wu, N., Guo, T., Wang, Y., Israel, L., Das, R., Petreas, M., 2015. High exposure of California firefighters to polybrominated diphenyl ethers. Environ. Sci. Technol. 49 (5), 2948–2958. https://doi.org/10.1021/es5055918.
Pedretti, N.F., Colicino, E., 2021. BWQS: Bayesian Weighted Quantile Sum Regression (R Package, version 1.0.0.
Pitter, G., Zare Jeddi, M., Barbieri, G., Gion, M., Fabricio, A.S.C., Daprà, F., Russo, F., Fletcher, T., Canova, C., 2020. Perfluoroalkyl substances are associated with elevated blood pressure and hypertension in highly exposed young adults. Environ. Health: A Global Access Science Source 19 (1), 1–11. https://doi.org/10.1186/s12940-020-00656-0.
Rossbach, B., Wollschläger, D., Letzel, S., Gottschalk, W., Muttray, A., 2020. Internal exposure of firefighting instructors to polycyclic aromatic hydrocarbons (PAH)

during live fire training. Toxicol. Lett. 331, 102–111. https://doi.org/10.1016/j.toxlet.2020.05.024. November 2019.
Rotander, A., Kärrman, A., Toms, L.M.L., Kay, M., Mueller, J.F., Gómez Ramos, M.J., 2015a. Novel fluorinated surfactants tentatively identified in firefighters using liquid chromatography quadrupole time-of-flight tandem mass spectrometry and a case-control approach. Environ. Sci. Technol. 49 (4), 2434–2442. https://doi.org/10.1021/es503653n.
Rotander, A., Toms, L.M.L., Aylward, L., Kay, M., Mueller, J.F., 2015b. Elevated levels of PFOS and PFHxS in firefighters exposed to aqueous film forming foam (AFFF). Environ. Int. 82, 28–34. https://doi.org/10.1016/j.envint.2015.05.005.
Rstudio Team, 2020. Rstudio. Integrated Development for R. Rstudio, PBC, Boston MA. http://www.rstudio.com/.
Ryter, S.W., Tyrrell, R.M., 2000. The heme synthesis and degradation pathways: role in oxidant sensitivity: heme oxygenase has both pro-and antioxidant properties. Free Radical Biol. Med. 28 (2), 289–309. https://doi.org/10.1016/S0891-5849(99)00223-3.
Řiháčková, K., Pindur, A., Komprdová, K., Pálešová, N., Kohoutek, J., Šenk, P., Navrátilová, J., Andrýsková, L., Hůlek, R., Ismael, M., Čupr, P., 2023. The exposure of Czech firefighters to perfluoroalkyl substances and polycyclic aromatic hydrocarbons: CELSPAC – FIREexpo case-control human biomonitoring study. Sci. Total Environ. 881 (April) https://doi.org/10.1016/j.scitotenv.2023.163298.
Sakr, C.J., Kreckmann, K.H., Green, J.W., Gillies, P.J., Reynolds, J.L., Leonard, R.C., 2007a. Cross-sectional study of lipids and liver enzymes related to a serum biomarker of exposure (ammonium perfluorooctanoate or APFO) as part of a general health survey in a cohort of occupationally exposed workers. J. Occup. Environ. Med. 49 (10), 1086–1096. https://doi.org/10.1097/JOM.0b013e318156eca3.
Sakr, C.J., Leonard, R.C., Kreckmann, K.H., Slade, M.D., Cullen, M.R., 2007b. Longitudinal study of serum lipids and liver enzymes in workers with occupational exposure to ammonium perfluorooctanoate. J. Occup. Environ. Med. 49 (8), 872–879. https://doi.org/10.1097/JOM.0b013e318124a93f.
Salihovic, S., Stubleski, J., Kärrman, A., Larsson, A., Fall, T., Lind, L., Lind, P.M., 2018. Changes in markers of liver function in relation to changes in perfluoroalkyl substances - a longitudinal study. Environ. Int. 117 (January), 196–203. https://doi.org/10.1016/j.envint.2018.04.052.
Seacat, A.M., Thomford, P.J., Hansen, K.J., Olsen, G.W., Case, M.T., Butenhoff, J.L., 2002. Subchronic Toxicity Studies on Perfluorooctanesulfonate Potassium Salt in Cynomolgus Monkeys. https://doi.org/10.1093/toxsci/68.1.249.
Sedlak, T.W., Saleh, M., Higginson, D.S., Paul, B.D., Juluri, K.R., Snyder, S.H., 2009. Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. Proc. Natl. Acad. Sci. U.S.A. 106 (13), 5171–5176. https://doi.org/10.1073/pnas.0813132106.
Sedlak, T.W., Snyder, S.H., 2004. Bilirubin benefits: cellular protection by a biliverdin reductase antioxidant cycle. Pediatrics 113 (6), 1776–1782. https://doi.org/10.1542/peds.113.6.1776.
Semmens, E.O., Domitrovich, J., Conway, K., Noonan, C.W., 2016. A cross-sectional survey of occupational history as a wildland firefighter and health. Am. J. Ind. Med. 59 (4), 330–335. https://doi.org/10.1002/ajim.22566.
Sen, P., Qadri, S., Luukkonen, P.K., Ragnarsdottir, O., McGlinchey, A., Jäntti, S., Juuti, A., Arola, J., Schlezinger, J.J., Webster, T.F., Orešič, M., Yki-Järvinen, H., Hyötyläinen, T., 2022. Exposure to environmental contaminants is associated with altered hepatic lipid metabolism in non-alcoholic fatty liver disease. J. Hepatol. 76 (2), 283–293. https://doi.org/10.1016/j.jhep.2021.09.039.
Shahsavani, S., Fararouei, M., Soveid, M., Hoseini, M., Dehghani, M., 2021. The association between the urinary biomarkers of polycyclic aromatic hydrocarbons and risk of metabolic syndromes and blood cell levels in adults in a Middle Eastern area. https://doi.org/10.1007/s40201-021-00722-w/Published.
Shrier, I., Platt, R.W., 2008. Reducing bias through directed acyclic graphs. BMC Med. Res. Methodol. 8, 1–15. https://doi.org/10.1186/1471-2288-8-70.
Soderberg, C., Stål, P., Askling, J., Glaumann, H., Lindberg, G., Marmur, J., Hultcrantz, R., 2010. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. Hepatology 51 (2), 595–602. https://doi.org/10.1002/hep.23314.
Soteriades, E.S., Kim, J., Christophi, C.A., Kales, S.N., 2019. Cancer incidence and mortality in firefighters: a state-of-the-art review and meta-analysis. Asian Pac. J. Cancer Prev. APJCP 20 (11), 3221–3231. https://doi.org/10.31557/APJCP.2019.20.11.3221.
Soteriades, E.S., Smith, D.L., Tsismenakis, A.J., Baur, D.M., Kales, S.N., 2011. Cardiovascular disease in US firefighters: a systematic review. Cardiol. Rev. 19 (4), 202–215. https://doi.org/10.1097/CRD.0b013e318215c105.
Speakman, J.R., Selman, C., 2003. Physical activity and resting metabolic rate. Proc. Nutr. Soc. 62 (3), 621–634. https://doi.org/10.1079/pns2003282.
Stanifer, J.W., Stapleton, H.M., Souma, T., Wittmer, A., Zhao, X., Boulware, L.E., 2018. Perfluorinated chemicals as emerging environmental threats to kidney health: a scoping review. Clin. J. Am. Soc. Nephrol. 13 (10), 1479–1492. https://doi.org/10.2215/CJN.04670418.
Steenland, K., Tinker, S., Frisbee, S., Ducatman, A., Vaccarino, V., 2009. Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. Am. J. Epidemiol. 170 (10), 1268–1278. https://doi.org/10.1093/aje/kwp279.
Stratakis, N., Conti, D. v, Jin, R., Margetaki, K., Valvi, D., Siskos, A.P., Maitre, L., Garcia, E., Varo, N., Zhao, Y., Roumeliotaki, T., Vafeiadi, M., Urquiza, J., Fernández-Barrés, S., Heude, B., Basagana, X., Casas, M., Fossati, S., Gražulevičienė, R., Uppal, K., McEachan, R.R.C., Papadopoulou, E., Robinson, O., Haug, L.S., Wright, J., Vos, M.B., Keun, H.C., Vrijheid, M., Berhane, K.T., McConnell, R., Chatzi, L., 2020. Prenatal exposure to perfluoroalkyl substances associated with increased susceptibility to liver injury in children. Hepatology 72 (5). https://doi.org/10.1002/hep.31483.
Tomaro, M.L., Del, A.M., Batlle, C., 2002. Bilirubin: its role in cytoprotection against oxidative stress. Int. J. Biochem. Cell Biol. 34 https://doi.org/10.1016/S1357-2725(01)00130-3.
Trowbridge, J., Gerona, R.R., Lin, T., Rudel, R.A., Buren, H., Morello-frosch, R., 2020. Exposure to perfluoroalkyl substances in a cohort of women firefighters and office workers in San Francisco. Environ. Sci. Technol. 54 (6), 3363–3374. https://doi.org/10.1021/acs.est.9b05490.Exposure.
Wagner, M., Bolm-Audorff, U., Hegewald, J., Fishta, A., Schlattmann, P., Schmitt, J., Seidler, A., 2015. Occupational polycyclic aromatic hydrocarbon exposure and risk of larynx cancer: a systematic review and meta-analysis. Occup. Environ. Med. 72 (3), 226–233. https://doi.org/10.1136/oemed-2014-102317.
Wang, Y., Zhao, H., Wang, T., Liu, X., Ji, Q., Zhu, X., Sun, J., Wang, Q., Yao, H., Niu, Y., Jia, Q., Su, W., Chen, W., 2019. Polycyclic aromatic hydrocarbons exposure and hematotoxicity in occupational population : a two-year follow-up study. Toxicol. Appl. Pharmacol. 378 (June), 114622 https://doi.org/10.1016/j.taap.2019.114622.
Weyand, E.H., Bevan, D.R., 1986. Benzo(a)pyrene disposition and metabolism in rats following intratracheal instillation. Cancer Res. 46 (11), 5655–5661.
Wielsøe, M., Long, M., Ghisari, M., Bonefeld-Jørgensen, E.C., 2015. Perfluoroalkylated substances (PFAS) affect oxidative stress biomarkers in vitro. Chemosphere 129, 239–245. https://doi.org/10.1016/j.chemosphere.2014.10.014.
Wingfors, H., Nyholm, J.R., Magnusson, R., Wijkmark, C.H., 2018. Impact of fire suit ensembles on firefighter pah exposures as assessed by skin deposition and urinary biomarkers. Annals of Work Exposures and Health 62 (2), 221–231. https://doi.org/10.1093/annweh/wxx097.
Xu, C., Liu, Q., Liang, J., Weng, Z., Xu, J., Jiang, Z., Gu, A., 2021. Urinary biomarkers of polycyclic aromatic hydrocarbons and their associations with liver function in adolescents. Environ. Pollut. 278, 116842 https://doi.org/10.1016/j.envpol.2021.116842.
Yamaguchi, M., Arisawa, K., Uemura, H., Katsuura-Kamano, S., Takami, H., Sawachika, F., Nakamoto, M., Juta, T., Toda, E., Mori, K., Hasegawa, M., Tanto, M., Shima, M., Sumiyoshi, Y., Morinaga, K., Kodama, K., Suzuki, T., Nagai, M., Satoh, H., 2013. Consumption of seafood, serum liver enzymes, and blood levels of PFOS and PFOA in the Japanese population. J. Occup. Health 55 (3), 184–194. https://doi.org/10.1539/joh.12-0264-OA.
Yang, Q., Qiu, X., Li, R., Ma, J., Li, K., Li, G., 2015. Polycyclic aromatic hydrocarbon (PAH) exposure and oxidative stress for a rural population from the North China Plain. Environ. Sci. Pollut. Control Ser. 22 (3), 1760–1769. https://doi.org/10.1007/s11356-014-3284-y.
Yoshikawa, T., Ruhr, L.P., FloRY, W., Giamalva, D., Church, D.F., Fryor, W.A., 1985. Toxicity of polycyclic aromatic hydrocarbons I. Effect of phenanthrene, pyrene, and their ozonized products on blood chemistry in rats toxicity of polycyclic aromatic hydrocarbons. I. Effect of phenanthrene, pyrene, and their ozonized products on blood chemistry in rats. Toxicol. Appl. Pharmacol. 79.
Zhu, J., Zhao, L.Y., Wang, X.X., Shi, Z., Zhang, Y., Wu, G., Zhang, S.Y., 2018. Identification of hepatotoxicity and renal dysfunction of pyrene in adult male rats. Environ. Toxicol. 33 (12), 1304–1311. https://doi.org/10.1002/tox.22638.

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HBM4EU-MOM: Prenatal methylmercury-exposure control in five countries through suitable dietary advice for pregnancy – Study design and characteristics of participants

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ABSTRACT

Background: Seafood is a major source of vital nutrients for optimal fetal growth, but at the same time is the main source of exposure to methylmercury (MeHg), an established neurodevelopmental toxicant. Pregnant women must be provided with dietary advice so as to include safely fish in their diet for nutrition and mercury control. The aim of this work is to present the design of a multicentre randomized control trial (RCT), which combines human biomonitoring (HBM) with dietary interventions using seafood consumption advice to pregnant women for MeHg control, and to collect information about other possible sources of exposure to mercury. It also presents the materials developed for the implementation of the study and the characteristics of the study participants, which were self-reported in the first trimester of pregnancy.

Methods: The "HBM4EU-MOM" RCT was performed in the frame of the European Human Biomonitoring Initiative (HBM4EU) in five coastal, high fish-consuming European countries (Cyprus, Greece, Spain, Portugal and Iceland). According to the study design, pregnant women (≥120/country, ≤20 weeks gestational age)

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provided a hair sample for total mercury assessment (THg) and personal information relevant to the study (e.g., lifestyle, pregnancy status, diet before and during the pregnancy, information on seafood and factors related to possible non-dietary exposures to mercury) during the first trimester of pregnancy. After sampling, participants were randomly assigned to "control" (habitual practices) or "intervention" (received the harmonized HBM4EU-MOM dietary advice for fish consumption during the pregnancy and were encouraged to follow it). Around child delivery, participants provided a second hair sample and completed another tailored questionnaire.

Results: A total of 654 women aged 18–45 years were recruited in 2021 in the five countries, primarily through their health-care providers. The pre-pregnancy BMI of the participants ranged from underweight to obese, but was on average within the healthy range. For 73% of the women, the pregnancy was planned. 26% of the women were active smokers before the pregnancy and 8% continued to smoke during the pregnancy, while 33% were passive smokers before pregnancy and 23% remained passively exposed during the pregnancy. 53% of the women self-reported making dietary changes for their pregnancy, with 74% of these women reporting making the changes upon learning of their pregnancy. Of the 43% who did not change their diet for the pregnancy, 74% reported that their diet was already balanced, 6% found it difficult to make changes and 2% were unsure of what changes to make. Seafood consumption did not change significantly before and during the first trimester of pregnancy (overall average ~8 times per month), with the highest frequency reported in Portugal (≥15 times per month), followed by Spain (≥7 times per month). During the first-trimester of pregnancy, 89% of the Portuguese women, 85% of the Spanish women and <50% of Greek, Cypriot and Icelandic women reported that they had consumed big oily fish. Relevant to non-dietary exposure sources, most participants (>90%) were unaware of safe procedures for handling spillage from broken thermometers and energy-saving lamps, though >22% experienced such an incident (>1 year ago). 26% of the women had dental amalgams. ~1% had amalgams placed and ~2% had amalgams removed during peri-pregnancy. 28% had their hair dyed in the past 3 months and 40% had body tattoos. 8% engaged with gardening involving fertilizers/pesticides and 19% with hobbies involving paints/pigments/dyes.

Conclusions: The study design materials were fit for the purposes of harmonization and quality-assurance. The harmonized information collected from pregnant women suggests that it is important to raise the awareness of women of reproductive age and pregnant women about how to safely include fish in their diet and to empower them to make proper decisions for nutrition and control of MeHg, as well as other chemical exposures.

1. Introduction

Seafood contains beneficial nutrients that are essential for foetal growth and development, as well as infant neurodevelopment during lactation. However, it is also a significant source of exposure to mercury that poses risks to the developing nervous system. Accordingly, controversial discussions and dissenting opinions are wide spread within the scientific community and also in public discourse.

Foetal neurodevelopment relies on important nutrients, which can be obtained through proper dietary habits (Hibbeln et al., 2007; Oken et al., 2008a). These nutrients include docosahexaenoic acid (3-DHA) for visual and cognitive development, n-3 long-chain polyunsaturated fatty acids (omega 3-PUFAs) for optimal brain development (Aparicio et al., 2021; Stratakis et al., 2020; Tressou et al., 2019), vitamin D for growth and development and selenium which plays a protective role against the neurotoxicity of methylmercury (Hibbeln et al., 2007; Oken et al., 2003, 2008a, 2008b, 2013; Davidson et al., 2010; Strain et al., 2012). While seafood is a primary source of these nutrients, it is also the primary source of exposure to methylmercury (MeHg) in Europe (Maulvault et al., 2015; Nguetseng et al., 2015; Višnjevec et al., 2014). Therefore, both the frequency of seafood consumption and the types of species consumed are critical factors in the risk/benefit balance (Hellberg et al., 2012; Li et al., 2020; Vilavert et al., 2017, Becker et al., 2007; Maulvault et al., 2015; Nguetseng et al., 2015; Višnjevec et al., 2014). Mercury (Hg) is a global pollutant due to its high persistence, long range transport, bioaccumulation and biomagnification (Driscoll et al., 2013; Liu et al., 2021; Pavithra et al., 2023). Despite the recent stringent Regulation 2017/852 of the European Parliament and of the Council on Hg and the entry into force of the United Nations (UN) Minamata Convention on Hg in 2017 (Minamata, 2019), Europeans remain exposed - primarily to legacy Hg and to Hg originating from sources outside the Union (Karel Houessionon et al., 2021; Li et al., 2022; Liu et al., 2021; Mng'ong'o et al., 2021; Ren et al., 2022; Višnjevec et al., 2014). Even low exposures to Hg may cause severe health effects including irreversible damage to the central nervous system during foetal development (Dórea, 2021; Re et al., 2022; Roe, 2022) (Bjørklund et al., 2022; Boucher et al., 2012; Garí et al., 2022) (Di Ciaula, 2021; Feng et al., 2020; Zhao et al., 2023; Zheng et al., 2023; Basu et al., 2018, 2023). Hg is a matter of concern to the global society, including in Europe, where the main source of exposure for the general population is seafood.

The feasibility study of the COPHES project/DEMOCOPHES (2010–2012) provided the first harmonized cross-border HBM data in Europe by measuring total mercury (THg) in scalp hair of women of reproductive age and of children aged 6–11 years. This study confirmed an association between Hg exposure and fish consumption, revealing that exposure is higher in countries where residents have a higher fish consumption. Notably, in DEMOCOPHES, high fish-consumers from Spain and Portugal had the highest Hg exposures out of 17 countries (5–7 times above the European average) (Hond et al., 2015; Castaño et al., 2015).

Many people in Europe remain unaware of effective ways to balance the risks/benefits of fish consumption. This applies also to vulnerable groups like pregnant women or women intending to get pregnant, as well as health professionals who care for/consult these women (e.g., obstetricians/gynaecologists, midwives and dieticians). Moreover, risk communication must be carefully balanced due to the well-known nutritional benefits of seafood consumption. Due to the presence of MeHg and other contaminants in seafood, inappropriate dietary advice may lead to the reduction of seafood consumption and compromise its nutritional value. A study form the US showed that a federal advisory recommending pregnant women to limit consumption of certain fish due to mercury resulted in reduced overall fish intake. This result highlights the importance of tailored dietary advice (Oken et al., 2003). A later US pilot randomized controlled trial to promote healthful fish consumption during pregnancy increased consumption of fish and DHA but not mercury (Oken et al., 2013). Though several European countries have developed official guidelines for pregnant women's seafood consumption, they are often not adequately communicated to the stakeholders' consumers, even in countries where the risk is high (Nunes et al., 2014; Taylor et al., 2018). Furthermore, several countries also lack suitable advice.

Therefore, exposure management knowledge is of utmost importance - especially for pregnant women in vulnerable regions of Europe. At the same time, the simultaneous assurance of the intake of nutrients prevailing through suitable dietary advice and concerning the consumption of seafood is also important (Hibbeln et al., 2019). This thesis is in agreement with the recommendation of the European Food Safety Authority (ESFA), which in 2015 called for Member States to examine their national situation and issue advice for vulnerable groups (EFSA, 2016). Recent reports in the scientific literature show that Human Biomonitoring in combination with the provision of suitable dietary advice to pregnant women is a powerful tool for controlling prenatal exposure to Hg while assuring the nutritional benefits provided by seafood. Such studies have been performed successfully in the United States and in Denmark (Kirk et al., 2017; Oken et al., 2013, Turyk et al., 2019).

The HBM4EU-MOM study ("Methylmercury-contrOl in expectant Mothers through suitable dietary advice for pregnancy") was conducted

in the context of the European Joint Programme HBM4EU (https://www.hbm4eu.eu/) (Ganzleben et al., 2017; Kolossa-Gehring et al., 2023). The study was operated at the science-policy interface to generate knowledge, which can directly be used for chemicals' policy and the improvement of environmental health (Kolossa-Gehring et al., 2023; Ganzleben et al., 2017). HBM4EU-MOM was designed and implemented in five coastal seafood-consuming European countries (Cyprus, Greece, Iceland, Portugal, Spain) and presents the main objectives: (a) to collect information about the basic characteristics of pregnant women in the five countries and lifestyle factors relevant to Hg exposures, (b) to evaluate the exposure of pregnant women to Hg during the first trimester of pregnancy and to investigate the associated factors with emphasis on seafood consumption, (c) to investigate the practices, attitudes and preferences of the women with regard to seafood consumption and to receive dietary information for pregnancy, (d) to design dietary recommendations for healthy seafood consumption during pregnancy emphasizing nutrition and Hg control, (e) to test the effect of the recommendations in a Randomized Control Trial, (f) to engage with the health-care providers of pregnant women, and (g) to communicate the results of the study to the participants, policy makers, health professionals, the wider public and the scientific community.

This work presents the study design, the harmonized procedures and materials developed in the frame of the study, as well as the characteristics of the participants, based on information collected during the first trimester of pregnancy. The results of Hg biomonitoring measurements and the impact of the dietary intervention will be presented in a different manuscript.

2. Methods

2.1. Study design

HBM4EU-MOM is a multicentre two-armed randomized controlled interventional trial implemented under the HBM4EU European partnership, with the overall aim to support "**M**ethylmercury-contr**O**l in expectant **M**others through suitable dietary advice for pregnancy" (Fig. 1). The study was conducted between October 2020 and March 2022, involving research organisations from 11 European countries and recruited pregnant women (N = 654, gestational age ≤20 weeks) from 5 countries (described below). At baseline (Phase 1), each participant had to provide a hair sample for total mercury assessment (THg) assessment and complete a standardized questionnaire with personal information relevant to the study aims. Afterwards, the participants were randomized distributed 1:1 to a "control group" and an "intervention group" and were blinded to the allocation. The control participants received the standard pregnancy care provided in their country of residence, whereas the intervention participants additionally received the HBM4EU-MOM seafood consumption advice (which was tailored to the seafood species consumed nationally) and were encouraged to follow it. A second round of sampling (Phase 2) took place post-intervention, at the end of the pregnancy, with each participant providing a second hair sample and completing another standardized questionnaire. The questionnaires are described below and are provided in the Supplementary material.

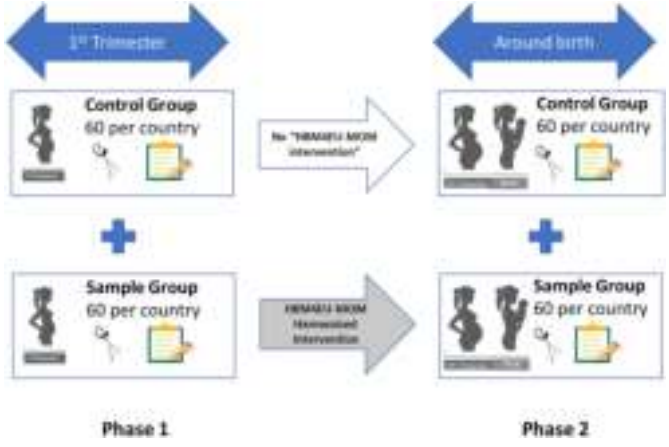

Fig. 1. The HBM4EU-MOM study design in a "nutshell".

2.2. Geographic coverage

HBM4EU-MOM harmonized and integrated five national cohorts from the following coastal countries in Europe: in the Southeast: Cyprus (CY) and Greece (EL); in the Southwest: Portugal (PT) and Spain (ES); in the Artic: Iceland (IS) (Fig. 2). The participating countries were selected to represent different coastal geographic regions, with hypothesized high and distinct seafood consumption patterns.

2.3. Study population

Inclusion criteria for participation were: women in singleton pregnancy; aged between 18 and 45 years; gestational age of up to 20 weeks; absence of health problems or conditions; sufficient fluency in the language of the national cohort (alternatively in Greek or English in the case of Cyprus); residency in the country of the national cohort for at least three years prior to recruitment; not excluding seafood from their diet; and willingness and ability to provide two hair samples.

Based on power calculations, the minimum estimated sample size at European level was 600 participants, equally distributed among the control and intervention groups, which amounted to a minimum of 120 participants in each of the five countries.

2.4. Ethics and personal data protection

The study was approved by the competent national ethics committees prior to the initiation of the recruitment of participants in the study (Table S1). All five countries completed internal declarations of ethics (Knudsen et al., 2023) within the HBM4EU project and submitted copies of the national ethics approvals to the coordinators for uploading on the European Commission Participant Portal. All study procedures and

Fig. 2. HBM4EU-MOM harmonized and integrated national cohorts from five coastal, fish-consuming European countries (Cyprus and Greece in the Southeast; Portugal and Spain in the Southwest; Iceland in the Artic).

materials (Table 1) complied with the General Data Protection Regulation of the European Union (GDPR). In IS, the study was further approved by the Icelandic Data Protection Agency. Informed consent was provided by all the pregnant women prior to fieldwork (e.g., completion of questionnaires and provision of hair samples). The certificates of informed consent included the provision to keep the hair samples in a biobank for a minimum period of two years.

2.5. Harmonization of procedures

A detailed protocol was developed based on the HBM4EU concepts (Fiddicke et al., 2015) and described the study characteristics (e.g., hypothesis, objectives, target population, recruitment strategy, biomarkers and analytical considerations, data management and statistical analysis, communication aspects, timeline). It is presented below and the harmonized materials developed for its implementation are presented in the supplement.

2.6. Questionnaires and communication materials

Tailored questionnaires were developed in the frame of the HBM4EU-MOM study for the purpose of collecting personal information from participants relative to the study aims. The questions assessed sociodemographic factors, pregnancy status, dietary practices before and during pregnancy with a dedicated section on seafood consumption (e.g., seafood species consumed, portions – standardized as "small", "medium" or "large" with the aid of photographs, frequency, seasonality, preferences and barriers to eating seafood). Moreover, they assessed preferences related to receiving dietary advice for pregnancy, lifestyle, dental amalgams, as well as possible occurrences of incidents of breakage of mercury-containing products (thermometers, energy-saving lamps) and whether participants knew how to properly deal with such occurrences.

To ensure the correct correspondence of seafood species across the five countries, the Latin names and photographs of different species were examined. While the master files of the questionnaires included all the seafood species from all five countries, the targeted questionnaire of each country was adapted according to the species of relevance to their population (e.g., the ones that are commonly consumed at country level) (Table 1 and Supplementary material B_Questionnaires).

The questionnaires were validated at European level through detailed discussions on each question among the partners. After verifying the harmonization of the national questionnaires in English, each country translated them into the corresponding national language(s) and validated them at national level through pilot testing. Instructions were elaborated for national fieldworkers, who received proper training. The countries were free to choose if they conduct the assisted interviews by completion of paper or electronic versions of the questionnaires.

Several tailored communication products were developed (Pack et al., 2023) to facilitate recruitment of participants and collection of informed consent (e.g., information leaflets, invitation letters, certificates of informed consent, reply cards, withdrawal forms, personal reports, etc). These materials were elaborated in a harmonized way at European level, based on HBM4EU templates (Pack et al., 2023). Limited flexibility was allowed for country adaptations according to the national situation and needs, while ensuring that the harmonization and comparability of the results would not be compromised. After verifying the harmonization of the national materials in English, each country translated them into the corresponding national language(s). Examples of the communication materials in English are presented in Tables 1 and in the Supplementary material B_ Communication Material. The communication materials included a harmonized framework for reporting personal results to the participants. All participants were provided with a personalized letter at the end of the study with their results, unless they explicitly declared in their certificate of informed consent that they did not wish to be informed. The personalized reporting of results included the THg measurements of each participant with interpretative information, the HBM4EU-MOM advice on safe seafood consumption during pregnancy and breastfeeding and guidelines for the safe handling of a potential Hg spillage at home from broken items. The materials developed and used in the frame of the study for the dietary intervention will be presented in another manuscript (under preparation).

2.7. Recruitment

Pregnant women who fulfilled the predefined inclusion criteria were invited to participate in the study according to the procedures described in Table S2. The preferable model was to recruit via health care providers of the women during routine antenatal visits in clinics. Each country adjusted its recruitment procedures as necessary to achieve the recruitment targets within the short timelines and the complications presented by the COVID-19 pandemic (e.g., lockdowns, social distancing, precautionary isolation of pregnant women).

2.8. Sampling and chemical analysis

A 'Train-the-Trainers' model was applied to ensure harmonized, standardized and quality-assured sampling of hair in all countries.

Study leaders and designated trainers of each country participated in an online training workshop due to the restrictions imposed by the COVID-19 pandemic. A video and a standard operating procedure (SOP) with detailed instructions for the hair sampling were created by CNSA-ISCIII team and made available to the national teams to use at country level. Subsequently, national training sessions of health care providers/fieldwork team members were organized as necessary in each country, to train the fieldwork team members and the engaged health-care providers. The national teams also received a SOP for hair sampling collection (Supplementary material B_Hair Sampling SOPs) together with a harmonized "Hair Sampling Questionnaire" (Table 1), to collect

Table 1
Study material developed and used in the frame of the HBM4EU-MOM study.

No.	Title	Supplementary materialB
1	HBM4EUmom_Invitation LetterGeneral(1a) and ControlGroup (1 b)	Communication Material
2	HBM4EUmom_InformationLeaflet	Communication Material
3	HBM4EUmom_ReplyCardYes (a) and No(b)	Communication Material
4	HBM4EUmom_ReminderLetter	Communication Material
5	HBM4EUmom_Letterof ThanksIneligible	Communication Material
6	HBM4EUmom_PreVisitLetter	Communication Material
7	HBM4EUmom_InformedConsent	Communication Material
8	HBM4EUmom_WithdrawalDocument	Communication Material
9	HBM4EUmom_PersonalResults	Communication Material
10	HBM4EUmom_1stPhasedQuestionnaire	Questionnaires
11	HBM4EUmom_2ndPhasedQuestionnaire	Questionnaires
12	HBM4EUmom_HairSamplingQuestionnaire	Questionnaires
13	HBM4EUmom_StandardOperatingProcedureforHairSamplingCollection	Hair Sampling SOP

relevant information in a uniform way (e.g., hair colour, length, treatment with products, etc). Hair samples were stored in dark, at room temperature, with no other specific conditions prior to analysis.

The laboratory chosen to perform the chemical analyses of all HBM4EU-MOM hair samples was CNSA-ISCIII in Spain. For this purpose, the countries and the laboratory co-signed a bilateral Material-Data Transfer Agreement (MDTA), using an internal HBM4EU template (Knudsen et al., 2023; Pack et al., 2023). An accredited method was applied, using direct Hg analysis via thermal decomposition-gold amalgamation atomic absorption spectroscopy (DMA-80 Direct Mercury Analyzer, Milestone, USA) (Esteban et al., 2015). The laboratory personnel were unaware of the allocation of samples to control/intervention groups during their processing and analysis.

2.9. Data management and statistical analysis

All samples and data were pseudonymised before or immediately after their collection and before any other action by awarding a unique code to each participant according to the harmonized guidelines for coding developed. The personal identification information of the participants (name and contacts) was only accessible to the coordinators of each national cohort and specific researchers.

For each of the five HBM4EU-MOM cohorts, national databases were constructed using a harmonized methodology of encoding the data from the chemical analysis and questionnaires on excel data sheets. The national databases were encrypted, submitted via a dedicated web platform (developed in-house at VITO, Belgium) and subjected to quality control (QC). Each cleaned national database was pooled to a central European database, managed by VITO. The corresponding authorized data users were provided with individual encrypted extracts, including specific variables needed for the analysis, from the central European database. These export "views" were provided in excel format, together with the appropriate sample metadata in a separate worksheet.

Descriptive statistics included the calculation of percentages of women by various groups referring to the general characteristics of the study population (e.g., education level, pregnancy-related variables, smoking, having amalgam fillings and tattoos, diet-related variables, information on the diet during pregnancy, etc). General characteristics of the study population were stratified by country. The implementation of the statistical analysis was performed in R programming environment (R-Project, 2009).

3. Results and discussion

3.1. Harmonized approach and materials

To our knowledge, HBM4EU-MOM is the first international, multicentre randomized control trial combining harmonized dietary interventions for safe seafood consumption in pregnancy with mercury biomonitoring of pregnant women. HBM4EU-MOM demonstrated the importance of a network of experts working closely together, developing and using standardized tools, which are fit for the purposes of quality assurance and harmonization of study protocols. The HBM4EU-MOM study was implemented in less than a year-and-a-half, which required highly effective time management and overall coordination, but still exerted a lot of pressure on the researchers. Because all the study materials needed to be available for submission to ethics committees, the time management for the preparatory phase was crucial for preventing unacceptable delays in the initiation of the fieldwork. A central unit to oversee the harmonization of the study was essential, so as to ensure the comparability of the results. At the same time, it was extremely important for the countries to have flexibility to adjust the implementation according to the national situations. The frame developed under HBM4EU (e.g. template communication materials and consent forms, sample transfer agreements, analytical and quality assurance tools, data transfer agreements, data management and statistical tools) served as the basis for developing tailored tools. The HBM4EU-MOM approach and materials (Table 1, Supplementary material B) served their purpose well and they may contribute to the collection of comparable information in future studies. The feasibility of assessing the exposure of European women of reproductive age to mercury using harmonized assessment of THg in hair in different countries, was first demonstrated in the frame of the DEMOCOPHES pilot study (Castaño et al., 2015; Hond et al., 2015). Three of the five HBMEU-MOM countries (ES, PT, CY) participated in DEMOCOPHES and transferred valuable expertise to the study (Castaño et al., 2015; Hond et al., 2015). In fact, the lessons-learned and materials developed (e.g., SOP, sampling questionnaire and training material) in DEMOCOPHES were highly valuable, which proves the usefulness of this kind of harmonized and validated materials in the European HBM arena. Those materials also contributed to the development of the WHO protocol for human biomonitoring surveys for the assessment of prenatal exposure to Hg using THg in maternal hair of women who just gave birth (WHO, 2020). In the HBM4EU-MOM study design, the second phase of maternal hair samplings at the time of child delivery, resembles the WHO approach so that the results from the control participants could be comparable to those collected globally using the WHO protocol.

3.2. Recruitment, samplings and retainment of participants

The population of pregnant women is vulnerable and difficult to engage in research trials (Mary Dawn Koenig et al., 2022; Van Delft et al., 2022; MacLachlan et al., 2021). The HBM4EU-MOM study employed successful recruitment and retention strategies to achieve the recruitment targets within tight timelines during the COVID-19 pandemic, and to control attrition so that the intervention and the second sampling phase could be completed. Overall, 654 participants were recruited between January and September 2021, with all five countries managing to reach or exceed the recruitment target of 120 participants (Table 2).

The recruitment procedures applied by each country are presented in Table S2. Our study plan envisioned the engagement health care providers of pregnant women for the recruitment and hair samplings, so as to build on the relationship of trust and health care between the pregnant women and their providers, to create learning opportunities for the health professionals and to facilitate more sustainable use of the results. Despite the pressures exerted by the pandemic on the health care systems, four of the five countries (CY, EL, ES and IS) succeeded in involving health professionals in the study and the national study teams provided to them training, support, encouragement and supervision. The type of engaged health-care providers depended on the structure of the health-care systems of the countries and they were primarily medical doctors and/or midwives. This model of recruitment was very successful. In the case of PT, the direct engagement of health professionals in the study was not possible and this caused delays and the need to explore different strategies.

To various degrees, two more approaches were used for recruitment in the countries: Researcher-led recruitment, where the research team directly engaged with potential participants in the clinics or other venues, and self-referrals of interested pregnant women in response to study advertising in social and other communication media.

With regard to the geographic distribution of the samples within the countries, CY had a national geographical coverage, EL and PT had national representation, but with 63–64% of participants recruited in a specific area, and ES and IS had a regional geographical coverage (Table S2). None of the geographical areas were known hotspots for mercury. When several health professionals in different clinics and geographic regions could be employed, the recruitment and samplings were achieved faster, but the burden on the national study team was greater due to more complicated management requirements.

Because HBM4EU-MOM was designed as a RCT with a dietary intervention phase and a second phase of post-intervention samplings,

Table 2
Basic characteristics of the study population.

	CY	EL	ES	IS	PT	All
Characteristics						
Country/Region	Cyprus	Greece	Spain/Fuenlabrada	Iceland/Reykjavik	Portugal	NA
Number of participants (n)	133	130	136	120	135	654
Sampling Period	February–May	January–July	March–August	May–October	June–November	January–November
Age (mean, min-max)	31(18–45)	33(24–43)	34(19–43)	30(20–42)	34(24–45)	32(18–45)
BMI, mean (SD)	23.7	23.4	24.7	26.4	23	24.1
	(18.2, 39.7)	(18.6, 35.8)	(16, 41.1)	(17.2, 43)	(17.4, 38.5)	(16, 43)
Residential degree of urbanization N(%)						
Cities	45.9	86.9	83.8	0	75.6	58.4
Towns/Suburbs	16.5	4.6	0	100	20	28.2
Rural area	37.6	8.5	14.7	0	4.4	13.0
No Information	0	0	1.5	0	0	0.3
Education level of the Participant (%)						
Low (ISCED 0–2)	1.5	0	25	3.3	0.7	6.3
Medium (ISCED 3–4)	11.3	10.8	32.4	27.5	8.2	17.9
High (ISCED ≥5)	86.5	88.5	42.7	52.5	91.1	72.5
No Information	0.8	0.8	0	16.7	0	3.4
Education level of the Participant's partner (%)						
Low (ISCED 0–2)	5.3	3.9	32.4	8.3	3	10.7
Medium (ISCED 3–4)	34.6	15.4	30.2	25.8	23.7	26
High (ISCED ≥5)	59.4	77	35.3	43.3	73.3	57.8
No Information	0.8	3.9	2.2	22.5	0	5.5
First-time pregnant (%)	54.1	60.8	51.5	35	51.9	50.9
No Information	0.8	0	0	16.7	0	3.2
Prior pregnancies (%)						
one	31.6	28.5	36	30	31.9	31.7
two	12	10.8	8.1	10.8	9.6	10.2
three	0	0	0.7	2.5	0	0.6
four	0	0	0.7	0.8	0	0.3
five	0.8	0	0	0	0	0.2
six	0.8	0	0	0	0	0.2
Planned pregnancy (%)						
No	39.8	26.2	16.2	14.2	8.9	21.1
Yes	58.6	62.3	81.6	68.3	91.1	72.6
No Information	1.5	11.5	2.2	17.5	0	6.3
Active smoker before pregnancy (%)						
No	57.9	63.8	62.5	81.7	85.2	70
Yes	41.4	35.4	35.3	2.5	14.8	26.3
No Information	0.8	0.8	2.2	15.8	0	3.7
Passive smoker before pregnancy (%)						
No	47.4	77.7	64	80.8	49.6	63.5
Yes	51.9	21.5	34.6	2.5	50.4	32.9
No Information	0.8	0.8	1.5	16.7	0	3.7
Active smoker during pregnancy, %						
No	89.5	89.2	83.1	82.5	96.3	88.2
Yes	9.8	9.2	16.2	1.7	3.7	8.3
No Information	0.8	1.5	0.7	15.8	0	3.5
Passive smoker during pregnancy (%)						
No	74.4	83.8	66.2	83.3	60.7	73.4
Yes	24.8	15.4	32.4	0.8	39.3	23.1
No Information	0.8	0.8	1.5	15.8	0	3.5
Amalgam fillings %						
No	71.4	59.2	75	57.5	76.3	68.2
Yes	24.1	40.8	22.8	18.3	22.2	25.7
No Information	4.5	0	2.2	24.2	1.5	6.1
Last placed %						
Less than 4 months	0	1.9	3.2	0	0	1.2
4–12 months	0	0	3.2	0	0	0.6
more than a year	100	98.1	93.5	100	100	98.2
Last removed %						
Less than 4 months	0	0	6.5	0	3.3	1.8
4–12 months	3.1	1.9	0	0	0	1.2
more than a year	43.8	98.1	38.7	59.1	46.7	62.5
Tattoo, %						
No	61.7	73.8	38.2	40	68.9	56.7
Yes	37.6	26.2	61.8	41.7	31.1	39.8
No Information	0.8	0	0	18.3	0	3.5

measures needed to be proactively employed to ensure that a sufficient number of participants would remain engaged with the study until its completion. To begin with, the national research teams tried to recruit more participants than the recruitment goal of 120 pregnant women per country. To retain the interest and engagement of participants and to encourage intervention participants to consume seafood according to the provided dietary advice, the countries used different approaches, such as free webinars and other means of providing information and guidance to participants related to pregnancy, lactation, nutrition and parenting. An attrition of less than 8% was observed and it was

attributed to occasional incidents of miscarriages and health-issues or loss of interest in the study. A very small number of participants did not provide the second hair sample because of time considerations and precautions due to the spread of COVID-19. This attrition is small and does not cause concerns for bias (Dumville et al., 2006; Close et al., 2016; Babic et al., 2019). The reasons for the observed attrition are comparable to those reported in other studies (Close et al., 2016).

3.3. General characteristics of the participants at baseline

The general characteristics of the study population are described in Table 2. The average age of the participants was 32 years and was similar across the five countries [range: 31–34]. The youngest participant was 18 years old and the oldest one was 45. Most participants (59%) resided in cities, 28% lived in towns and suburbs, while 13% lived in rural areas.

3.3.1. Educational level

Most participants (72%) were highly educated (attained high level tertiary education of ISCED ≥5), while 18% attained upper secondary to post-secondary non-tertiary education (ISCED 3–4). Only 6.3% had a low education level (ISCED 0–2) (Table 2, Fig. 3). The distribution of the educational levels of the participants' partners was wider, with 58% having a high education level (ISCED ≥5), 26% a medium level (ISCED 3–4) and 11% a low education level (ISCED 0–2). The spread of educational levels of HBM4EU-MOM participants and their partners was compared to that of the general European population aged 18–45 years, as reported by EUROSTAT for the year 2021 (EUROSTAT, 2022). Overall, the HBM4EU-MOM population is skewed to higher educational levels. The overrepresentation of highly educated participants was most pronounced in PT, EL and CY. In the case of ES, there was better representation of low and medium education levels than in the other countries. Previous studies have shown that mercury exposures are higher in people of higher educational levels, putatively because of higher seafood consumption (WHO, 2019). Such possible associations will also be explored for HBM4EU-MOM participants.

3.3.2. Diet

Maternal pre-pregnancy body mass index is a measure of maternal health and nutrition, which is the key to meeting the nutrient demands of pregnancy and vital for foetal development and infant health. Both high and low pre-pregnancy body mass index (BMI) have been associated with suboptimal foetal growth and risk of pregnancy complications (Gudipally et al., 2023; Zong et al., 2022; Tang et al., 2021). Several studies of European women show an increasing trend in maternal obesity incidence over time (Heslehurst et al., 2007). Maternal pre-pregnancy BMI is classified (Zong et al., 2022) as underweight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2) or obese (≥30 kg/m^2). The average pre-pregnancy BMI of HBM4EU-MOM participants was within the healthy range and so were the national averages of CY, EL, ES and PT (Table 2), but the average BMI value in IS was within the overweight category. It is also noteworthy that in all countries except for EL, there were participants who were underweight and that in all five countries there were obese participants. These results indicate a need in all the participating countries to better inform women about the importance of maintaining a healthy BMI when planning for pregnancy.

Because the maternal diet during the periconceptional period and pregnancy is vital for the health of both the mother and the child (Rodríguez-Bernal et al., 2013), the WHO (WHO, 2001) and many national governments issue dietary guidelines for pregnant women (WHO, 2001; Linou, 2014). More than half of the HBM4EU-MOM participants reported that they made dietary changes for their pregnancy (Fig. 4). Despite the fact that most of the women said that their pregnancy was planned (Table 2), the timing of the changes for most women was when they found out that they were pregnant (Table 3). Very few women said that they made dietary changes before they got pregnant. The women who did not make any dietary changes for the pregnancy mostly believed that their diet was already balanced, while a few found it difficult to make changes or did not know what changes to make. These results indicate that more attention should be given to awareness raising and education of women about the importance of their diet for both nutrition and exposure control. Ideally, women should prepare themselves for a healthy pregnancy before conception. Several food contaminants are eliminated very slowly from the body and therefore changing the diet at the onset of pregnancy will not prevent the embryo from getting exposed during a critical developmental phase. This is the case for MeHg, whose half-life in seafood-eating humans is estimated to be between 39 and 70 days (Gad, 2014; WHO, 1990).

According to data of the Food and Agriculture Organization of the United Nations (FAO, 2022), three of the five countries participating in HBM4EU-MOM are among the top per-capita consumers of seafood in the world. In 2019, the estimated average per-capita consumption of aquatic food worldwide was 20.5 kg/capita/year and in Europe was 21.1 kg/capita/year. High- and low-income countries exceeded these averages (26.5 and 28.1 kg/capita/year, respectively), whereas lower-middle and low-income countries were below the averages (15.2 and 5.4 kg/capita/year, respectively). IS was the top consumer at 91.2 kg/capita/year, PT was third at 57.2 kg/capita/year and ES was sixth at 42.3 kg/g. EL and CY have lower consumption than the other three countries, but remain among the top seafood consumers in Europe.

HBM4EU-MOM results showed that seafood was consumed by the participating women at almost constant frequency (7.9 times/month or 1.8 times/months) both before and during pregnancy, in all five countries. Mild changes are noted in the before/during pregnancy average consumption frequencies within each country, but at the level of individual participants, a range of behaviours is observed (Fig. 5). As

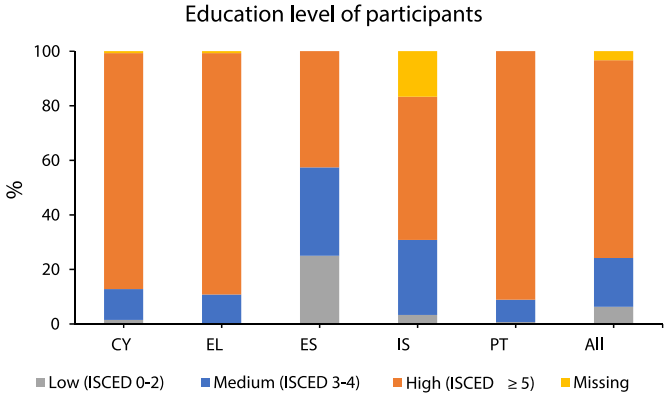

Fig. 3. Educational level of the participants, showing that most of the recruited women are highly educated (ISCED ≥5).

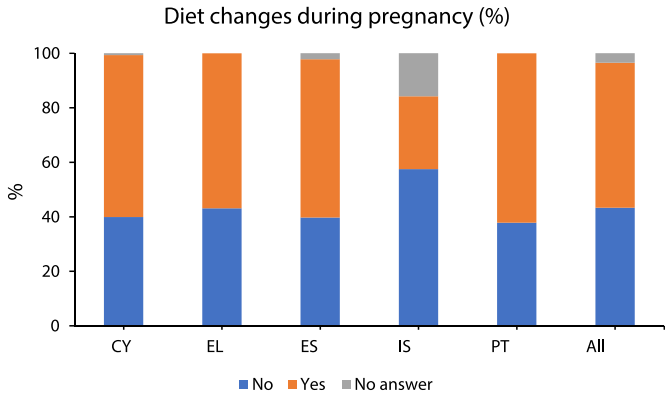

Fig. 4. Dietary changes made by the participants for the pregnancy.

Table 3
Dietary changes made for the pregnancy by the study population (N = 654).

	CY	EL	ES	IS	PT	All
Made dietary changes for this pregnancy (%)						
No	39.9	43.1	39.7	57.5	37.8	43.3
Yes	59.4	56.9	58.1	26.7	62.2	53.2
No answer	0.8	0.0	2.2	15.8	0.0	3.5
If yes, when (%)						
3 months before the pregnancy	3.8	1.4	3.8	6.3	2.4	3.2
1–3 months before the pregnancy	2.5	14.9	2.5	6.3	1.2	5.2
Just before the pregnancy	2.5	9.5	11.4	0.0	11.9	8.1
When I learned I was pregnant	70.9	68.9	76.0	84.4	77.4	74.4
Refuse to answer	0.0	1.4	0.0	0.0	0.0	0.3
Other	20.3	4.1	6.3	3.1	7.1	8.9
If no, why (%)						
It is difficult to make changes	3.9	8.9	9.3	–	2.0	6.1
My diet was already balanced	59.6	71.4	83.3	–	82.4	74.2
I did not think I had to make any changes	23.1	16.1	5.6	–	13.7	14.6
I did not know what changes to make	5.8	0.0	0.0	–	2.0	1.9
Refuse to answer	1.9	0.0	0.0	–	0.0	0.5
Other	5.8	3.6	1.9	–	0.0	2.8

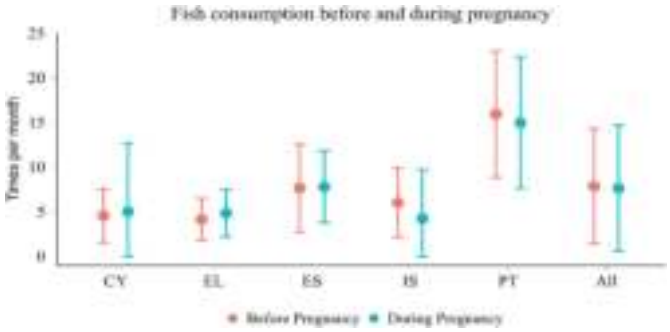

Fig. 5. Self-declared frequency of seafood consumption (in times per month) before and during the pregnancy, for each country. 'All' refers to the pooled samples from all countries. The circles present the mean consumption frequency, the vertical lines present the range of values and the T-bars indicate the minimum and maximum frequencies.

hypothesized, the frequency of consumption presents high geographic variability. Portuguese participants reported the highest average frequency of consumption both before (15.9 times/month or 3.7 times/week) and during pregnancy (15.0 times/month or 3.5 times/week) and some of the Portuguese women consumed seafood more than 5 times per week. Spanish participants presented the second highest average consumption, at 1.8 times per week both before and during pregnancy. It is noteworthy that despite the very high per capital seafood consumption in IS, Icelandic women consumed seafood at 1.4 times per week before the pregnancy and this dropped to an average consumption of 1.0 times per week during the pregnancy. The Greek and Cypriot participants had lower and similar consumption frequencies (around 1 time/week), both before and during the pregnancy.

The concentration of MeHg can vary considerably across different species of seafood and their habitats. Likely, the nutritional value of seafood presents high variability. Smaller fish, at the lower level of food webs, usually contain more unsaturated fatty acids and lower MeHg concentrations. Large and predatory fish usually contain less nutritional value and higher concentrations of MeHg. For this reason, suitable advice for seafood consumption must contain recommendations of species to prefer or avoid, in addition to frequency of consumption. The HBM4EU-MOM dietary intervention was based on this premise and in agreement with EFSA's statement on the benefits of seafood consumption compared to the risks of methylmercury in seafood (EFSA, 2015). According to EFSA, pregnant women would have to consume 1–4 servings of seafood to reach the nutritional benefits based on the dietary reference value (DRV) of n-3 long-chain polyunsaturated fatty acids (PUFAs). However, if seafood species with high methylmercury content are consumed, the tolerable weekly intake (TWI) for MeHg can be reached with <1–2 servings and before attaining the DRV. Because of the variety of seafood species consumed across Europe, EFSA recommended that each country considers the national species consumed for the development of appropriate recommendations.

The types of seafood consumed by the pregnant women participating in HBM4EU-MOM over the four months before joining the study (e.g., right before conception and at early gestation) are presented in Table 4. It is notable that big oily fish are consumed in all countries and collectively by 62% of the participants. The highest prevalence is in PT (consumed by 89%), followed by ES (85%), with the other three countries trailing at <50%. The most consumed category in all countries was that of the white fish. These results indicate that women of childbearing age need to be better informed about including seafood in their diet in a safe way.

3.3.3. *Non-dietary sources of exposure to Hg*

Pregnant women and their foetuses may get exposed to other forms of Hg from non-dietary sources, such as accidental spills from mercury-containing products (Sarigiannis et al., 2012) or placement/removal of dental amalgams. The occurrence of dental amalgam fillings in the pregnant women are presented in Table 2. The highest prevalence presented was in EL (41%), followed by a range of 22–24% in CY, ES, PT, and the lowest one in IS (18%). In line with the commitments to the Minamata Convention on Mercury, the Regulation (EU) 2017/852 (Minamata, 2019) on mercury states that amalgam should not be used on pregnant women except when deemed strictly necessary by the dental practitioner based on the specific medical needs of the patient. It is noteworthy however, that a small number of participants had dental amalgams placed (1%) or removed (2%) during the peri-pregnancy period (within 4 months from their participation in HBM4EU-MOM). This indicates a need to raise the awareness of dentists and pregnant women.

Citing Hg toxicity, the European Union is phasing out general-purpose fluorescent lighting across Europe in 2023 in the frame of the Restriction of Hazardous Substances (RoHS) Directive. Thermometers containing Hg have been banned in the European Union since 2009 (REACH, 2009). Our findings, however, show that several European pregnant women still experience occurrences of breakage of these products and that they are not aware of safe procedures to respond to such an incident (Table 5). Because people may have both types of products stocked away or in use. Thus, it is important to continue raising awareness to safety guidelines for proper disposal and response to accidental spillage.

3.3.4. *Other possible chemical exposures*

Maternal exposure to tobacco smoke during pregnancy has been

Table 4
Categories of seafood consumed by women during the 4 months preceding baseline sampling, which took place in the first-trimester of pregnancy.

	CY	EL	ES	IS	PT	ALL
White Fish Consumption %						
Yes	83.5	94.6	96.3	75.8	100	90.4
No	15.8	5.4	3.7	6.7	0	6.3
Small oily Fish Consumption %						
Yes	51.1	89.2	83.8	55.8	93.3	75.1
No	48.1	10.8	15.4	25.8	6.7	21.3
Big oily Fish Consumption %						
Yes	48.1	43.8	85.3	42.5	88.9	62.4
No	51.1	56.2	14.7	38.3	11.1	33.9
Other Sea Food Consumption %						
Yes	89.5	90	92.6	0	94.1	74.8
No	9.8	10	7.4	0	5.2	6.6

Table 5
Occurrences of broken mercury thermometers and energy-saving bulbs within participants' residences and self-reported knowledge of appropriate response measures (data collected at baseline sampling, which took place in the first-trimester of pregnancy).

	CY	EL	ES	IS	PT	All
Broken mercury-containing thermometer (%)						
Do not know	1.5	0	1.5	29.2	5.9	7.2
No	87.2	60.8	75.7	62.5	66.7	70.8
Yes	11.3	39.2	22.8	8.3	27.4	22
Yes/Less than 1 year ago	0	4.6	0.7	0.8	0.7	1.4
Yes/More than 1 year ago	11.3	33.8	22.1	7.5	26.7	20.5
Able to react	9	24.6	2.9	0.8	8.1	9.2
Broken energy-saving lamp (%)						
Do not know	4.5	0	4.4	37.5	14.8	11.8
No	85	96.2	83.4	59.2	674	78.4
Yes	10.5	3.8	12.5	3.3	17.8	9.8
Yes/Less than 1 year ago	1.6	1.6	2.9	0	1.5	1.5
Yes/More than 1 year ago	9	2.3	9.6	3.3	16.3	8.3
Able to react	6	0	0.7	0.8	16.3	4.9

linked to several adverse health effects for both the fetus and the mother. As a result of awareness raising campaigns and regulations, a general reduction in cigarette was achieved worldwide. Surprisingly, however, many pregnant women continue to smoke and many more remain exposed to second hand smoke (Beck et al., 2023; Lange et al., 2018). Overall, 26% of HBM4EU-MOM participants were smokers before the pregnancy and 8.3% continued to smoke during the pregnancy, with the highest prevalence observed in Spain (16%). This prevalence is higher than the 8.1% reported in Europe by Bednarczuk et al. (2020). A significant percentage of women (23%) remained exposed to second-hand smoke during pregnancy, ranging from 39% in Portugal, 32% in Spain, 25% in Cyprus and 15% in Greece to only 0.8% in Iceland. These data suggest that further efforts are needed in the four Southern European countries to prevent fetal and maternal exposure to tobacco smoke.

The HBM4EU-MOM study provided an opportunity to collect information from pregnant women about lifestyle practices, which may lead to various chemical exposures.

Many participants (40% overall) reported that they had a tattoo, ranging from 26% in EL to 62% in ES. This is a relatively underexplored exposure source, which may be linked to exposures to various chemical substances, such as polycyclic aromatic hydrocarbons, primary aromatic amines and metals (Negi et al., 2022). Collectively, 28% of the women had their hair dyed within 3 months from sampling (Table S3). This practice varied geographically, ranging on average from 0% in IS to 47% in ES. A natural hair dye was used most frequently in CY and EL (77% and 82%, respectively), whereas in ES and PT a chemical one was often used (70% and 81%, respectively). Only few participants from CY (2.3%), ES (1.5%) and PT (0.7%) underwent a chemical hair structure treatment within three months from the sampling of hair. The safety of many of the substances used in hair dyes is assessed by the expert panel's Cosmetic Ingredient Review. Although many substances are considered safe for users at the levels used in hair dyes, there are conflicting data on a large number of formulations (He et al., 2022).

Participants from all five countries engaged with gardening involving the use of fertilizers or pesticides (overall mean: 8%; range of national means: 2–12%) and with activities involving paints, pigments or dyes (overall mean: 19%; range of national means: 9–24%). Activities such as leather tanning, recycling of electrical parts and smelting, welding and soldering were performed by only a negligible number of participants (Table S4).

HBM studies can support policy decisions for safer chemicals management and public health protections. The engagement of pregnant women and their health care providers in studies such as HBM4EU-MOM also creates opportunities for disease prevention and improved health through personalized actions. However, our experiences show that the public and the medical sector should be better educated about human biomonitoring as a force in citizen empowerment and health promotion. Enhancing this understanding will also facilitate enrolment in HBM studies.

4. Conclusions

The HBM4EU-MOM study developed and made available validated harmonized procedures and materials for the assessment of prenatal exposures to mercury and the associated factors in different countries. The harmonized information collected regarding the basic characteristics of pregnant European women and their lifestyle practices, suggests the following: (a) it is important to raise the awareness of women of reproductive age and pregnant women about how to safely include fish in their diet and to empower them to make proper decisions for nutrition and control of MeHg, (b) prenatal and women's exposure to tobacco smoke remains a concern in Europe and targeted awareness campaigns are necessary for prevention and control, (c) more generally, women of reproductive age and pregnant women must be better informed about lifestyle choices to prevent harmful chemical exposure and about the importance of maintaining a healthy BMI when planning for pregnancy.

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Data availability statement

Not applicable.

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Abbreviations

BMI	Body Mass Index
BE	Belgium
BP	Before pregnancy
CNSA-ISCIII	*National Centre for Environmental Health*-Instituto de Salud Carlos III
CY	Cyprus
DRV	Dietary Reference Value

DP	During pregnancy
3-DHA	3-docosahexaenoic acid
EC	European Commission
EFSA	European Food Safety Authority
EL	Greece
ES	Spain
GM	Geometric Mean
HBM	Human Biomonitoring
HBM4EU-MOM	"Methylmercury-control in expectant mothers through suitable dietary advice for pregnancy"
IS	Iceland
ISCED	International Standard Classification of Education
MOH-CY	Ministry of Health -Cyprus – State General Laboratory
PT	Portugal
PUFA	Polyunsaturated Fatty Acids
RCT	Randomized control trial
RoHS	Restriction of Hazardous Substances
QA/QC	Quality Assurance/Quality Control
SOP	Standard Operating Procedure
THg	Total mercury
TWI	Tolerable Weekly Intake
VITO	Vlaamse Instelling voor Technologisch Onderzoek- Flemish Institute for Technological Research
WHO	World Health Organization

Appendix A. Supplementary data

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References

Aparicio, E., Martín-Grau, C., Bedmar, C., Orus, N.S., Basora, J., Arija, V., 2021. Maternal factors associated with levels of fatty acids, specifically n-3 pufa during pregnancy: eclipses study. Nutrients 13, 1–19.

Babic, A., Tokalic, R., Amílcar Silva Cunha, J., Novak, I., Suto, J., Vidak, M., Miosic, I., Vuka, I., Poklepovic Pericic, T., Puljak, L., 2019. Assessments of attrition bias in Cochrane systematic reviews are highly inconsistent and thus hindering trial comparability. BMC Med. Res. Methodol. 19, 76.

Basu, N., Horvat, M., Evers, D.C., Zastenskaya, I., Weihe, P., Tempowski, J., 2018. A State-Of-The-Science Review of Mercury Biomarkers in Human Populations Worldwide between 2000 and 2018, vol. 126, 106001.

Basu, N., Bastiansz, A., Dórea, J.G., Fujimura, M., Horvat, M., Shroff, E., Weihe, P., Zastenskaya, I., 2023. Our evolved understanding of the human health risks of mercury. Ambio.

Beck, A.L., Bräuner, E.V., Hauser, R., Lim, Y.-H., Uldbjerg, C.S., Juul, A., 2023. Maternal Exposure to Cigarette Smoke during Pregnancy and Testicular Cancer in Offspring: A Systematic Review and Meta-Analysis, vol. 13, p. 618.

Becker, W., D, P.O., P.-G, A.K., 2007. Risks and Benefits of Fish Consumption. NATIONAL FOOD ADMINISTRATION, Sweden.

Bednarczuk, N., Milner, A., Greenough, A., 2020. The Role of Maternal Smoking in Sudden Fetal and Infant Death Pathogenesis, vol. 11.

Bjørklund, G., Antonyak, H., Polishchuk, A., Semenova, Y., Lesiv, M., Lysiuk, R., Peana, M., 2022. Effect of methylmercury on fetal neurobehavioral development: an overview of the possible mechanisms of toxicity and the neuroprotective effect of phytochemicals. Arch. Toxicol. 96, 3175–3199.

Boucher, O., Jacobson, S.W., Plusquellec, P., Dewailly, É., Ayotte, P., Forget-Dubois, N., Jacobson, J.L., Muckle, G., 2012. Prenatal methylmercury, postnatal lead exposure, and evidence of attention deficit/hyperactivity disorder among Inuit children in Arctic Québec. Environ. Health Perspect. 120, 1456–1461.

Castaño, A., Cutanda, F., Esteban, M., Pärt, P., Navarro, C., Gómez, S., Rosado, M., López, A., López, E., Exley, K., Schindler, B.K., Govarts, E., Casteleyn, L., Kolossa-Gehring, M., Fiddicke, U., Koch, H., Angerer, J., Hond, E.D., Schoeters, G., Sepai, O., Horvat, M., Knudsen, L.E., Aerts, D., Joas, A., Biot, P., Joas, R., Jiménez-Guerrero, J. A., Diaz, G., Pirard, C., Katsonouri, A., Cerna, M., Gutleb, A.C., Ligocka, D., Reis, F. M., Berglund, M., Lupsa, I.-R., Halzlová, K., Charlier, C., Cullen, E., Hadjipanayis, A., Krsková, A., Jensen, J.F., Nielsen, J.K., Schwedler, G., Wilhelm, M., Rudnai, P., Középesy, S., Davidson, F., Fischer, M.E., Janasik, B., Namorado, S., Gurzau, A.E., Jajcaj, M., Mazej, D., Tratnik, J.S., Larsson, K., Lehmann, A., Crettaz, P., Lavranos, G., Posada, M., 2015. Fish consumption patterns and hair mercury levels in children and their mothers in 17 EU countries. Environ. Res. 141, 58–68.

Close, C., Sinclair, M., Mccullough, J.E.M., Liddle, S.D., Hughes, C.M., 2016. Factors affecting recruitment and attrition in randomised controlled trials of complementary and alternative medicine for pregnancy-related issues. Evid. base Compl. Alternative Med. 2016, 6495410.

Davidson, P.W., Leste, A., Benstrong, E., Burns, C.M., Valentin, J., Sloane-Reeves, J., Huang, L.-S., Miller, W.A., Gunzler, D., Van Wijngaarden, E., Watson, G.E., Zareba, G., Shamlaye, C.F., Myers, G.J., 2010. Fish consumption, mercury exposure, and their associations with scholastic achievement in the Seychelles Child Development Study. Neurotoxicology 31, 439–447.

Di Ciaula, A., 2021. Bioaccumulation of toxic metals in children exposed to urban pollution and to cement plant emissions. Exposure and Health 13, 681–695.

Dórea, J.G., 2021. Neurodevelopment and exposure to neurotoxic metal(loid)s in environments polluted by mining, metal scrapping and smelters, and e-waste recycling in low and middle-income countries. Environ. Res. 197, 111124.

Driscoll, C.T., Mason, R.P., Chan, H.M., Jacob, D.J., Pirrone, N., 2013. Mercury as a global pollutant: sources, pathways, and effects. Environ. Sci. Technol. 47, 4967–4983.

Dumville, J.C., Torgerson, D.J., Hewitt, C.E., 2006. Reporting Attrition in Randomised Controlled Trials, vol. 332, pp. 969–971.

Efsa, S.C., 2015. Statement on the Benefits of Fish/seafood Consumption Compared to the Risks of Methylmercury in Fish/seafood, vol. 13, p. 3982.

Efsa, 2016. Summary of the 2015 data. Collection on Contaminant Occurrence Data 13, 1048E.

Esteban, M., Schindler, B.K., Jiménez, J.A., Koch, H.M., Angerer, J., Rosado, M., Gómez, S., Casteleyn, L., Kolossa-Gehring, M., Becker, K., Bloemen, L., Schoeters, G., Hond, E.D., Sepai, O., Exley, K., Horvat, M., Knudsen, L.E., Joas, A., Joas, R., Aerts, D., Biot, P., Borošová, D., Davidson, F., Dumitrascu, I., Fischer, M.E., Grander, M., Janasik, B., Jones, K., Kašparová, L., Larssen, T., Naray, M., Nielsen, F., Hohenblum, P., Pinto, R., Pirard, C., Plateel, G., Tratnik, J.S., Wittsiepe, J., Castaño, A., 2015. Mercury analysis in hair: comparability and quality assessment within the transnational COPHES/DEMOCOPHES project. Environ. Res. 141, 24–30.

Eurostat, 2022. Regions in Europe, 2022 interactive edition. https://ec.europa.eu/eurostat/cache/digpub/regions/.

Fao, 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. FAO, Rome. https://doi.org/10.4060/cc0461en.

Feng, L., Zhang, C., Liu, H., Li, P., Hu, X., Wang, H., Chan, H.M., Feng, X., 2020. Impact of Low-Level Mercury Exposure on Intelligence Quotient in Children via Rice Consumption. Ecotoxicology and Environmental Safety, p. 202.

Fiddicke, U., Becker, K., Schwedler, G., Seiwert, M., Joas, R., Joas, A., Biot, P., Aerts, D., Casteleyn, L., Dumez, B., Castaño, A., Esteban, M., Angerer, J., Koch, H.M., Schoeters, G., Hond, E.D., Sepai, O., Exley, K., Knudsen, L.E., Horvat, M., Bloemen, L., Katsonouri, A., Hadjipanayis, A., Cerna, M., Krsková, A., Jensen, J.F., Nielsen, J.K.S., Rudnai, P., Közepésy, S., Gutleb, A.C., Fischer, M.E., Ligocka, D., Kamińska, J., Reis, M.F., Namorado, S., Lupsa, I.-R., Gurzau, A.E., Halzlová, K., Mazej, D., Tratnik, J.S., Rivas, T.C., Gómez, S., Berglund, M., Larsson, K., Lehmann, A., Crettaz, P., Dewolf, M.-C., Burns, D., Kellegher, A., Kolossa-Gehring, M., 2015. Lessons learnt on recruitment and fieldwork from a pilot European human biomonitoring survey. Environ. Res. 141, 15–23.

Gad, S.C., 2014. Methylmercury. In: WEXLER, P. (Ed.), Encyclopedia of Toxicology, third ed. Academic Press, Oxford.

Ganzleben, C., Antignac, J.-P., Barouki, R., Castaño, A., Fiddicke, U., Klánová, J., Lebret, E., Olea, N., Sarigiannis, D., Schoeters, G.R., Sepai, O., Tolonen, H., Kolossa-Gehring, M., 2017. Human biomonitoring as a tool to support chemicals regulation in the European Union. Int. J. Hyg Environ. Health 220, 94–97.

Garí, M., Grzesiak, M., Krekora, M., Kaczmarek, P., Jankowska, A., Król, A., Kaleta, D., Jerzyńska, J., Janasik, B., Kuraś, R., Tartaglione, A.M., Calamandrei, G., Hanke, W., Polańska, K., 2022. Prenatal Exposure to Neurotoxic Metals and Micronutrients and Neurodevelopmental Outcomes in Early School Age Children from Poland, vol. 204. Environmental Research.

Gudipally, M., Farooq, F., Basany, K., Haggerty, C.L., Tang, G., Kusneniwar, G.N., Jammy, G.R., Bunker, C.H., Reddy, P.S., 2023. Impact of prepregnancy body mass index on adverse pregnancy outcomes: analysis from the Longitudinal Indian Family hEalth cohort study. AJOG Global Reports 3, 100134.

He, L., Michailidou, F., Gahlon, H.L., Zeng, W., 2022. Hair dye ingredients and potential health risks from exposure to hair dyeing. Chem. Res. Toxicol. 35, 901–915.

Hellberg, R.S., Dewitt, C.A.M., Morrissey, M.T., 2012. Risk-benefit analysis of seafood consumption. A Review 11, 490–517.

Heslehurst, N., Ells, L., Simpson, H., Batterham, A., Wilkinson, J., Summerbell, C., 2007. Trends in Maternal Obesity Incidence Rates, Demographic Predictors, and Health Inequalities in 36 821 Women over a 15-year Period, vol. 114, pp. 187–194.

Hibbeln, J.R., Davis, J.M., Steer, C., Emmett, P., Rogers, I., Williams, C., Golding, J., 2007. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. Lancet 369, 578–585.

Hibbeln, J.R., Spiller, P., Brenna, J.T., Golding, J., Holub, B.J., Harris, W.S., Kris-Etherton, P., Lands, B., Connor, S.L., Myers, G., Strain, J.J., Crawford, M.A., Carlson, S.E., 2019. Relationships between seafood consumption during pregnancy and childhood and neurocognitive development: two systematic reviews. Prostaglandins Leukot. Essent. Fatty Acids 151, 14–36.

Hond, E.D., Govarts, E., Willems, H., Smolders, R., Casteleyn, L., Kolossa-Gehring, M., Schwedler, G., Seiwert, M., Fiddicke, U., Castaño, A., Esteban, M., Angerer, J., Koch, H.M., Schindler, B.K., Sepai, O., Exley, K., Bloemen, L., Horvat, M., Knudsen, L.E., Joas, A., Joas, R., Biot, P., Aerts, D., Koppen, G., Katsonouri, A., Hadjipanayis, A., Krskova, A., Maly, M., Mørck, T.A., Rudnai, P., Kozepesy, S., Mulcahy, M., Mannion, R., Gutleb, A.C., Fischer, M.E., Ligocka, D., Jakubowski, M., Reis, M.F., Namorado, S., Gurzau, A.E., Lupsa, I.-R., Halzlova, K., Jajcaj, M., Mazej, D., Tratnik, J.S., López, A., Lopez, E., Berglund, M., Larsson, K., Lehmann, A., Crettaz, P., Schoeters, G., 2015. First steps toward harmonized human biomonitoring in Europe: demonstration project to. Perform Human Biomonitoring on a European Scale 123, 255–263.

Karel Houessionon, M.G., Ouendo, E.M.D., Bouland, C., Takyi, S.A., Kedote, N.M., Fayomi, B., Fobil, J.N., Basu, N., 2021. Environmental heavy metal contamination from electronic waste (E-waste) recycling activities worldwide: a systematic review from 2005 to 2017. Int. J. Environ. Res. Publ. Health 18.

Kirk, L.E., Jørgensen, J.S., Nielsen, F., Grandjean, P., 2017. Public health benefits of hair-mercury analysis and dietary advice in lowering methylmercury exposure in pregnant women. Scand. J. Publ. Health 45, 444–451.

Knudsen, L.E., Tolonen, H., Scheepers, P.T.J., Loots, I., Vorkamp, K., Hajeb, P., Sepai, O., Gilles, L., Splanemann, P., Weise, P., Kolossa-Gehring, M., 2023. Implementation and coordination of an ethics framework in HBM4EU – experiences and reflections. Int. J. Hyg Environ. Health 248, 114098.

Kolossa-Gehring, M., Pack, L.K., Hülck, K., Gehring, T., 2023. HBM4EU from the Coordinator's perspective: lessons learnt from managing a large-scale EU project. Int. J. Hyg Environ. Health 247, 114072.

Lange, S., Probst, C., Rehm, J., Popova, S., 2018. National, regional, and global prevalence of smoking during pregnancy in the general population: a systematic review and meta-analysis. Lancet Global Health 6, e769–e776.

Li, N., Wu, X., Zhuang, W., Xia, L., Chen, Y., Wu, C., Rao, Z., Du, L., Zhao, R., Yi, M., Wan, Q., Zhou, Y., 2020. Fish consumption and multiple health outcomes: umbrella review. Trends Food Sci. Technol. 99, 273–283.

Li, C., Wang, H., Liao, X., Xiao, R., Liu, K., Bai, J., Li, B., He, Q., 2022. Heavy metal pollution in coastal wetlands: a systematic review of studies globally over the past three decades. J. Hazard Mater. 424, 127312.

Linou, A., 2014. National dietary guidelines for women, including women in pregnancy, lactation& menopause. http://www.diatrofikoiodigoi.gr/?Page=summary-women.

Liu, S., Wang, X., Guo, G., Yan, Z., 2021. Status and environmental management of soil mercury pollution in China: a review. J. Environ. Manag. 277.

Maclachlan, A., Crawford, K., Shinwell, S., Nixon, C., Henderson, M., 2021. Recruiting hard-to-reach pregnant women at high psychosocial risk: strategies and costs from a randomised controlled trial. Trials 22, 402.

Mary Dawn Koenig, L.T.-H., Demartelly, Victoria, Labomascus, Bazil, Ojinjideka Hemphill, Nefertiti, Welke, Lauren, Lacey, Pezley, Ruchob, Rungnapa, Hirsch, Bruni, Furlette-Koski, Melissa, Kessee, Nicollette, Ferrans, Carol Estwing, 2022. Recruitment and Retention of Urban Pregnant Women to a Clinical Study Administering an Oral Isotope Dietary Tracer, vol. 3, pp. 652–660.

Maulvault, A.L., Anacleto, P., Barbosa, V., Sloth, J.J., Rasmussen, R.R., Tediosi, A., Fernandez-Tejedor, M., Van Den Heuvel, F.H.M., Kotterman, M., Marques, A., 2015. Toxic elements and speciation in seafood samples from different contaminated sites in Europe. Environ. Res. 143, 72–81.

Minamata, 2019. Minamata Convention on Mercury. United Nations Environment Programme.

Mng'ong'o, M., Munishi, L.K., Ndakidemi, P.A., Blake, W., Comber, S., Hutchinson, T.H., 2021. Toxic metals in East African agro-ecosystems: key risks for sustainable food production. J. Environ. Manag. 294, 112973.

Negi, S., Bala, L., Shukla, S., Chopra, D., 2022. Tattoo Inks Are Toxicological Risks to Human Health: A Systematic Review of Their Ingredients, Fate inside Skin, Toxicity Due to Polycyclic Aromatic Hydrocarbons, Primary Aromatic Amines, Metals, and Overview of Regulatory Frameworks, vol. 38, pp. 417–434.

Nguetseng, R., Fliedner, A., Knopf, B., Lebreton, B., Quack, M., Rüdel, H., 2015. Retrospective monitoring of mercury in fish from selected European freshwater and estuary sites. Chemosphere 134, 427–434.

Nunes, E., Cavaco, A., Carvalho, C., 2014. Children's health risk and benefits of fish consumption: risk indices based on a diet diary follow-up of two weeks. J. Toxicol. Environ. Health 77, 103–114.

Oken, E., Kleinman, K.P., Berland, W.E., Simon, S.R., Rich-Edwards, J.W., Gillman, M. W., 2003. Decline in fish consumption among pregnant women after a national mercury advisory. Obstet. Gynecol. 102, 346–351.

Oken, E., Østerdal, M.L., Gillman, M.W., Knudsen, V.K., Halldorsson, T.I., Strøm, M., Bellinger, D.C., Hadders-Algra, M., Michaelsen, K.F., Olsen, S.F., 2008a. Associations of maternal fish intake during pregnancy and breastfeeding duration with attainment of developmental milestones in early childhood: a study from the Danish National Birth Cohort. Am. J. Clin. Nutr. 88, 789–796.

Oken, E., Radesky, J.S., Wright, R.O., Bellinger, D.C., Amarasiriwardena, C.J., Kleinman, K.P., Hu, H., Gillman, M.W., 2008b. Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 Years in a US cohort. Am. J. Epidemiol. 167, 1171–1181.

Oken, E., Guthrie, L.B., Bloomingdale, A., Platek, D.N., Price, S., Haines, J., Gillman, M. W., Olsen, S.F., Bellinger, D.C., Wright, R.O., 2013. A pilot randomized controlled trial to promote healthful fish consumption during pregnancy: the Food for Thought Study. Nutr. J. 12, 33.

Pack, L.K., Gilles, L., Cops, J., Tolonen, H., Van Kamp, I., Esteban-López, M., Pedraza-Díaz, S., Lacasaña, M., Alzaga, B.G., Lermen, D., Bartel-Steinbach, M., Katsonouri, A., Fiddicke, U., Castaño, A., Kolossa-Gehring, M., 2023. A step towards harmonising human biomonitoring study setup on European level: materials provided and lessons learnt in HBM4EU. Int. J. Hyg Environ. Health 249, 114118.

Pavithra, K.G., Sundarrajan, P., Kumar, P.S., Rangasamy, G., 2023. Mercury sources, contaminations, mercury cycle, detection and treatment techniques: a review. Chemosphere 312, 137314.

R-Project, 2009. R Developement Core Team A language and environment for statistical computing. http://www.R-project.org.

Re, D.B., Yan, B., Calderón-Garcidueñas, L., Andrew, A.S., Tischbein, M., Stommel, E.W., 2022. A perspective on persistent toxicants in veterans and amyotrophic lateral sclerosis: identifying exposures determining higher ALS risk. J. Neurol. 269, 2359–2377.

Reach, 2009. Annex XVII to the REACH Regulation. *Entry no. 18a in Regulation EC No. 552/2009 REACH*.

Ren, S., Song, C., Ye, S., Cheng, C., Gao, P., 2022. The spatiotemporal variation in heavy metals in China's farmland soil over the past 20 years: a meta-analysis. Sci. Total Environ. 806, 150322.

Rodríguez-Bernal, C.L., Ramón, R., Quiles, J., Murcia, M., Navarrete-Muñoz, E.M., Vioque, J., Ballester, F., Rebagliato, M., 2013. Dietary intake in pregnant women in a Spanish Mediterranean area: as good as it is supposed to be? Publ. Health Nutr. 16, 1379–1389.

Roe, K., 2022. An alternative explanation for alzheimer's disease and Parkinson's disease initiation from specific antibiotics, gut microbiota dysbiosis and neurotoxins. Neurochem. Res. 47, 517–530.

Sarigiannis, D.A., Karakitsios, S.P., Antonakopoulou, M.P., Gotti, A., 2012. Exposure analysis of accidental release of mercury from compact fluorescent lamps (CFLs). Sci. Total Environ. 435–436, 306–315.

Strain, J.J., Davidson, P.W., Thurston, S.W., Harrington, D., Mulhern, M.S., Mcafee, A.J., Van Wijngaarden, E., Shamlaye, C.F., Henderson, U., Watson, G.E., Zareba, G., Cory-Slechta, D.A., Lynch, M., Wallace, J.M.W., Mcsorley, E.M., Bonham, M.P., Stokes-Riner, A., Sloane-Reeves, J., Janciuras, J., Wong, R., Clarkson, T.W., Myers, G.J., 2012. Maternal PUFA status but not prenatal methylmercury exposure is associated with children's language functions at age five years in the Seychelles,12. J. Nutr. 142, 1943–1949.

Stratakis, N., Conti, D.V., Borras, E., Sabido, E., Roumeliotaki, T., Papadopoulou, E., Agier, L., Basagana, X., Bustamante, M., Casas, M., Farzan, S.F., Fossati, S., Gonzalez, J.R., Grazuleviciene, R., Heude, B., Maitre, L., Mceachan, R.R.C., Theologidis, I., Urquiza, J., Vafeiadi, M., West, J., Wright, J., Mcconnell, R., Brantsaeter, A.L., Meltzer, H.M., Vrijheid, M., Chatzi, L., 2020. Association of fish consumption and mercury exposure during pregnancy with metabolic health and inflammatory biomarkers in children. JAMA Netw. Open 3, e201007.

Tang, J., Zhu, X., Chen, Y., Huang, D., Tiemeier, H., Chen, R., Bao, W., Zhao, Q., 2021. Association of maternal pre-pregnancy low or increased body mass index with adverse pregnancy outcomes. Sci. Rep. 11, 3831.

Taylor, C.M., Emmett, P.M., Emond, A.M., Golding, J., 2018. A review of guidance on fish consumption in pregnancy: is it fit for purpose? Publ. Health Nutr. 21, 2149–2159.

Tressou, J., Buaud, B., Simon, N., Pasteau, S., Guesnet, P., 2019. Very low inadequate dietary intakes of essential n-3 polyunsaturated fatty acids (PUFA) in pregnant and lactating French women: the INCA2 survey. Prostaglandins Leukot. Essent. Fatty Acids 140, 3–10.

Turyk, M., Korinek, J., Souther, L., Baker, B., McCann, P., 2019. Dietary counseling on risks and benefits of fish consumption and mercury testing in reproductive age women in a health clinic setting. Environ. Epidemiol. 3, 402. https://doi.org/10.1097/01.EE9.0000610492.30800.c6.

Van Delft, K., S.-T, N., Thakar, R., Sultan, A.H., 2022. Recruitment and Retention of Urban Pregnant Women to a Clinical Study Administering an Oral Isotope Dietary Tracer, vol. 3, pp. 652–660.

Vilavert, L., Borrell, F., Nadal, M., Jacobs, S., Minnens, F., Verbeke, W., Marques, A., Domingo, J.L., 2017. Health risk/benefit information for consumers of fish and shellfish: FishChoice, a new online tool. Food Chem. Toxicol. 104, 79–84.

Višnjevec, A.M., Kocman, D., Horvat, M., 2014. Human Mercury Exposure and Effects in Europe, vol. 33, pp. 1259–1270.

WHO, 1990. International Program on Chemical Safety. Environmental Methylmercury: Health Criteria 101. World Health Organization, Geneva, 1990. *[cited 2012 Oct 2]*. http://www.inchem.org/documents/ehc/ehc/ehc101.htm.

WHO, 2001. Healthy Eating during Pregnancy and Breastfeeding (Booklet for Mothers). World Health Organization – 2001.

WHO, 2019. Environmental Health Inequalities in Europe. Second assessment report. WHO Regional Office for Europe, Copenhagen, 2019. Licence: CC BY-NC-SA 3.0 IGO.. *ISBN: 9789289054157,* Available online at: https://apps.who.int/iris/bitstream/handle/10665/325176/9789289054157-eng.pdf.

WHO, 2020. The First Survey Protocol. World Health Organization, Regional Office for Europe, Copenhagen, 2018. Licence: CC BY-NC-SA 3.0 IGO. https://apps.who.int/iris/bitstream/handle/10665/334181/WHO-EURO-2020-1069-40815-55163-eng.pdf. (Accessed 27 March 2023).

Zhao, G., Liu, S.J., Gan, X.Y., Li, J.R., Wu, X.X., Liu, S.Y., Jin, Y.S., Zhang, K.R., Wu, H.M., 2023. Analysis of whole blood and urine trace elements in children with autism spectrum disorders and autistic behaviors. Biol. Trace Elem. Res. 201, 627–635.

Zheng, K., Zeng, Z., Tian, Q., Huang, J., Zhong, Q., Huo, X., 2023. Epidemiological evidence for the effect of environmental heavy metal exposure on the immune system in children. Sci. Total Environ. 868, 161691.

Zong, X.N., Wang, H., Yang, L., Guo, Y., Zhao, M., Magnussen, C.G., Xi, B., 2022. Maternal Pre-pregnancy Body Mass Index Categories and Infant Birth Outcomes: A Population-Based Study of 9 Million Mother–Infant Pairs, vol. 9.

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Human biomonitoring of deoxynivalenol (DON) - Assessment of the exposure of young German adults from 1996 - 2021

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ABSTRACT

The mycotoxin deoxynivalenol (DON) is a frequently found contaminant in cereals and cereal-based products. As a German contribution to the European Joint Programme HBM4EU, we analysed the total DON concentration (tDON) in 24-h urine samples from the German Environmental Specimen Bank (ESB). In total, 360 samples collected in 1996, 2001, 2006, 2011, 2016, and 2021 from young adults in Muenster (Germany), were measured by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) after enzymatic deconjugation of the glucuronide metabolites.

tDON was found in concentrations above the lower limit of quantification (0.3 μg/L) in 99% of the samples. Medians of the measured concentrations and the daily excretion were 4.3 μg/L and 7.9 μg/24 h, respectively. For only nine participants, urinary tDON concentrations exceeded the provisional Human biomonitoring guidance value (HBM GV) of 23 μg/L.

Urinary tDON concentrations were significantly higher for male participants. However, 24-h excretion values normalized to the participant's body weight did not exhibit any significant difference between males and females and the magnitude remained unchanged over the sampling years with exception of the sampling year 2001.

Daily intakes were estimated from excretion values. Exceedance of the tolerable daily intake (TDI) of 1 μg/kg bw per day was observed for less than 1% of all participants. TDI exceedances were only present in the sampling year 2001 and not in more recent sampling years while exceedance of the HBM guidance value was also observed in 2011 and 2021.

1. Introduction

Deoxynivalenol (DON) is a mycotoxin produced primarily by fungi of the genus *Fusarium*. *Fusarium* fungi belong to the most prevalent toxin-producing fungi infecting cereal crops pre harvest in northern temperate regions (SCF, 2002). Therefore, DON is a frequent contaminant in cereals and cereal-based products. The general population is exposed to this toxin mainly via ingestion of contaminated foods (EFSA Panel on Contaminants in the Food Chain, 2017; Marin et al., 2013). At workplaces, inhalation of bioaerosols and dusts and dermal contact with contaminated material are relevant routes of exposure (Degen, 2011; Fromme et al., 2016; Viegas et al., 2018). General toxicity and immunotoxicity are considered to be critical effects of DON. The Scientific Committee on Food (SCF) of the European Commission derived a tolerable daily intake (TDI) of 1 μg/kg bw per day based on the no observed adverse effect level of 100 μg/kg bw per day and an uncertainty factor of 100 (SCF, 2002). Adverse effects that were taken into account cover growth retardation, increased susceptibility to infections and reproductive effects as most sensitive endpoints observed in animal studies in mice and rats by Iverson et al. (1996), Tryphonas et al. (1986) and Khera et al. (1984), respectively. Since 2017, the TDI is used as group TDI for the sum of DON and co-occurring metabolites of DON (acetylated and modified forms) in food that are largely converted to DON during mammalian digestion (EFSA Panel on Contaminants in the

Abbreviations: bw, body weight; DGlcA, DON-glucuronides; D3GlcA, DON-3-glucuronide; D15GlcA, DON-15-glucuronide; DON, deoxynivalenol; DOM-1, De-epoxy-deoxynivalenol; NOAEL, no-observed-adverse-effect level; ESB, Environmental Specimen Bank; HBM, human biomonitoring; HPLC-MS/MS, high performance liquid chromatography-tandem mass spectrometry; IAC, immunoaffinity chromatography; TDI, tolerable daily intake; tDON, total DON.

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Table 1
Characteristics of the study population.

Sampling year	N (m/f)	Age [years] AM (range)	bw [kg] AM (range)	24-h urine volume [mL] AM (range)	Creatinine [g/L] AM (range)
1996	60 (30/30)	24.5 (20–29)	68.8 (45–98)	1441 (540–2525)	1.18 (0.49–2.21)
2001	60 (30/30)	23.6 (20–29)	70.1 (44–124)	1698 (510–2800)	1.07 (0.30–2.43)
2006	60 (30/30)	24.0 (20–29)	69.3 (47–112)	1884 (345–4287)	0.91 (0.29–2.52)
2011	60 (30/30)	23.0 (20–29)	71.5 (48–102)	1886 (766–3049)	0.86 (0.25–2.07)
2016	60 (30/30)	23.5 (20–29)	71.5 (48–100)	2122 (569–3207)	0.72 (0.32–2.29)
2021	60 (30/30)	23.0 (20–28)	67.7 (50–94)	2142 (1042–4918)	0.71 (0.26–1.71)
ΣAll	360	23.6 (20–29)	69.8 (44–124)	1862 (345–4918)	0.91 (0.25–2.52)
ΣMale	180	23.9 (20–29)	78.1 (53–124)	1883 (569–4918)	1.07 (0.25–2.43)
ΣFemale	180	23.2 (20–29)	61.5 (44–102)	1841 (345–4410)	0.75 (0.26–2.52)

N: number of samples; m: male; f: female; AM: arithmetic mean; bw: body weight.

Food Chain, 2017). Based on the TDI, a provisional HBM guidance value of 23 μg DON/L urine was derived for 24-h urine samples (Apel et al., 2023).

Furthermore, in Europe the EU regulation (EC) No 1881/2006 give maximum levels for DON in foodstuffs such as cereals, cereal flour, pasta and bread (European Commission, 2006). DON concentrations exceeding these maximum levels occur and are reported for less than 2% of the analysed foodstuffs samples (EFSA, 2013). In humans, ingested DON is absorbed, distributed, metabolized and excreted to urine rapidly (Mengelers et al., 2019). Approximately 64% of a single oral dose of DON were recovered in human urine within 24 h after administration (Vidal et al., 2018). Conjugation of DON to glucuronic acid is the major metabolic pathway, and DON-15-glucuronide (D15GlcA) is the main conjugation product besides DON-3-glucuronide (D3GlcA) (Warth et al., 2013). In human biomonitoring studies, DON-glucuronides (DGlcA) accounted in average for 66%–91% of the total DON (tDON, sum of free DON plus glucuronides) excreted (Brera et al., 2015; Turner et al., 2011a; Vidal et al., 2018; Warth et al., 2012). De-epoxy-deoxynivalenol (DOM-1) is a further DON metabolite found less frequently in human urine samples (EFSA Panel on Contaminants in the Food Chain, 2017). It is suggested that DOM-1 derives from DON metabolism by intestinal microbiota (Gratz et al., 2013). While tDON correlates well with dietary exposure to DON, DOM-1 is considered to be an unsuitably biomarker of exposure (EFSA Panel on Contaminants in the Food Chain, 2017).

Within the European Human Biomonitoring Initiative (HBM4EU) (Ganzleben et al., 2017; Kolossa-Gehring et al., 2023), a large scale EU project operating at the science-policy interface, substances were systematically prioritized with the aim to harmonise human biomonitoring procedures and collect recent exposure data (Ougier et al., 2021). The resulting highly quality assured and comparable data supply the basis for their use to improve policies and control their effectiveness at national and European scale.

Due to its widespread occurrence and concerns of possible adverse effects on human health, DON has been prioritized in the 2nd HBM4EU prioritisation round and set on the 2nd priority list of substances (HBM4EU, 2018). The combined analytical determination of free DON and DGlcA in urine after enzymatic deconjugation of the glucuronides with *β*-glucuronidase has been chosen and validated as HBM method to assess the DON exposure (Esteban López et al., 2021; Gilles et al., 2022; HBM4EU, 2022). In this study, we applied this method to analyse 360 24-h urine samples from the German Environmental Specimen Bank (ESB) collected from 1996 to 2021. The results provide information on both the recent and the past internal exposure to DON, and hence enable the investigation of the DON exposure over the course of time. Based on the results we estimate the daily intake and assess the exposure level.

2. Materials and methods

2.1. Study population

In total, 360 24-h urine samples collected from student volunteers in Muenster (containing only one first-morning void) were analysed in this study. The samples were aliquoted, precooled down to −160 °C and then stored under cryogenic conditions over liquid nitrogen in the ESB (Kolossa-Gehring et al., 2012; Lermen et al., 2014).

Per sampling year (1996, 2001, 2006, 2011, 2016, 2021), samples of 60 participants with an equal distribution of males and females were analysed. Table 1 summarizes the characteristics of the study population as well as information on 24-h urine volumes and creatinine contents. All participants provided informed consent. The study protocol of the ESB was approved by the ethics committees of the Medical Association of Westphalia-Lippe (until 2011) and of the Medical Association of the Saarland (since 2011).

2.2. Analytical method

Analytical determination of total deoxynivalenol was carried out according to Berger et al. with one modification and involved enzymatic cleavage of glucuronide conjugates with *β*-glucuronidase from *Escherichia coli* (*E. coli*) followed by immunoaffinity chromatography purification (IAC). Purified and concentrated samples were analysed by HPLC-MS/MS using fully ^{13}C-labeled DON as internal standard and a blank urine sample for a matrix-matched calibration that was obtained from a volunteer without consumption of cereals or cereal-based products. The lower limit of quantification (LLOQ) of the applied method was 0.3 μg/L.

DON StarR immunoaffinity columns (Romer Labs, Tulln, Austria) used in our previous study were unavailable. Therefore, DON Star immunoaffinity columns (Romer Labs, Tulln, Austria) were used for IAC purification. While DOM-1 binds to DON StarR (previous study), DON Star columns showed no cross-reactivity for this DON metabolite. No differences were observed for DON.

Creatinine was measured photometrically according to the Jaffé method (Lermen et al., 2014).

2.3. Statistical analysis

IBM SPSS Statistics 26 was used for statistical analysis. tDON concentrations below LLOQ were assigned with the value half of the LLOQ. To compare sexes and sampling years, non-parametric tests (Mann-Whitney-*U* test, Kruskal–Wallis test) were applied. Bonferroni-correction was used for post-hoc tests.

Correlations were evaluated using non-parametric Spearman rank correlation analysis. A significance level of $\alpha = 0.05$ was assigned for statistical significance.

Descriptive statistics are represented as means, percentiles (50th, 90th) and ranges.

Urinary tDON concentrations are given without creatinine adjustment. Daily urinary tDON excretions are shown normalized to the body weight and without normalization.

For latter see supplementary information.

Table 2
Urinary tDON concentrations. Values are given in μg/L.

	Sampling year	1996	2001	2006	2011	2016	2021	Σ1996–2021
All participants	N > LLOQ	60 (100%)	59 (98%)	60 (100%)	60 (100%)	60 (100%)	58 (97%)	357 (99%)
	AM	5.09	13.1	4.90	6.81	4.05	4.97	6.48
	GM	3.85	8.80	3.83	5.37	2.47	3.16	4.19
	Median	3.93	9.11	4.01	5.52	2.26	3.36	4.33
	P90	10.4	26.0	9.79	14.2	10.3	9.37	14.2
	Range	0.45–23.0	<LLOQ–99.1	0.84–18.8	0.38–24.2	0.5–21.3	<LLOQ–24.9	<LLOQ–99.1
Males	N > LLOQ	30 (100%)	29 (97%)	30 (100%)	30 (100%)	30 (100%)	30 (100%)	179 (99%)
	AM	6.19	16.3	5.98	7.25	4.04	7.21	7.51
	GM	4.57	9.88	4.77	5.61	2.56	5.10	5.01
	Median	5.24	11.7	4.78	5.91	2.34	5.52	5.50
	P90	12.6	42.9	10.5	16.0	10.2	17.8	16.50
	Range	0.45–23.0	<LLOQ–99.1	0.92–18.8	1.51–24.2	0.73–19.4	0.73–24.9	<LLOQ–99.1
Females	N > LLOQ	30 (100%)	30 (100%)	30 (100%)	30 (100%)	30 (100%)	28 (93%)	178 (99%)
	AM	3.98	9.83	3.81	6.36	4.06	2.73	5.13
	GM	3.24	7.85	3.07	5.13	2.39	1.97	3.51
	Median	3.33	7.3	2.94	5.03	2.07	2.28	3.54
	P90	7.29	22.6	7.86	13.5	10.6	7.03	11.1
	Range	1.06–19.2	1.72–26.4	0.84–10.4	0.38–16.9	0.5–21.3	<LLOQ–8.66	<LLOQ–26.4

N: number of samples; LLOQ: lower limit of quantification (0.3 μg/L); AM: arithmetic mean; GM: geometric mean; P90: 90th percentile.

Table 3
Urinary DON concentrations in human biomonitoring studies.

Country	Collection year(s)	N (m/f)	Study Population	Urine type	Positive, N (%)	Median [μg/L] (range)	Reference
Germany	1996–2021	360 (180/180)	adults	24-h	357 (99)	tDON 4.33 (<0.3–99.1)	This study
Germany	2013	50 (23/27)	adults	1stmorning	50 (100)	tDON 7.35 (1.06–38.4)	Ali et al. (2016)
Italy	2011	52 (26/26)	children and adults	1stmorning	50 (96)	tDON 10.3 (<1.5–67.4)	Solfrizzo et al. (2014)
Norway	2014	230 (92/138)	children and adults	1stmorning	229 (99)	tDON 6.17 (<0.015–86.9)	Brera et al. (2015)
Portugal	2015–2016	94 (48/46)	adults	24-h	59 (63)	DON 2.51 (<1.0–36.3)	Martins et al. (2019)
					35 (37)	D3GlcA <1.0 (<1.0–34.7)	
					48 (51)	D15GlcA 1.73 (<0.9–204)	
Belgium	2013–2014	239 (106/133)	adults	1stmorning	89 (37)	DON 1.7 (<0.2–130)	Heyndrickx et al. (2015)
					184 (77)	D3GlcA 4.4 (<0.2–126)	
					238 (100)	D15GlcA 31.2 (1.1–461)	
South Africa		53 (0/53)	adults	1stmorning	53 (100)	tDON 8.95 (<0.5–353)	Shephard et al. (2013)
China	1997–1998	60 (0/60)	adults	spot	58 (97)	tDON 4,8[a] (<0.5–29.9)	Turner et al. (2011b)
Bangladesh	2013	62 (31/31)	adults	1stmorning	17 (27)	tDON <0,3 (<0,3–1.78)	Ali et al. (2016)
Pakistan	2014	264 (153/111)	children and adults	spot	54 (20)	tDON <0.5 (<0.5–1.25)	Xia et al. (2020)

N, number of samples; m: male; f: female; 1stmorning, first-morning void urine sample; [a] mean.

2.4. Daily intake estimation

Daily intakes of tDON were estimated from the daily excretion of tDON normalized to the body weight and urinary excretion ratios of 72% and 64% as reported by Turner et al. (2010) and Vidal et al. (2018).

3. Results and discussion

3.1. Urinary concentrations of tDON

tDON was found in concentrations above the LLOQ (0.3 μg/L) in 357 of the 360 urine samples. Table 2 summarizes the urinary tDON concentrations differentiated for male and female participants and year of sampling.

For nine of the 360 urine samples, tDON concentrations exceeded the provisional HBM guidance value of 23 μg/L. Most of the exceedances (7 out of 9) were observed in 2001. In 2011 and 2021 only one participant exceeded the guidance value. Male participants had significantly higher urinary tDON concentrations than female participants (Mann-Whitney-*U* test, $p < .001$). Higher urinary tDON concentrations for males were also reported by Heyndrickx et al. (2015). In contrast, Solfrizzo et al. (2014) observed no differences between the sexes.

After normalization to creatinine, no significant differences between the sexes were observed anymore in our study. This can most probably be explained by significantly higher urinary creatinine concentrations of males in comparison to females (Lermen et al., 2019). The median (maximum) values observed after creatinine adjustment are 5.52 (45.5) μg/g creatinine and 5.54 (29.0) μg/g creatinine for males and females, respectively.

In previous human biomonitoring (HBM) studies (Table 3), urinary DON concentration were determined in first-morning void urine, spot urine and 24-h urine samples. Furthermore, free DON and DON glucuronides were analysed separately as well as combined after enzymatic deconjugation. In the studies of Brera et al. (2015), Solfrizzo et al. (2014) and Xia et al. (2020), children were included additionally in the study population and Shephard et al. (2013) requested their study participants to consume a traditional maize-based evening meal prior urine collection. These differences hamper the comparison of the obtained HBM data. However, the frequency in which DON was observed and the urinary tDON levels found in this study are comparable to previously published data from other European HBM studies and higher compared to levels found in South Asian HBM studies. This observation is in line with the different dietary habits. DON is mainly a contaminant in wheat, barley, maize and products derived thereof whose consumption is lower in South Asia (Ali et al., 2016; Turner et al., 2011b).

3.2. Daily excretion values of tDON

While no differences are observed for the 24-h urine volume (Table 1) of females and males (AM: 1841 *vs.* 1883 mL/24 h; 90th percentiles: 2749 *vs.* 2725 mL/24 h), males exhibit higher urinary tDON concentrations (medians: 3.54 *vs.* 5.50 μg/L; 90th percentiles: 11.1 *vs.*

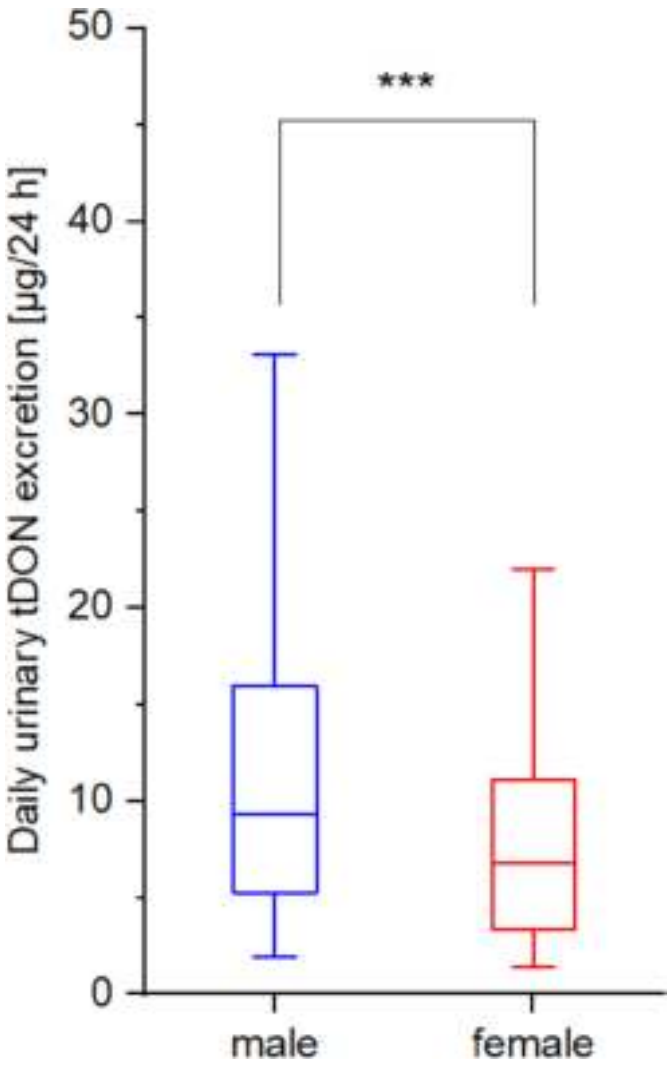

Fig. 1. Boxplot of daily urinary tDON excretion. The boxes and boundaries of the whiskers represent the 25th/75th and 5th/95th percentiles, respectively. The horizontal lines indicate the median values. ***: There was a statistically significant difference in daily urinary tDON excretion between the sexes, $p <$ .001 (Mann-Whitney-*U* test).

16.50 µg/L; Table 2). This contributes to a significant higher daily tDON excretion in men than in woman (Mann-Whitney-*U* test, p < .001, Fig. 1). Medians (90th percentile) were 9.30 (23.5) and 6.76 (17.9) µg/24 h, respectively.

However, when daily tDON excretion is normalized to participant's body weight (Table 4) no significant differences are observed between male and female participants (medians: 11.9 *vs.* 11.0 ng/24 h x kg bw; 90th percentiles: 34.5 *vs.* 28.3 ng/24 h x kg bw) indicating an equal magnitude of exposure of both sexes in our study population. Table 4 summarizes the statistics for the daily urinary tDON excretion normalized to body weight.

3.3. Daily DON intake assessment

One goal of our HBM study was the estimation of the daily DON intake. Urinary excretion ratios of 72.3% (95% CI 59.1–85.5%) and 64.0 ± 22.8% were reported by Turner et al. (2010) and Vidal et al. (2018), respectively. Considering these urinary excretion factors, we estimated the daily DON intake from the daily tDON excretion normalized to participant's body weight. Table 5 summarizes the estimated daily intake for a low level of exposure (10th percentile), an average level of exposure (median) and a high level of exposure (90th percentile). The TDI for DON is 1 µg/kg bw per day. Depending on the excretion factor applied, the estimated daily intake exceeds the TDI for two and three of all participants, respectively (all from sampling year 2001).

In HBM studies from Belgium, Italy, Austria, Portugal and Spain, exceedance of the TDI was observed for 29% (Heyndrickx et al., 2015), 6% (Solfrizzo et al., 2014), 33% (Warth et al., 2012), 10% (Martins et al., 2019) and 8% (Rodríguez-Carrasco et al., 2014) of the participants, respectively. The percentage of TDI exceedance observed in our study is far lower (0.6–0.8%). This observation is in line with the study results of Ali et al. (2016), who did not observe an exceedance of the TDI for a smaller study population from Germany (N = 50). Differences in exposure in different countries could be explained most likely by different dietary habits (Heyndrickx et al., 2015). However, a comparison of the results and the assessment of the DON exposure in the different countries is only possible to a limited extend due to different approaches used to estimate the daily DON intake.

Although a urinary collection period of 24 h is recommended for the estimation of the daily DON intake due to rapid renal DON excretion (Mengelers et al., 2019), first-morning void urine samples were used in the majority of the HBM studies shown here. In addition, different assumptions on the daily urine production and different urinary excretion ratios were applied. Heyndrickx et al. (2015), Martins et al. (2019), and Warth et al. (2012) used an urinary excretion ratio of 72% that was determined by Turner et al. (2010) based on first-morning void urine samples of adults from the United Kingdom. In contrast, Solfrizzo et al. (2014) applied an crudely estimated urinary excretion ratio of 50% (Turner et al., 2008).

As mentioned above, the urinary tDON concentration in this study exceeded the provisional HBM guidance value in nine samples. In fact, sex-specific differences that were observed in this study, the urine volume as well as the body weight are not taken into account if the HBM guidance value is applied. However, all of the samples where the

Table 5
Estimated daily intake of DON, expressed in µg/kg bw per day. P10, 10th percentile; P50, median; P90, 90th percentile; Max, maximum value.

Excretion factor	P10	P50	P90	Max
0.72	0.05	0.16	0.42	1.80
0.64	0.06	0.18	0.47	2.02

Table 4
Statistics for the daily urinary tDON excretion normalized to body weight. Values are given in ng/kg bw per day.

	Sampling year	1996	2001	2006	2011	2016	2021	Σ1996–2021
All participants	N > LLOQ	60 (100%)	59 (98%)	60 (100%)	60 (100%)	60 (100%)	58 (97%)	357 (99%)
	AM	101	267	116	165	114	136	150
	GM	77	202	98	136	71	96	106
	Median	85	216	111	151	56	103	113
	P90	176	501	205	334	296	297	304
	Range	6–350	6*–1300	15–281	17–548	7–585	3*–529	3*–1300

N: number of samples; LLOQ: lower limit of quantification; AM: arithmetic mean; GM: geometric mean; P90: 90th percentile; *Volume-based DON concentration of the corresponding 24-h urine sample is <LLOQ.

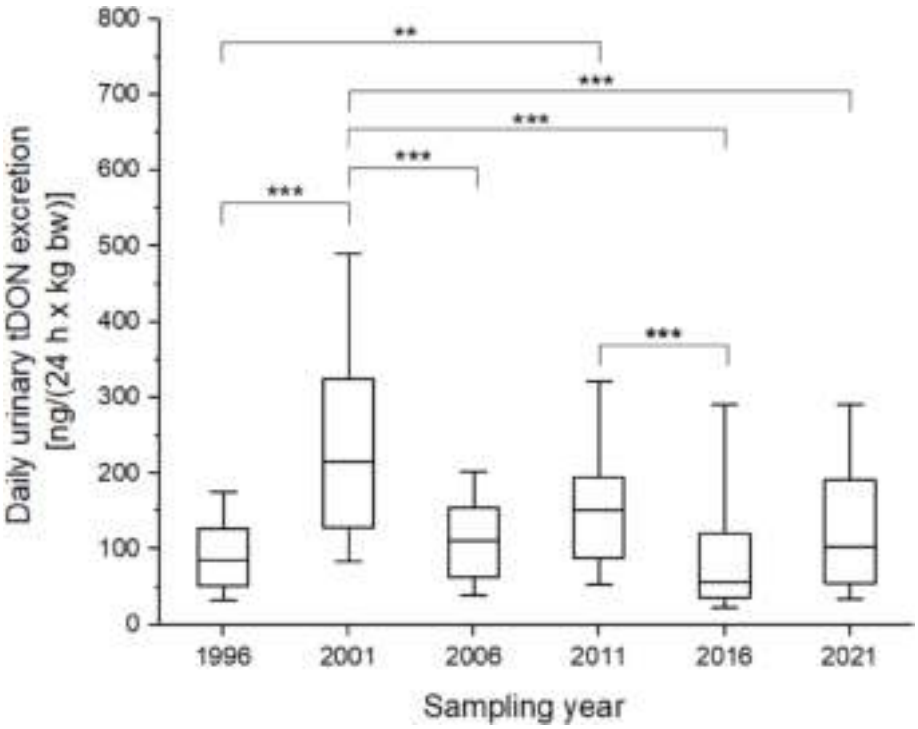

Fig. 2. Boxplot of daily urinary tDON excretion normalized to body weight. The boxes and boundaries of the whiskers represent the 25th/75th and 10th/90th percentiles, respectively. The horizontal lines indicate the median values. ***: Significant difference, $p < .001$. **: Significant difference, $p < .005$.

estimated daily intake exceeded the TDI were identified using the HBM guidance value. Thus, the HBM guidance value, that was derived recently, is suited to directly identify body burdens presenting an appreciable risk to health.

3.4. Development of tDON levels over time

The ESB study design enables monitoring changes of exposure over time to assess effects or a necessity of policy measures. In previous studies, decreasing trends could be observed e.g. for lead, mercury and phthalates (Bartel-Steinbach et al., 2022; Koch et al., 2017; Lermen et al., 2021). While for other substances like 1,2-Cyclohexane dicarboxylic acid diisononyl ester (DINCH), an alternative for phthalate plasticizers, an increasing trend could be shown (Kasper-Sonnenberg et al., 2019; Lemke et al., 2021).

To assess changes of the DON exposure level, results on tDON were assigned to the respective sampling year. However, a clear time trend could not be observed regardless of the parameter tested.

A weak negative correlation was observed between the urinary tDON concentration and the sampling year ($r = -0.158$, $p < .001$). This observation is most probably caused by a significant increase of the urine volume excreted within 24 h ($r = 0.345$, $p < .001$). The latter has been analysed in detail by Lermen et al. (2019) for samples of the ESB.

For the daily tDON excretion normalized to body weight, there were significant differences between the sampling years (Kruskal–Wallis test, $p < .001$). Highest amounts of tDON excreted were observed in 2001 (Fig. 2). The daily tDON excretion was significantly lower in the previous (1996) as well as the following sampling years (2006, 2016 and 2021).

Due to the widespread contamination of cereal-based food with DON, ingestion is the main route of exposure to DON in the general population. To actively decrease the presence in foodstuff, maximum values for DON were set at European level in 2006 (European Commission, 2006). Since a similar level of excreted tDON is observed for all sampling years with exception of 2001, our data do not clearly reflect this policy measure. However, for 2006 and the following sampling years an exceeding of the TDI was not observed in our study population. Reasons for the exceptionally high concentrations measured in 2001 could not be evaluated based on the limited data available.

4. Summary and conclusion

To the best of our knowledge, we report the first systematic investigation of the DON exposure for a larger study population ($N = 360$) over a period of 25 years. tDON could be quantified in 99% of the ESB samples, proving almost all participants were exposed to DON. However, exceedance of the TDI was observed solely for less than 1% of the participants. With exception of 2001, data evaluation revealed an equal magnitude of exposure over all sampling years.

The data give information about the dietary background exposure and show the ubiquitous exposure towards DON. The shown possibility of exceedance of health based guidance values strengthens the importance for further information to identify and assess additional DON exposure like occupational exposure at workplaces e.g. in agriculture, food production and waste management and to evaluate if higher exposed subgroups exist in the population.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2023.114198.

References

Ali, N., Blaszkewicz, M., Degen, G.H., 2016. Assessment of deoxynivalenol exposure among Bangladeshi and German adults by a biomarker-based approach. Toxicol. Lett. 258, 20–28. https://doi.org/10.1016/j.toxlet.2016.06.006.

Apel, P., Lamkarkach, F., Lange, R., Sissoko, F., David, M., Rousselle, C., Schoeters, G., Kolossa-Gehring, M., 2023. Human biomonitoring guidance values (HBM-GVs) for priority substances under the HBM4EU initiative – new values derivation for deltamethrin and cyfluthrin and overall results. Int. J. Hyg Environ. Health 248, 114097. https://doi.org/10.1016/j.ijheh.2022.114097.

Bartel-Steinbach, M., Lermen, D., Gwinner, F., Schäfer, M., Göen, T., Conrad, A., Weber, T., von Briesen, H., Kolossa-Gehring, M., 2022. Long-term monitoring of mercury in young German adults: time trend analyses from the German Environmental Specimen Bank, 1995–2018. Environ. Res. 207, 112592 https://doi.org/10.1016/j.envres.2021.112592.

Berger, M., Marske, L., Monien, B., Siodlaczek, S., Göen, T., Hartwig, A., MAK Commision, 2023. Deoxynivalenol - Bestimmung von Deoxynivalenol und Deepoxy-Deoxynivalenol in Urin mittels LC-MS/MS. MAK Collect. Occup. Health Saf (in press).

Brera, C., de Santis, B., Debegnach, F., Miano, B., Moretti, G., Lanzone, A., Del Sordo, G., Buonsenso, D., Chiaretti, A., Hardie, L., White, K., Lise Brantsæter, A., Knutsen, H., Sundstøl Eriksen, G., Sandvik, M., Wells, L., Allenf, S., Sathyapalanf, T., 2015. Experimental study of deoxynivalenol biomarkers in urine. EFSA support 12, 818E. https://doi.org/10.2903/sp.efsa.2015.EN-818 publ.

Degen, G.H., 2011. Tools for investigating workplace-related risks from mycotoxin exposure. World Mycotoxin J. 4, 315–327. https://doi.org/10.3920/WMJ2011.1295.

EFSA, 2013. Deoxynivalenol in food and feed: occurrence and exposure. EFSA J. 11, 3379. https://doi.org/10.2903/j.efsa.2013.3379.

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2017. Risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. EFSA J. 15, 4718. https://doi.org/10.2903/j.efsa.2017.4718.

Esteban López, M., Göen, T., Mol, H., Nübler, S., Haji-Abbas-Zarrabi, K., Koch, H.M., Kasper-Sonnenberg, M., Dvorakova, D., Hajslova, J., Antignac, J.-P., Vaccher, V., Elbers, I., Thomsen, C., Vorkamp, K., Pedraza – Díaz, S., Kolossa-Gehring, M., Castaño, A., 2021. The European human biomonitoring platform - design and implementation of a laboratory quality assurance/quality control (QA/QC) programme for selected priority chemicals. Int. J. Hyg Environ. Health 234, 113740. https://doi.org/10.1016/j.ijheh.2021.113740.

European Commission, 2006. Commission Regulation (EC) No 1881/2006, Setting Maximum Levels for Certain Contaminants in Foodstuffs. Official Journal of the European Union.

Fromme, H., Gareis, M., Völkel, W., Gottschalk, C., 2016. Overall internal exposure to mycotoxins and their occurrence in occupational and residential settings – an overview. Int. J. Hyg Environ. Health 219, 143–165. https://doi.org/10.1016/j.ijheh.2015.11.004.

Ganzleben, C., Antignac, J.-P., Barouki, R., Castaño, A., Fiddicke, U., Klánová, J., Lebret, E., Olea, N., Sarigiannis, D., Schoeters, G.R., Sepai, O., Tolonen, H., Kolossa-Gehring, M., 2017. Human biomonitoring as a tool to support chemicals regulation in the European Union. Int. J. Hyg Environ. Health 220, 94–97. https://doi.org/10.1016/j.ijheh.2017.01.007.

Gilles, L., Govarts, E., Rodriguez Martin, L., Andersson, A.-M., Appenzeller, B.M.R., Barbone, F., Castaño, A., Coertjens, D., Den Hond, E., Dzhedzheia, V., Eržen, I., López, M.E., Fábelová, L., Fillol, C., Franken, C., Frederiksen, H., Gabriel, C., Haug, L.S., Horvat, M., Halldórsson, T.I., Janasik, B., Holcer, N.J., Kakucs, R., Karakitsios, S., Katsonouri, A., Klánová, J., Kold-Jensen, T., Kolossa-Gehring, M., Konstantinou, C., Koponen, J., Lignell, S., Lindroos, A.K., Makris, K.C., Mazej, D., Morrens, B., Murínová, Ľ.P., Namorado, S., Pedraza-Diaz, S., Peisker, J., Probst-Hensch, N., Rambaud, L., Rosolen, V., Rucic, E., Rüther, M., Sarigiannis, D., Tratnik, J.S., Standaert, A., Stewart, L., Szigeti, T., Thomsen, C., Tolonen, H., Eiríksdóttir, Á., Van Nieuwenhuyse, A., Verheyen, V.J., Vlaanderen, J., Vogel, N., Wasowicz, W., Weber, T., Zock, J.-P., Sepai, O., Schoeters, G., 2022. Harmonization of human biomonitoring studies in Europe: characteristics of the HBM4EU-aligned studies participants. Int. J. Environ. Res. Publ. Health 19, 6787. https://doi.org/10.3390/ijerph19116787.

Gratz, S.W., Duncan, G., Richardson, A.J., 2013. The human fecal microbiota metabolizes deoxynivalenol and deoxynivalenol-3-glucoside and may Be responsible for urinary deepoxy-deoxynivalenol. Appl. Environ. Microbiol. 79 https://doi.org/10.1128/AEM.02987-12.

HBM4EU, 2018. Deliverable report D 4.5 - second list of HBM4EU priority substances and chemical substance group leaders for 2019-2021. https://www.hbm4eu.eu/work-packages/deliverable-4-5-second-list-of-hbm4eu-priority-substances-and-chemical-substance-group-leaders-for-2019-2021/. (Accessed 21 November 2022).

HBM4EU, 2022. Substance report - mycotoxins. https://www.hbm4eu.eu/wp-content/uploads/2022/07/Mycotoxins_Substance-report.pdf. (Accessed 21 November 2022).

Heyndrickx, E., Sioen, I., Huybrechts, B., Callebaut, A., De Henauw, S., De Saeger, S., 2015. Human biomonitoring of multiple mycotoxins in the Belgian population: results of the BIOMYCO study. Environ. Int. 84, 82–89. https://doi.org/10.1016/j.envint.2015.06.011.

Iverson, F., Armstrong, C., Nera, E., Truelove, J., Fernie, S., Scott, P., Stapley, R., Hayward, S., Gunner, S., 1996. Chronic Feeding Study of deoxynivalenol in B6C3F1 male and female mice. Teratog. Carcinog. Mutagen. 15, 283–306. https://doi.org/10.1002/tcm.1770150606.

Kasper-Sonnenberg, M., Koch, H.M., Apel, P., Rüther, M., Pälmke, C., Brüning, T., Kolossa-Gehring, M., 2019. Time trend of exposure to the phthalate plasticizer substitute DINCH in Germany from 1999 to 2017: biomonitoring data on young adults from the Environmental Specimen Bank (ESB). Int. J. Hyg Environ. Health 222, 1084–1092. https://doi.org/10.1016/j.ijheh.2019.07.011.

Khera, K.S., Arnold, D.L., Whalen, C., Angers, G., Scott, P.M., 1984. Vomitoxin (4-deoxynivalenol): effects on reproduction of mice and rats. Toxicol. Appl. Pharmacol. 74, 345–356. https://doi.org/10.1016/0041-008X(84)90288-6.

Koch, H.M., Rüther, M., Schütze, A., Conrad, A., Pälmke, C., Apel, P., Brüning, T., Kolossa-Gehring, M., 2017. Phthalate metabolites in 24-h urine samples of the German Environmental Specimen Bank (ESB) from 1988 to 2015 and a comparison with US NHANES data from 1999 to 2012. Int. J. Hyg Environ. Health 220, 130–141. https://doi.org/10.1016/j.ijheh.2016.11.003.

Kolossa-Gehring, M., Becker, K., Conrad, A., Schröter-Kermani, C., Schulz, C., Seiwert, M., 2012. Environmental surveys, specimen bank and health related environmental monitoring in Germany. Int. J. Hyg Environ. Health 215, 120–126. https://doi.org/10.1016/j.ijheh.2011.10.013.

Kolossa-Gehring, M., Pack, L.K., Hülck, K., Gehring, T., 2023. HBM4EU from the Coordinator's perspective: lessons learnt from managing a large-scale EU project. Int. J. Hyg Environ. Health 247, 114072. https://doi.org/10.1016/j.ijheh.2022.114072.

Lemke, N., Murawski, A., Lange, R., Weber, T., Apel, P., Dębiak, M., Koch, H.M., Kolossa-Gehring, M., 2021. Substitutes mimic the exposure behaviour of REACH regulated phthalates – a review of the German HBM system on the example of plasticizers. Int. J. Hyg Environ. Health 236, 113780. https://doi.org/10.1016/j.ijheh.2021.113780.

Lermen, D., Bartel-Steinbach, M., Gwinner, F., Conrad, A., Weber, T., von Briesen, H., Kolossa-Gehring, M., 2019. Trends in characteristics of 24-h urine samples and their relevance for human biomonitoring studies – 20 years of experience in the German Environmental Specimen Bank. Int. J. Hyg Environ. Health 222, 831–839. https://doi.org/10.1016/j.ijheh.2019.04.009.

Lermen, D., Schmitt, D., Bartel-Steinbach, M., Schröter-Kermani, C., Kolossa-Gehring, M., von Briesen, H., Zimmermann, H., 2014. A new approach to standardize multicenter studies: mobile lab technology for the German environmental Specimen Bank. PLoS One 9, e105401. https://doi.org/10.1371/journal.pone.0105401.

Lermen, D., Weber, T., Göen, T., Bartel-Steinbach, M., Gwinner, F., Mueller, S.C., Conrad, A., Rüther, M., von Briesen, H., Kolossa-Gehring, M., 2021. Long-term time trend of lead exposure in young German adults – evaluation of more than 35 Years of data of the German Environmental Specimen Bank. Int. J. Hyg Environ. Health 231, 113665. https://doi.org/10.1016/j.ijheh.2020.113665.

Marin, S., Ramos, A.J., Cano-Sancho, G., Sanchis, V., 2013. Mycotoxins: occurrence, toxicology, and exposure assessment. Food Chem. Toxicol. 60, 218–237. https://doi.org/10.1016/j.fct.2013.07.047.

Martins, C., Vidal, A., De Boevre, M., De Saeger, S., Nunes, C., Torres, D., Goios, A., Lopes, C., Assuncao, R., Alvito, P., 2019. Exposure assessment of Portuguese population to multiple mycotoxins: the human biomonitoring approach. Int. J. Hyg Environ. Health 222, 913–925. https://doi.org/10.1016/j.ijheh.2019.06.010.

Mengelers, M., Zeilmaker, M., Vidal, A., De Boevre, M., De Saeger, S., Hoogenveen, R., 2019. Biomonitoring of deoxynivalenol and deoxynivalenol-3-glucoside in human volunteers: renal excretion profiles. Toxins 466.

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.

Rodríguez-Carrasco, Y., Moltó, J.C., Mañes, J., Berrada, H., 2014. Exposure assessment approach through mycotoxin/creatinine ratio evaluation in urine by GC–MS/MS. Food Chem. Toxicol. 72, 69–75. https://doi.org/10.1016/j.fct.2014.07.014.

SCF (Scientific Committee on Food), 2002. Opinion of the Scientific Committee on Food on Fusarium Toxins. Part 6: Group Evaluation of T-2 Toxin, HT-2 Toxin, Nivalenol and Deoxynivalenol. SCF/CS/CNTM/MYC/27 Final.

Shephard, G.S., Burger, H.-M., Gambacorta, L., Gong, Y.Y., Krska, R., Rheeder, J.P., Solfrizzo, M., Srey, C., Sulyok, M., Visconti, A., Warth, B., van der Westhuizen, L., 2013. Multiple mycotoxin exposure determined by urinary biomarkers in rural subsistence farmers in the former Transkei, South Africa. Food Chem. Toxicol. 62, 217–225. https://doi.org/10.1016/j.fct.2013.08.040.

Solfrizzo, M., Gambacorta, L., Visconti, A., 2014. Assessment of Multi-Mycotoxin Exposure in Southern Italy by Urinary Multi-Biomarker Determination, vol. 6, pp. 523–538. https://doi.org/10.3390/toxins6020523.

Tryphonas, H., Iverson, F., Ying, S., Nera, E.A., McGuire, P.F., O'Grady, L., Clayson, D.B., Scott, P.M., 1986. Effects of deoxynivalenol (vomitoxin) on the humoral and cellular immunity of mice. Toxicol. Lett. 30, 137–150. https://doi.org/10.1016/0378-4274(86)90096-2.

Turner, P.C., Hopton, R.P., White, K.L.M., Fisher, J., Cade, J.E., Wild, C.P., 2011a. Assessment of deoxynivalenol metabolite profiles in UK adults. Food Chem. Toxicol. 49, 132–135. https://doi.org/10.1016/j.fct.2010.10.007.

Turner, P.C., Ji, B.T., Shu, X.O., Zheng, W., Chow, W.H., Gao, Y.T., Hardie, L.J., 2011b. A biomarker survey of urinary deoxynivalenol in China: the Shanghai Women's Health Study. Food Addit. Contam. 28, 1220–1223. https://doi.org/10.1080/19440049.2011.584070.

Turner, P.C., Rothwell, J.A., White, K.L.M., Gong, Y., Cade, J.E., Wild, C.P., 2008. Urinary deoxynivalenol is correlated with cereal intake in individuals from the United Kingdom. Environ. Health Perspect. 116, 21–25. https://doi.org/10.1289/ehp.10663.

Turner, P.C., White, K.L.M., Burley, V.J., Hopton, R.P., Rajendram, A., Fisher, J., Cade, J. E., Wild, C.P., 2010. A comparison of deoxynivalenol intake and urinary deoxynivalenol in UK adults. Biomarkers 15, 553–562. https://doi.org/10.3109/1354750X.2010.495787.

Vidal, A., Claeys, L., Mengelers, M., Vanhoorne, V., Vervaet, C., Huybrechts, B., De Saeger, S., De Boevre, M., 2018. Humans significantly metabolize and excrete the mycotoxin deoxynivalenol and its modified form deoxynivalenol-3-glucoside within 24 hours. Sci. Rep. 8, 5255. https://doi.org/10.1038/s41598-018-23526-9.

Viegas, S., Viegas, C., Oppliger, A., 2018. Occupational exposure to mycotoxins: current knowledge and prospects. Ann. Work Expo. Health 62, 923–941. https://doi.org/10.1093/annweh/wxy070.

Warth, B., Sulyok, M., Berthiller, F., Schuhmacher, R., Krska, R., 2013. New insights into the human metabolism of the Fusarium mycotoxins deoxynivalenol and zearalenone. Toxicol. Lett. 220, 88–94. https://doi.org/10.1016/j.toxlet.2013.04.012.

Warth, B., Sulyok, M., Fruhmann, P., Berthiller, F., Schuhmacher, R., Hametner, C., Adam, G., Fröhlich, J., Krska, R., 2012. Assessment of human deoxynivalenol exposure using an LC–MS/MS based biomarker method. Toxicol. Lett. 211, 85–90. https://doi.org/10.1016/j.toxlet.2012.02.023.

Xia, L., Routledge, M.N., Rasheed, H., Ismail, A., Dong, Y., Jiang, T., Gong, Y.Y., 2020. Biomonitoring of aflatoxin B1 and deoxynivalenol in a rural Pakistan population using ultra-sensitive LC-MS/MS method. Toxins 12, 591. https://doi.org/10.3390/toxins12090591.

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Levels of per- and polyfluoroalkyl substances (PFAS) in Norwegian children stratified by age and sex - Data from the Bergen Growth Study 2

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ABSTRACT

Background and aim: Due to the persistence, bioaccumulation and potential adverse health effects, there have been restrictions and phase out in the production of certain per- and polyfluoroalkyl substances (PFAS) since the early 2000s. Published serum levels of PFAS during childhood are variable and may reflect the impact of age, sex, sampling year and exposure history. Surveying the concentrations of PFAS in children is vital to provide information regarding exposure during this critical time of development. The aim of the current study was therefore to evaluate serum concentrations of PFAS in Norwegian schoolchildren according to age and sex.

Material and methods: Serum samples from 1094 children (645 girls and 449 boys) aged 6–16 years, attending schools in Bergen, Norway, were analyzed for 19 PFAS. The samples were collected in 2016 as part of the Bergen Growth Study 2. Statistical analyses included Student t-test, one-way ANOVA and Spearman's correlation analysis of log-transformed data.

Results: Of the 19 PFAS examined, 11 were detected in the serum samples. Perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS) and perfluorononaoic acid (PFNA) were present in all samples with geometric means of 2.67, 1.35, 0.47 and 0.68 ng/mL, respectively. In total, 203 children (19%) had PFAS levels above the safety limits set by the German Human Biomonitoring Commission. Significantly higher serum concentrations were found in boys compared to girls for PFOS, PFNA, PFHxS and perfluoroheptanesulfonic acid (PFHpS). Furthermore, serum concentrations of PFOS, PFOA, PFHxS and PFHpS were significantly higher in children under the age of 12 years than in older children.

Conclusions: PFAS exposure was widespread in the sample population of Norwegian children analyzed in this study. Approximately one out of five children had PFAS levels above safety limits, indicating a potential risk of negative health effects. The majority of the analyzed PFAS showed higher levels in boys than in girls and decreased serum concentrations with age, which may be explained by changes related to growth and maturation.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a diverse group of synthetic chemicals consisting of several thousands of different substances. Since the 1940s, PFAS have been applied in a variety of industries worldwide due to their useful properties of being chemically

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and thermally stable, as well as being repellant to water, oil, and dirt. For these reasons, PFAS are commonly used in numerous products, including impregnation of paper, textile, and leather, and in firefighting foams, cosmetics, and non-stick kitchenware (Glüge et al., 2020). PFAS have high resistance to degradation, leading to accumulation in the environment. Furthermore, several of the compounds accumulate in the food chain and in humans (Conder et al., 2008, Russell et al., 2013). The fluorinated carbon length is related to the bioaccumulation of PFAS, although other factors may also affect the bioaccumulation potential. In general, perfluoroalkyl carboxylic acids with seven or more fluorinated carbons, e.g. perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA), and perfluoroalkane sulfonates with six or more fluorinated carbons, e.g. perfluorohexanesulfonic acid (PFHxS) and perfluorooctanesulfonic acid (PFOS), are considered bioaccumulative (Conder et al., 2008; Ng and Hungerbühler 2014).

Exposure of PFAS in the general population occurs primarily through ingestion of contaminated food and drinking water, but exposure through inhalation or ingestion of contaminated dust and dermal exposure can also make substantial contributions (EFSA, 2020; Fromme et al., 2009; Haug et al., 2011; Poothong et al., 2020; Trudel et al., 2008). In addition, there is a transplacental transfer of PFAS from the mother to the fetus during pregnancy, and infants are exposed through breastfeeding (Appel et al., 2022; Blomberg et al., 2023; Fromme et al., 2010). PFAS exposure has been confirmed in blood samples from children and adults in numerous countries (EFSA, 2020; Winkens et al., 2017).

Several studies have shown an association between exposure to PFAS and adverse health effects in humans, including reduced vaccine response in children, reduced birth weight, dyslipidemia, and certain types of cancers (Panieri et al., 2022, Rappazzo et al., 2017). In 2020, the European Food Safety Authority (EFSA) set a tolerable weekly intake (TWI) of 4.4 ng/kg body weight per week for the sum of the four PFAS PFOA, PFNA, PFHxS and PFOS (EFSA, 2020). Reduction in vaccination response in children was the basis for setting a cutoff for TWI, as this was the most sensitive health outcome in human cohorts, and the association was considered causal due to similar effects in experimental animal studies. The current dietary exposure to these PFAS exceeds the TWI for large parts of the European population, including Norwegian citizens (EFSA, 2020; VKM Report 2022). Chronic exposure at the TWI corresponds to serum concentrations of 6.9 ng/mL for the sum of the four above-mentioned PFAS in adults (EFSA, 2020). The German Human Biomonitoring Commission has derived health related guidance values for the evaluation of internal exposures to PFOA and PFOS separately. The Human Biomonitoring (HBM)-I values in blood plasma are defined as 2 ng PFOA/mL and 5 ng PFOS/mL in all age groups. These values represent the concentrations below which, according to current knowledge, no adverse health effects are expected to occur (The German Environment Agency, 2020).

The European Commission has recently established maximum levels for certain PFAS in drinking water and food (The European Commission, 2020; 2022). PFOA, PFOS and their precursors are encompassed by the Stockholm Convention on Persistent Organic Pollutants, and further restrictions on PFAS are under development in Europe (European Chemicals Agency, 2023; Stockholm Convention on persistent organic pollutants, 2019). However, despite future restrictions or bans on more PFAS and their precursors, they will persist in the environment and may affect humans for generations to come.

Norwegian data showed a steady increase in the concentration of PFAS in serum samples from the general population of adults between 1977 to the mid-1990s (Haug et al., 2009a). Further, there was a clear decline in the PFOA, perfluoroheptanesulfonic acid (PFHpS) and PFOS concentrations from around the year 2000, which is consistent with the phase-out of these compounds. A clear increase was also observed for PFHxS, PFNA, perfluorodecanoic acid (PFDA) and perfluoroundecanoic acid (PFUnDA) until the early 1990s. Pooled serum samples from children aged 5–14 years, collected in 1976, 1987, 1998 and 2007, were also included in the study. The PFAS concentrations in these children showed similar trends as for the adults (Haug et al., 2009a). The Norwegian findings for PFOA and PFOS are in line with the reported trends in North America and Europe (Land et al., 2018). In contrast, biomonitoring data from China, where the production has continued, indicate a further increase in serum concentrations of PFOA and PFOS after the year 2000. Concentrations of longer-chained perfluoroalkyl carboxylic acids (PFCAs), including PFNA, PFDA and PFUnDA, are generally increasing in all parts of the world (Land et al., 2018). Published serum levels of PFAS during childhood vary strongly and may reflect the impacts of body weight changes and energy needs, sex, sampling year, and history of exposure due to changes in the production of PFAS (Haug et al., 2018; Nøst et al., 2014; Winkens et al., 2017).

The Bergen Growth Study 2 (BGS2) is a puberty reference study conducted in Norway in 2016. As part of this cross-sectional study, blood samples were collected from 1094 children aged 6–16 years to study hormonal changes in puberty, and exposure to endocrine disruptive chemicals, including PFAS (Júlíusson et al., 2023). Previous data on PFAS exposure in Norwegian schoolchildren were limited to either pooled samples or narrow age groups (Averina et al., 2018; Haug et al., 2009a; Richterová et al., 2023). The aim of the current study was therefore to map PFAS exposure in a critical developmental window by determining serum concentrations in Norwegian children according to a wide age range of 6–16 years, and sex.

2. Materials and methods

2.1. Study design and sampling

Serum samples from 1094 children (645 girls and 449 boys) aged 6–16 years were collected in January–June 2016 as a part of the Bergen Growth Study 2 (BGS2). The BGS2 is a cross-sectional study of growth and pubertal development in Norwegian children. The children were recruited from six randomly selected public schools in Bergen – the second largest city in Norway. All children in the selected schools were invited to participate in the study, and all children who agreed to participate were included. The overall participation rate was 43% and ranged from 29% to 53% across schools. Numbers of participants by age and sex are presented in Supplementary Table 1. The selected schools in BGS2 covered varied socioeconomic backgrounds, but children of parents with an academic degree were overrepresented (80.4%) compared to the general population of Bergen (40.8%, Statistics Norway, 2023). The proportion of children with parents of Norwegian, European, and non-European origin was 80.7%, 7.6% and 11.7%, respectively. Based on the criteria for weight status defined by the International Obesity Task Force (Cole et al., 2000), 7.4% of the participants were children with underweight, 11.2% with overweight and 2.1% with obesity (Júlíusson et al., 2023). An experienced biomedical laboratory scientist collected blood samples for hormonal, genetic and endocrine disruptive chemicals analysis, including PFAS analysis, by venipuncture from an antecubital vein. Further details of the study design and measurement protocol of the BGS2 have recently been described (Júlíusson et al., 2023).

2.2. Analysis of PFAS

Nineteen PFAS in total, including perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, PFDA, PFUnDA, perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorobutanesulfonic acid (PFBS), PFHxS, PFHpS, PFOS, perfluorodecanesulfonic acid (PFDS), perfluorooctanesulfonamide (PFOSA), N-methylperfluorooctanesulfonamide (MeFOSA) and N-ethylperfluorooctanesulfonamide (EtFOSA), were analyzed at the Norwegian Institute of Public Health using high-performance tandem mass spectrometry, by a previously described

method (Haug et al., 2009b). In brief, the method involves transferring 150 μL serum to a centrifugation tube, adding internal standards and methanol to make up a total volume of 150 μL methanol, before mixing using a whirl mixer. The samples are then centrifuged, and the supernatant is transferred to a glass autosampler vial added 500 μL 0.1 M formic acid and mixed on a whirl mixer. The samples are analyzed by injection of 80 μL extract on a column switching liquid chromatography (LC) system coupled to a triple quadrupole mass spectrometer (MS). For quantification of PFOS, the total area of the linear and branched isomers is integrated. The limit of quantification (LOQ) was 0.1 ng/mL for PFBA, 0.2 ng/mL for PFTeDA and PFDS, and 0.05 ng/mL for the remaining 16 PFAS.

The laboratory participates regularly in interlaboratory comparisons, was through an extensive process (Nübler et al., 2022), and was approved for measuring a range of PFAS in the HBM4EU project (HBM4EU, 2022). Procedure blanks and in-house controls, as well as two samples from the interlaboratory comparisons in the EU project HBM4EU, were analyzed along with the samples. The procedure blanks (n = 66) did not contain PFAS above the limit of quantification, except for PFOA and PFNA in a few samples in concentrations close to LOQ. The relative standard deviations (RSDs) for the in-house controls (n = 42) varied between 10 and 15%. For the interlaboratory comparison samples (n = 11 per sample), the mean % differences from the assigned values, except PFPeA, were in the range of −14 to +6%, demonstrating high quality of the analyses. The results of PFPeA were deemed unsatisfactory due to a high deviation from the assigned values.

2.3. Statistical analysis

The distribution of all PFAS in serum failed the Shapiro-Wilk test of normality due to positive skewness. The natural logarithm of the values was used in further analysis. Concentrations below the LOQ were set to LOQ divided by the square root of two for analysis (Duffek et al., 2020; Papadopoulou et al., 2016). Statistical analyses were performed using IBM SPSS Statistics version 26. The geometric mean concentration (GM) and 95% confidence interval (CI) were calculated by back transforming the geometric mean and the CI of the log-transformed data to the original scale of measurement. The children were grouped according to age as 6–7 years, 8–9 years, 10–11 years, 12–13 years, and 14–16 years. Statistical significance of the differences in geometric means between age groups was tested with one-way ANOVA, and Student t-test was used to compare PFAS concentrations between sexes. Further, Spearman's correlation analysis was used to explore correlations among different PFAS. For PFHpA and PFHpS, detected vs. non-detected were compared using the Pearson's chi-squared test.

2.4. Ethical considerations

This study was approved by the Norwegian Regional Committee for Medical and Health Research Ethics West (reference number 2015/128). Written informed consent was obtained from a parent or legal guardian of each participating child, and from the participants themselves when they were 12 years of age and older. The children received age-appropriate information ahead of participation. Assent from the participants themselves was an additional requirement for inclusion. A cinema voucher was given as an incentive to participate in the study.

3. Results

3.1. PFAS concentrations in serum

The distributions of PFAS concentrations in serum samples from the 1094 children (645 girls and 449 boys) included in BGS2, are shown in Table 1. PFOS, PFOA, PFHxS and PFNA were detected in all samples. PFOS was the substance showing the highest serum concentrations, with a GM of 2.67 ng/mL, followed by PFOA (1.35 ng/mL), PFNA (0.68 ng/mL) and PFHxS (0.47 ng/mL). The concentration of PFDA, PFUnDA and PFHpS was substantially lower. For PFBA, PFHxA, PFBS, PFDS, PFOSA, MeFOSA and EtFOSA serum concentrations were below the LOQ in all children. PFPeA was detected in a small number of samples, but the measurements were deemed unreliable according to the quality assurance system and are therefore not reported (see section Analysis of PFAS for further information). Only PFAS that were quantifiable in at least 50% of the samples were included in further analyses.

In total, 132 (12%) and 90 (8%) out of the 1094 children had serum concentrations above the HBM I values of 2 ng/mL for PFOA and 5 ng/mL for PFOS respectively, and 203 (19%) children had concentrations above HBM I values for PFOA, PFOS or both. Furthermore, 241 children (22%) had serum concentrations above 6.9 ng/mL (level corresponding to the EFSA TWI) for the sum of PFOA, PFNA, PFHxS and PFOS. The highest proportion of children exceeding the limit of 6.9 ng/mL was found in the youngest age groups, respectively 181 out of 705 children (26%) below 12 years of age, versus 60 out of 389 children (15%) above 12 years of age. Fig. 1 shows a scatter plot of the sum of PFOA, PFNA, PFHxS and PFOS in all 1094 children by age and sex. Supplementary Table 2 gives a more detailed presentation of the proportion of children exceeding the PFAS safety limits by age and sex.

3.2. Correlation among PFAS in serum

There was a significant positive correlation among all the seven PFAS, as shown in Table 2. The strongest correlation was for PFDA with PFUnDA (Spearman correlation coefficient: 0.782), and for PFDA with

Table 1
Serum concentrations (ng/mL) of PFAS in 1094 children aged 6–16 years.

	LOQ	N > LOQ	%>LOQ	P2.5	P50	P97.5	Max	AM	GM	95%CI GM
PFHpA	0.05	301	27	< LOQ	< LOQ	0.17	0.40	0.05	< LOQ	<LOQ-0.116
PFOA	0.05	1094	100	0.6	1.38	2.60	3.95	1.44	1.35	0.664–2.761
PFNA	0.05	1094	100	0.28	0.66	1.93	4.49	0.77	0.68	0.259–1.783
PFDA	0.05	1082	99	0.06	0.16	0.36	0.74	0.17	0.15	0.062–0.384
PFUnDA	0.05	976	89	< LOQ	0.11	0.32	1.03	0,13	0.11	<LOQ-0.347
PFDoDA	0.05	62	6	< LOQ	< LOQ	0.08	0.27	< LOQ	< LOQ	<LOQ-0.058
PFTrDA	0.05	58	5	< LOQ	< LOQ	0.07	0.21	< LOQ	< LOQ	<LOQ-0.051
PFTeDA	0.20	2	0.2	< LOQ	< LOQ	< LOQ	0.25	< LOQ	< LOQ	
PFHxS	0.05	1094	100	0.20	0.47	1.64	11.04	0.58	0.49	0.171–1.284
PFHpS	0.05	602	55	< LOQ	0,05	0.14	0.29	0.06	0.05	< LOQ-0.126
PFOS	0.05	1094	100	0.08	2.66	6.83	18.5	2.96	2.67	1.098–6.475
Σ4PFAS				20.62	5.41	11.4	22.4	5.76	5.38	2.64–11.0

LOQ = limit of quantification; AM = arithmetic mean; GM = geometric mean (back-transformed arithmetic mean of the natural logarithm of concentrations); 95%CI = 95% confidence interval; PFHpA = perfluoroheptanoic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononaoic acid; PFDA = perfluorodecanoic acid; PFUnDA = perfluoroundecanoic acid; PFDoDA = perfluorododecanoic acid; PFTrDA = perfluorotridecanoic acid; PFTeDA = perfluorotetradecanoic acid; PFHxS = perfluorohexanesulfonic acid; PFHpS = perfluoroheptanesulfonic acid; PFOS = perfluorooctanesulfonic acid; Σ4PFAS = sum of PFOA, PFNA, PFHxS and PFOS.

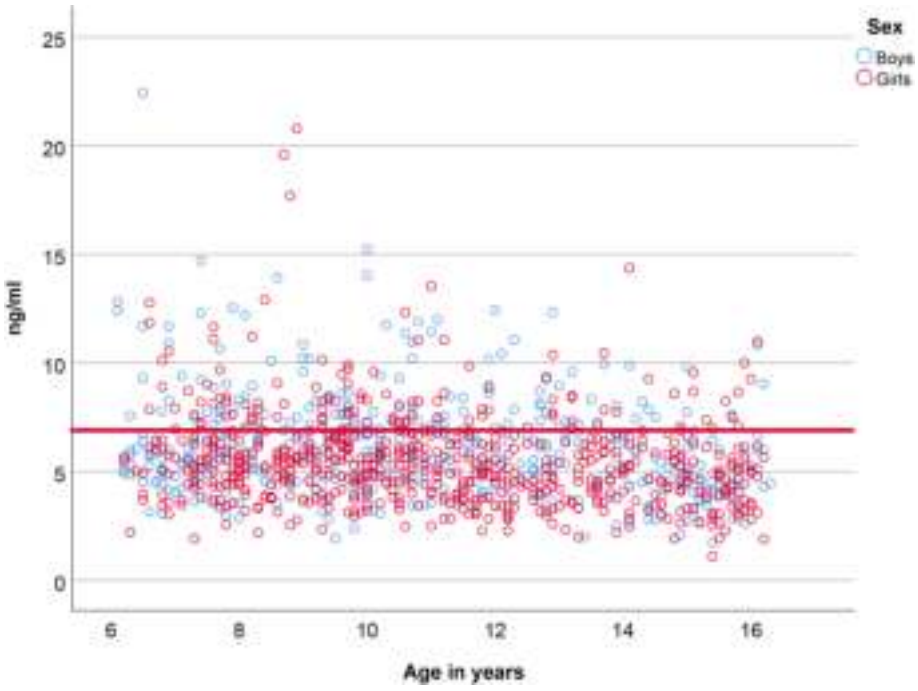

Fig. 1. Sum of PFOA, PFNA, PFHxS and PFOS serum concentrations
Scatter plot showing the sum of perfluorooctanoic acid (PFOA), perfluorononaoic acid (PFNA), perfluorohexanesulfonic acid (PFHxS) and perfluorooctanesulfonic acid (PFOS) in all 1094 children by age and sex. The horizontal line at 6.9 ng/mL is the estimated serum concentration in adults resulting from chronic exposure at the tolerable weekly intake of 4.4 ng/kg body weight per week for the sum of the four PFAS, set by the European Food Safety Authority.

Table 2
Spearman's correlations among PFAS in serum (n = 1094).

	PFNA	PFDA	PFUnDA	PFHxS	PFHpS	PFOS
PFOA	0.442	0.535	0.301	0.415	0.372	0.457
PFNA		0.462	0.403	0.235	0.296	0.342
PFDA			0.782	0.308	0.367	0.621
PFUnDA				0.301	0.348	0.608
PFHxS					0.525	0.604
PFHpS						0.595

PFOA = perfluorooctanoic acid; PFNA = perfluorononaoic acid; PFDA = perfluorodecanoic acid; PFUnDA = perfluoroundecanoic acid; PFHxS = perfluorohexanesulfonic acid; PFHpS = perfluoroheptanesulfonic acid; PFOS = perfluorooctanesulfonic acid. All correlations are significant at the 0.01 level (2-tailed).

PFOS (Spearman correlation coefficient: 0.621).

3.3. PFAS concentrations by age and sex

Serum concentrations of PFOS, PFOA, PFDA, PFHxS, PFHpS and PFUnDA were significantly higher in children under the age of 12 years than in children that were above 12 years (Fig. 2). For several of the PFAS the decreasing age trend started about two years earlier in girls compared to boys.

Serum concentrations of PFNA, PFHxS, PFHpS and PFOS were significantly higher in boys compared to girls in all age groups combined (Table 3). After stratification by age group, there were no significant differences between the sexes in the youngest age group, (6–7 years), for any of the PFAS. For PFOA, significantly higher values in boys than girls were observed in the age group 12–13 years specifically. PFDA was the only PFAS that showed significantly lower levels in boys compared to girls, more specifically in the age group 14–16 years (Fig. 2). GM by age and sex are presented in Supplementary Table 3. In addition, detected versus non-detected were examined for PFHpA and PFHpS. A higher proportion of boys had detected levels of PFHpS compared to girls (63% vs. 50%), and a higher proportion of children under 12 years of age had detected levels of PFHpS compared to older children (62% vs. 43%), both significant at the 0.05 level. No significant differences were found for PFHpA.

4. Discussion

Through the Bergen Growth Study 2, we assessed PFAS exposure data from 1094 children aged 6–16 years, living in Bergen, Norway. PFOS, PFOA, PFNA and PFHxS were detected in all samples, which demonstrated widespread exposure in the sample population of Norwegian children analyzed in this study. PFOS had the highest serum concentration, followed by PFOA, PFNA and PFHxS. About one out of five children had serum concentrations above levels considered safe. Furthermore, serum concentrations were generally higher in the youngest age groups compared to the oldest. In addition, there were significant differences between boys and girls, in particular for PFNA, PFHxS, PFHpS and PFOS. The significant positive correlations between the PFAS point to similar exposure sources and pathways, e.g., contaminated food, which based on prior studies has been considered the main source of exposure (EFSA, 2020).

A previous Norwegian study in pregnant women found that variables related to socioeconomic status (i.e., higher maternal education and household income) were related to higher levels of various PFAS (Brantsæter et al., 2013). Higher PFAS concentrations were also found in European teenagers from households with a higher educational level (Richterová et al., 2023). Because children of parents with a higher level educational level were overrepresented in the current study, the PFAS levels might be higher in children participating in BGS2 compared to non-participants and the general population of children in Bergen.

The PFAS levels in the current study are comparable to those of other European studies that have analyzed samples from the same period and age range (Haug et al., 2018; Richterová et al., 2023). Previous publications on PFAS exposure in Norwegian schoolchildren have been limited to either pooled samples or narrow age ranges (Averina et al., 2018; Haug et al., 2009a; Richterová et al., 2023). The Norwegian national data of 12-year-old children included in the European Human Biomonitoring Initiative (HBM4EU) aligned studies, show similar concentrations compared to our data, i.e., PFOA of 1.28 vs 1.35 ng/mL, PFNA of 0.44 vs 0.68 ng/mL, PFHxS of 0.48 vs 0.47 ng/mL and PFOS of 2.79 vs 2.67 ng/mL (Richterová et al., 2023). In our study, the proportion of children exceeding serum levels of 6.9 ng/mL for the sum of PFOA, PFNA, PFHxS and PFOS was 22% in total and 15% for children above 12 years of age. In comparison, 14% of children aged 12–18 years

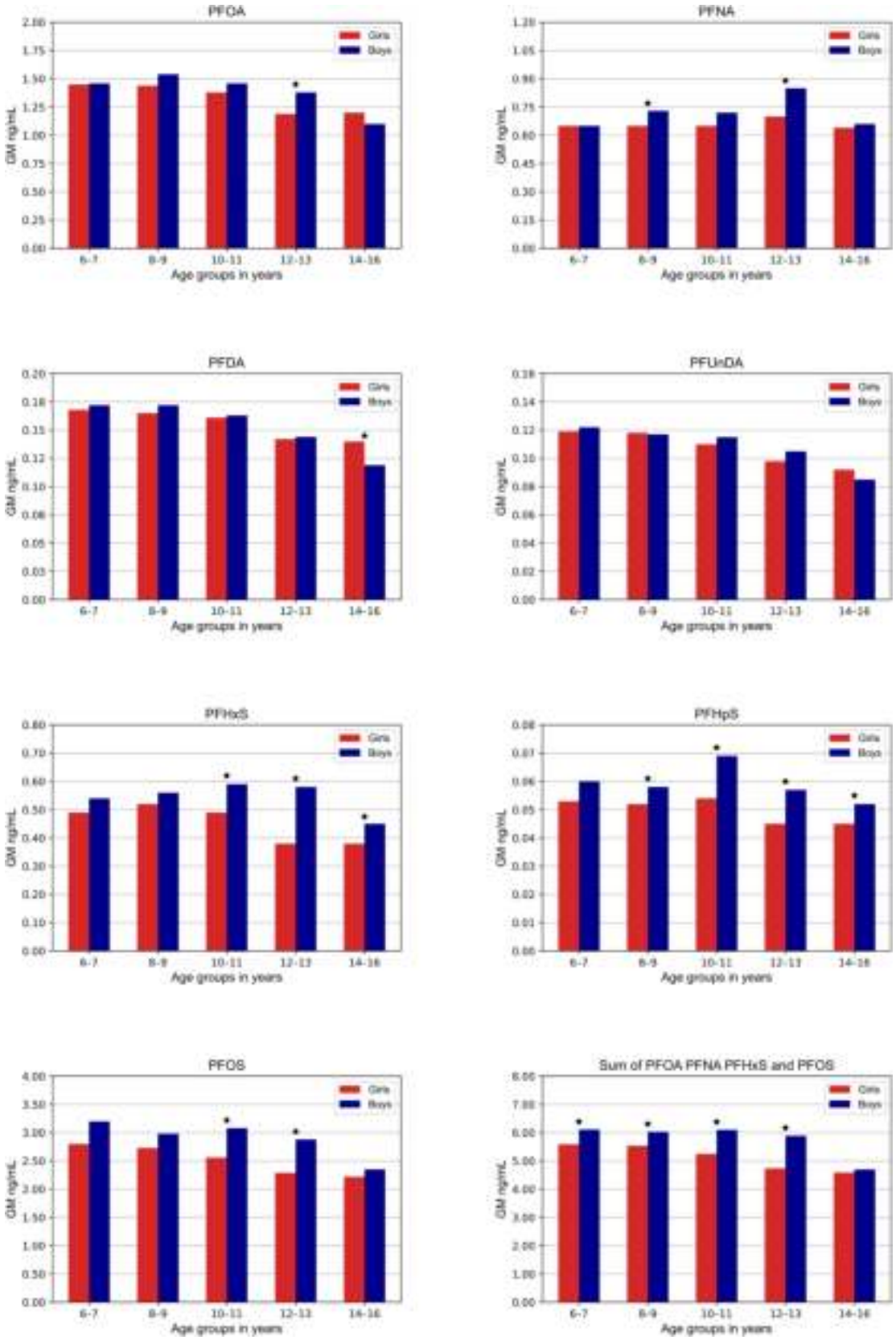

Fig. 2. Geometric mean concentrations for PFAS by age and sex
GM = geometric mean (back-transformed arithmetic mean of the natural logarithm of concentrations); PFOA = perfluorooctanoic acid; PFNA = perfluorononaoic acid; PFDA = perfluorodecanoic acid; PFUnDA = perfluoroundecanoic acid; PFHxS = perfluorohexanesulfonic acid; PFHpS = perfluoroheptanesulfonic acid; PFOS = perfluorooctanesulfonic acid. The stars denote p-values <0.05 for sex differences.

Table 3
Geometric mean concentrations (ng/mL) and 95% confidence intervals (CI) for PFAS by sex.

	Boys GM (95%CI) (n = 449)	Girls GM (95%CI) (n = 645)	P value
PFOA	1.38 (0.70–2.73)	1.33 (0.64–2.78)	0.107
PFNA	0.71 (0.27–1.90)	0.66 (0.25–1.70)	0.007*
PFDA	0.15 (0.06–0.37)	0.16 (0.06–0.39)	0.748
PFUnDA	0.11 (0.03–0.35)	0.11 (0.03–0.34)	0.814
PFHxS	0.54 (0.20–1.44)	0.45 (0.17–1.24)	<0.001*
PFHpS	0.06 (0.02–0.15)	0.05 (0.02–0.11)	<0.001*
PFOS	2.89 (1.20–6.98)	2.52 (1.05–6.05)	<0.001*
Σ4PFAS	5.74 (2.84–11.58)	5.15 (2.54–10.45)	<0.001*

GM = geometric mean (back-transformed arithmetic mean of the natural logarithm of concentrations); PFOA = perfluorooctanoic acid; PFNA = perfluorononaoic acid; PFDA = perfluorodecanoic acid; PFUnDA = perfluoroundecanoic acid; PFHxS = perfluorohexanesulfonic acid; PFHpS = perfluoroheptanesulfonic acid; PFOS = perfluorooctanesulfonic acid. Σ4PFAS = sum of PFOA, PFNA, PFHxS and PFOS. Stars denote p-values that are significant at the 0.05 level.

in the HBM4EU studies had serum concentrations above 6.9 ng/mL (Richterová et al., 2023). The proportion of children in the HBM4EU studies exceeding the health-based guidance value of EFSA was calculated per country, and ranged from 1% in Spain, to the maximum of 24% in France (Lobo Vicente et al., 2023).

EFSA showed results from toxicokinetic (TK) modeling of PFOA and PFOS serum concentration under constant exposure conditions that indicated a decline in serum concentration after the breastfeeding period until age 5–6 years, followed by a gradual increase over time until it stabilized after age 30–40 years. The impact of breastfeeding for 12 months on serum concentrations disappeared around the age of 10 years according to the model used by EFSA (EFSA, 2020). Our data showed higher serum concentrations in children under 12 years of age compared to children in the older age groups, which deviates from EFSA's TK model. Both duration of breastfeeding and dietary intake will affect the expected change in serum concentration with age. In Norway, breastfeeding rates are high compared to several other European countries (Theurich et al., 2019). In addition, Norwegian national survey data show that many women continue breastfeeding after the first twelve months (Norwegian Institute of Public Health 2020; Revheim et al., 2023). Longer breastfeeding duration leads to later decrease in serum concentrations. In the current study, we have not accessed data on food intake or breastfeeding. Inverse associations between PFAS concentrations and age in schoolchildren have been reported in previous publications, and can, at least partly, be explained by growth dilution (Koponen et al., 2018; Nyström et al., 2022).

Reported sex differences in biomonitoring data in children are variable (Winkens et al., 2017). In our study, the majority of the PFAS showed higher levels in boys compared to girls. Published data from adults generally show higher PFAS levels in men compared to women, which is primarily attributed to blood loss via menstruation, transfer of PFAS to the fetus during pregnancy, and excretion via breast milk (Winkens et al., 2017). Pregnancy and breastfeeding are not relevant for our study population. However, menstruation can contribute as shown in previous studies for PFOS (Nyström et al., 2022; Wong et al., 2014). In addition, females have lower energy intake per kilogram of body weight compared to males, implying a lower PFAS intake per kilogram of body weight (Shomaker et al., 2010). As girls are entering puberty earlier than boys, their growth spurt and following growth dilution will occur earlier. This, in addition to menstruation, could explain the different age patterns between the sexes. Future research should include further exploration of the relationships of PFAS in relation to growth and maturation.

A limitation of the present study is that we only included children living in Bergen. Our findings may therefore not be representative for the entire Norwegian population of children. However, our data correspond well with the national data from Norwegian children in the HBM4EU studies (Richterová et al., 2023). As this is a cross-sectional study, the observed differences between age groups could be due to changes related to growth and maturation, behavioral factors, as well as differences in exposure levels and duration. On the other hand, a cross-sectional design with sample collection during a narrow time frame might be more beneficial than a longitudinal design, given that exposure levels have changed during the last decades. Finally, a cross-sequential design might give a more complete picture. Strengths of our study include a relatively large number of participants and a wide range of PFAS assessed using a sensitive analytical method. All blood samples were collected during a narrow time frame which avoids interference of the sample time point when comparing age groups or sexes.

5. Conclusions

In the current study, 11 of the 19 analyzed PFAS were detected in the serum samples of children recruited through the BGS2. The children covered a wide age range, from 6 to 16 years of age, and PFAS exposure was stratified by age and sex. PFOS, PFOA, PFHxS and PFNA were present in all samples demonstrating widespread exposure in the sample population of Norwegian children analyzed in this study. Approximately one out of five children had serum concentrations above safety levels, indicating a potential risk of negative health effects. The majority of the analyzed PFAS showed higher levels in boys than in girls, and higher levels in the youngest age groups compared to the oldest. Future analyses should include examining the relationships between PFAS, growth and maturation.

Declarations of interest

None.

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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.2023.114199.

References

Appel, M., Forsthuber, M., Ramos, R., Widhalm, R., Granitzer, S., Uhl, M., Hengstschläger, M., Stamm, T., Gundacker, C., 2022. The transplacental transfer efficiency of per- and polyfluoroalkyl substances (PFAS): a first meta-analysis. J. Toxicol. Environ. Health B Crit. Rev. 25 (1), 23–42. https://doi.org/10.1080/10937404.2021.2009946.

Averina, M., Brox, J., Huber, S., Furberg, A.S., 2018. Perfluoroalkyl substances in adolescents in northern Norway: lifestyle and dietary predictors. The Tromsø study, Fit Futures 1. Environ. Int. 114, 123–130. https://doi.org/10.1016/j.envint.2018.02.031.

Blomberg, A.J., Norén, E., Haug, L.S., Lindh, C., Sabaredzovic, A., Pineda, D., Jakobsson, K., Nielsen, C., 2023. Estimated transfer of perfluoroalkyl substances (PFAS) from maternal serum to breast milk in women highly exposed from contaminated drinking water: a study in the ronneby mother-child cohort. Environ. Health Perspect. 131 (1), 17005 https://doi.org/10.1289/EHP11292.

Brantsæter, A.L., Whitworth, K.W., Ydersbond, T.A., Haug, L.S., Haugen, M., Knutsen, H. K., Thomsen, C., Meltzer, H.M., Becher, G., Sabaredzovic, A., Hoppin, J.A., Eggesbø, M., Longnecker, M.P., 2013. Determinants of plasma concentrations of perfluoroalkyl substances in pregnant Norwegian women. Environ. Int. 54, 74–84. https://doi.org/10.1016/j.envint.2012.12.014.

Cole, T.J., Bellizzi, M.C., Flegal, K.M., Dietz, W.H., 2000. Establishing a standard definition for child overweight and obesity worldwide: international survey. Br. Med. J. 320 (7244), 1240–1243. https://doi.org/10.1136/bmj.320.7244.1240.

Conder, J.M., Hoke, R.A., De Wolf, W., Russell, M.H., Buck, R.C., 2008. Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. Environ. Sci. Technol. 42 (4), 995–1003. https://doi.org/10.1021/es070895g.

Duffek, A., Conrad, A., Kolossa-Gehring, M., Lange, R., Rucic, E., Schulte, C., Wellmitz, J., 2020. Per- and polyfluoroalkyl substances in blood plasma - results of the German Environmental Survey for children and adolescents 2014-2017 (GerES V). Int. J. Hyg Environ. Health 228, 113549. https://doi.org/10.1016/j.ijheh.2020.113549.

European Chemicals Agency, 2023. Per- and Polyfluoroalkyl Substances (PFASs). https://echa.europa.eu/hot-topics/perfluoroalkyl-chemicals-pfas. accecced: 20. February.23.

European Commission, 2020. Commission staff working document. Poly- and perfluoroalkyl substances (PFAS). https://ec.europa.eu/environment/pdf/chemicals/2020/10/SWD_PFAS.pdf accecced: 20.February.23.

European Commission, 2022. Commission regulation (EU) 2022/2388 amending Regulation (EC) No 1881/2006 as regards maximum levels of perfluoroalkyl substances in certain foodstuffs. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R2388 (accecced: 20.02.23.

Fromme, H., Tittlemier, S.A., Völkel, W., Wilhelm, M., Twardella, D., 2009. Perfluorinated compounds–exposure assessment for the general population in Western countries. Int. J. Hyg Environ. Health 212 (3), 239–270. https://doi.org/10.1016/j.ijheh.2008.04.007.

Fromme, H., Mosch, C., Morovitz, M., Alba-Alejandre, I., Boehmer, S., Kiranoglu, M., Faber, F., Hannibal, I., Genzel-Boroviczény, O., Koletzko, B., Völkel, W., 2010. Pre- and postnatal exposure to perfluorinated compounds (PFCs). Environ. Sci. Technol. 44 (18), 7123–7129. https://doi.org/10.1021/es101184f.

Glüge, J., Scheringer, M., Cousins, I.T., DeWitt, J.C., Goldenman, G., Herzke, D., Lohmann, R., Ng, C.A., Trier, X., Wang, Z., 2020. An overview of the uses of per- and polyfluoroalkyl substances (PFAS). Enviro. Sci. Process.Impacts 22 (12), 2345–2373. https://doi.org/10.1039/d0em00291g.

Haug, L.S., Thomsen, C., Becher, G., 2009a. Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. Environ. Sci. Technol. 43 (6), 2131–2136. https://doi.org/10.1021/es802827u.

Haug, L.S., Thomsen, C., Becher, G., 2009b. A sensitive method for determination of a broad range of perfluorinated compounds in serum suitable for large-scale human biomonitoring. J. Chromatogr. A 1216 (3), 385–393. https://doi.org/10.1016/j.chroma.2008.10.113.

Haug, L.S., Huber, S., Becher, G., Thomsen, C., 2011. Characterisation of human exposure pathways to perfluorinated compounds–comparing exposure estimates with biomarkers of exposure. Environ. Int. 37 (4), 687–693. https://doi.org/10.1016/j.envint.2011.01.011.

Haug, L.S., Sakhi, A.K., Cequier, E., Casas, M., Maitre, L., Basagana, X., Andrusaityte, S., Chalkiadaki, G., Chatzi, L., Coen, M., de Bont, J., Dedele, A., Ferrand, J., Grazuleviciene, R., Gonzalez, J.R., Gutzkow, K.B., Keun, H., McEachan, R., Meltzer, H.M., Petraviciene, I., Robinson, O., Saulnier, P.J., Slama, R., Sunyer, J., Urquiza, J., Vafeiadi, M., Wright, J., Vrijheid, M., Thomsen, C., 2018. In-utero and childhood chemical exposome in six European mother-child cohorts. Environ. Int. 121 (Pt 1), 751–763. https://doi.org/10.1016/j.envint.2018.09.056.

HBM4EU, 2022. The European Human Biomonitoring Initiative. Online library. https://www.hbm4eu.eu/online-library/.

Júlíusson, P.B., Bruserud, I.S., Oehme, N.H.B., Madsen, A., Forthun, I.H., Balthasar, M.R., Rosendahl, K., Viste, K., Jugessur, A., Schell, L.M., Bjerknes, R., Roelants, M., 2023. Deep phenotyping of pubertal development in Norwegian children: the Bergen Growth Study 2. Ann. Hum. Biol. https://doi.org/10.1080/03014460.2023.2174272 (in press).

Koponen, J., Winkens, K., Airaksinen, R., Berger, U., Vestergren, R., Cousins, I.T., Karvonen, A.M., Pekkanen, J., Kiviranta, H., 2018. Longitudinal trends of per- and polyfluoroalkyl substances in children's serum. Environ. Int. 121 (Pt 1), 591–599. https://doi.org/10.1016/j.envint.2018.09.006.

Land, M., de Wit, C.A., Bignert, A., Cousins, I.T., Herzke, D., Johansson, J.H., Martin, J. W., 2018. What is the effect of phasing out long-chain per- and polyfluoroalkyl substances on the concentrations of perfluoroalkyl acids and their precursors in the environment? A systematic review. Environ. Evid. 7, 4. https://doi.org/10.1186/s13750-017-0114-y.

Lobo Vicente, J., Ganzleben, C., Gasol, R., Marnane, I., Gilles, L., Buekers, J., Bessems, J., Colles, A., Gerofke, A., David, M., Barouki, R., Uhl, M., Sepai, O., Loots, I., Crabbé, A., Coertjens, D., Kolossa-Gehring, M., Schoeters, G., 2023. HBM4EU results support the chemicals' strategy for sustainability and the zero-pollution action plan. Int. J. Hyg Environ. Health 248, 114111. https://doi.org/10.1016/j.ijheh.2023.114111.

Ng, C.A., Hungerbühler, K., 2014. Bioaccumulation of perfluorinated alkyl acids: observations and models. Environ. Sci. Technol. 48 (9), 4637–4648. https://doi.org/10.1021/es404008g.

Statistics Norway, 2023. SSB (Statistisk Sentralbyrå). Personer 16 år og over, etter region, kjønn, alder, utdanningsnivå, statistikkvariabel og år. https://www.ssb.no/statbank/table/08921 accecced: 30.March.23.

Norwegian Institute of Public Health, 2020. Småbarnskost 3. Landsomfattende undersøkelse av kostholdet blant 2-åringer i Norge. https://www.fhi.no/publ/2020/smabarnskost-3/ accecced: 20.February.23.

Nøst, T.H., Vestergren, R., Berg, V., Nieboer, E., Odland, J.Ø., Sandanger, T.M., 2014. Repeated measurements of per- and polyfluoroalkyl substances (PFASs) from 1979 to 2007 in males from Northern Norway: assessing time trends, compound correlations and relations to age/birth cohort. Environ. Int. 67, 43–53. https://doi.org/10.1016/j.envint.2014.02.011.

Nübler, S., Esteban López, M., Castaño, A., Mol, H.G.J., Haji-Abbas-Zarrabi, K., Schäfer, M., Müller, J., Hajslova, J., Dvorakova, D., Antignac, J.P., Koch, H.M., Haug, L.S., Vorkamp, K., Göen, T., 2022. Interlaboratory Comparison Investigations (ICIs) and External Quality Assurance Schemes (EQUASs) for human biomonitoring of perfluoroalkyl substances (PFASs) in serum as part of the quality assurance programme under HBM4EU. Sci. Total Environ. 847, 157481 https://doi.org/10.1016/j.scitotenv.2022.157481.

Nyström, J., Benskin, J.P., Plassmann, M., Sandblom, O., Glynn, A., Lampa, E., Gyllenhammar, I., Moraeus, L., Lignell, S., 2022. Demographic, life-style and physiological determinants of serum per- and polyfluoroalkyl substance (PFAS) concentrations in a national cross-sectional survey of Swedish adolescents. Environ. Res. 208, 112674 https://doi.org/10.1016/j.envres.2022.112674.

Panieri, E., Baralic, K., Djukic-Cosic, D., Buha Djordjevic, A., Saso, L., 2022. PFAS molecules: a major concern for the human health and the environment. Toxics 10 (2), 44. https://doi.org/10.3390/toxics10020044.

Papadopoulou, E., Sabaredzovic, A., Namork, E., Nygaard, U.C., Granum, B., Haug, L.S., 2016. Exposure of Norwegian toddlers to perfluoroalkyl substances (PFAS): the association with breastfeeding and maternal PFAS concentrations. Environ. Int. 94, 687–694. https://doi.org/10.1016/j.envint.2016.07.006.

Poothong, S., Papadopoulou, E., Padilla-Sánchez, J.A., Thomsen, C., Haug, L.S., 2020. Multiple pathways of human exposure to poly- and perfluoroalkyl substances (PFASs): from external exposure to human blood. Environ. Int. 134, 105244 https://doi.org/10.1016/j.envint.2019.105244.

Rappazzo, K.M., Coffman, E., Hines, E.P., 2017. Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature. Int. J. Environ. Res. Publ. Health 14 (7), 691. https://doi.org/10.3390/ijerph14070691.

Revheim, I., Balthasar, M.R., Akerkar, R.R., Stangenes, K.M., Almenning, G., Nygaard, E., Markestad, T., Øverland, S., Roelants, M., Juliusson, P.B., 2023. Trends in the prevalence of breastfeeding up to 6 months of age using structured data from routine child healthcare visits. Acta paediatrica (Oslo, Norway 112 (1), 100–105. https://doi.org/10.1111/apa.16367, 1992.

Richterová, D., Govarts, E., Fábelová, L., Rausová, K., Rodriguez Martin, L., Gilles, L., Remy, S., Colles, A., Rambaud, L., Riou, M., Gabriel, C., Sarigiannis, D., Pedraza-Diaz, S., Ramos, J.J., Kosjek, T., Snoj Tratnik, J., Lignell, S., Gyllenhammar, I., Thomsen, C., Haug, L.S., Kolossa-Gehring, M., Vogel, N., Franken, C., Vanlarebeke, N., Bruckers, L., Stewart, L., Sepai, O., Schoeters, G., Uhl, M., Castaño, A., Esteban López, M., Göen, T., Palkovičová Murínová, Ľ., 2023. PFAS levels and determinants of variability in exposure in European teenagers - results from the HBM4EU aligned studies (2014-2021). Int. J. Hyg Environ. Health 247, 114057. https://doi.org/10.1016/j.ijheh.2022.114057.

Russell, M.H., Nilsson, H., Buck, R.C., 2013. Elimination kinetics of perfluorohexanoic acid in humans and comparison with mouse, rat and monkey. Chemosphere 93 (10), 2419–2425. https://doi.org/10.1016/j.chemosphere.2013.08.060.

Schrenk, D., Bignami, M., Bodin, L., Chipman, J.K., Del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L.R., Leblanc, J.C., Nebbia, C.S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Vleminckx, C., Wallace, H., Barregård, L., Ceccatelli, S., Cravedi, J. P., Schwerdtle, T., EFSA, 2020. Panel on Contaminants in the Food Chain (EFSA CONTAM Panel), n.d.. Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA J. Eur. Food Saf. Auth. 18 (9), e06223. doi: 10.2903/j.efsa.2020.6223.

Shomaker, L.B., Tanofsky-Kraff, M., Savastano, D.M., Kozlosky, M., Columbo, K.M., Wolkoff, L.E., Zocca, J.M., Brady, S.M., Yanovski, S.Z., Crocker, M.K., Ali, A., Yanovski, J.A., 2010. Puberty and observed energy intake: boy, can they eat. Am. J. Clin. Nutr. 92 (1), 123–129. https://doi.org/10.3945/ajcn.2010.29383.

Stockholm Convention on persistent organic pollutants, 2019. All POPs listed in the Stockholm Convention. http://pops.int/TheConvention/ThePOPs/AllPOPs/tabid/2509/Default.aspx. (Accessed 20 February 2023).

The German Environment Agency, 2020. Human biomonitoring commission (HBM commission). https://www.umweltbundesamt.de/en/topics/health/commissions-working-groups/human-biomonitoring-commission-hbm-commission accecced: 20.February.23.

Theurich, M.A., Davanzo, R., Busck-Rasmussen, M., Díaz-Gómez, N.M., Brennan, C., Kylberg, E., Bærug, A., McHugh, L., Weikert, C., Abraham, K., Koletzko, B., 2019. Breastfeeding rates and programs in Europe: a survey of 11 national breastfeeding committees and representatives. J. Pediatr. Gastroenterol. Nutr. 68 (3), 400–407. https://doi.org/10.1097/MPG.0000000000002234.

Trudel, D., Horowitz, L., Wormuth, M., Scheringer, M., Cousins, I.T., Hungerbühler, K., 2008. Estimating consumer exposure to PFOS and PFOA. Risk Anal. : an official publication of the Society for Risk Analysis 28 (2), 251–269. https://doi.org/10.1111/j.1539-6924.2008.01017.x.

VKM Report, 2022. Norwegian Scientific Committee for Food and Environment. Benefit and risk assessment of fish in the Norwegian diet. https://vkm.no/english/riskassessments/allpublications/benefitandriskassessmentoffishinthenorwegiandiet.4.7b65040716afa427d7ec5d3a.html accessed 20.February.23.

Winkens, K., Vestergren, R., Berger, U., Cousins, I.T., 2017. Early life exposure to per- and polyfluoroalkyl substances (PFASs): a critical review. Emerging Contaminants 3 (Issue 2), 55–68. https://doi.org/10.1016/j.emcon.2017.05.001.

Wong, F., MacLeod, M., Mueller, J.F., Cousins, I.T., 2014. Enhanced elimination of perfluorooctane sulfonic acid by menstruating women: evidence from population-based pharmacokinetic modeling. Environ. Sci. Technol. 48 (15), 8807–8814. https://doi.org/10.1021/es500796y.

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The association between long-term exposure to outdoor artificial light at night and poor sleep quality among Chinese veterans: A multi-city study

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ABSTRACT

Background: A handful of previous studies have reported the association between exposure to outdoor artificial light at night (ALAN) and sleep problems. However, evidence for such association is limited in low- and middle-income countries. This study aimed to examine the association between outdoor ALAN exposure and sleep quality in veterans across different regions of China.

Methods: Within the network of the Chinese Veteran Clinical Research Platform, we selected 7258 participants from 277 veteran communities in 18 cities across China during December 2009 and December 2011, using a multi-stage stratified cluster sampling strategy. Face-to-face interviews with the participants were conducted by trained investigators. We used the Pittsburgh Sleep Quality Index (PSQI) to assess participants' sleep quality. We defined poor sleep quality as a PSQI global score >7. The 3-year average exposure to outdoor ALAN prior to the baseline interview was calculated using satellite imagery data, according to participants' geolocation information. The association of ALAN exposure with sleep quality was examined using the mixed-effects logistic regression models with natural cubic splines.

Results: The exposure-response curve for sleep quality associated with ALAN exposure was nonlinear, with a threshold value of 49.20 nW/cm^2/sr for the 3-year average exposure to outdoor ALAN prior to the baseline interview. Higher ALAN exposure above the threshold was associated with increased risk of poor sleep quality. After adjusting for potential confounders, the odds ratios (and 95%CI, 95% confidence intervals) were 1.15 (0.97, 1.36) and 1.45 (1.17, 1.78) at the 75th and 95th percentiles of ALAN against the threshold. The association of ALAN exposure with poor sleep quality was more pronounced in veterans with depression than those without. Higher OR of poor sleep quality at the 75th percentile of ALAN against the threshold was observed in veterans with depression than those without [2.09 (1.16, 3.76) vs. 1.09 (0.92, 1.30)].

Conclusions: Long-term exposure to outdoor ALAN was associated with higher risk of poor sleep quality in Chinese veterans. Effective outdoor ALAN management may help to reduce the burden of sleep disorders in Chinese veterans.

Abbreviations: ALAN, artificial light at night; CES-D, Center for Epidemiological Survey Depression; CI, confidence interval; CVCR, Chinese Veteran Clinical Research; DMSP-OLS, Defense Meteorological Satellite Program's Operational Linescan System; NO2, nitrogen dioxide; NREM, non-rapid eye movement; OR, odds ratio; PM2.5, particulate matter with aerodynamic diameters ≤2.5 μm; PSQI, Pittsburgh Sleep Quality Index; REM, rapid eye movement; SD, standard deviation; SE, standard error.

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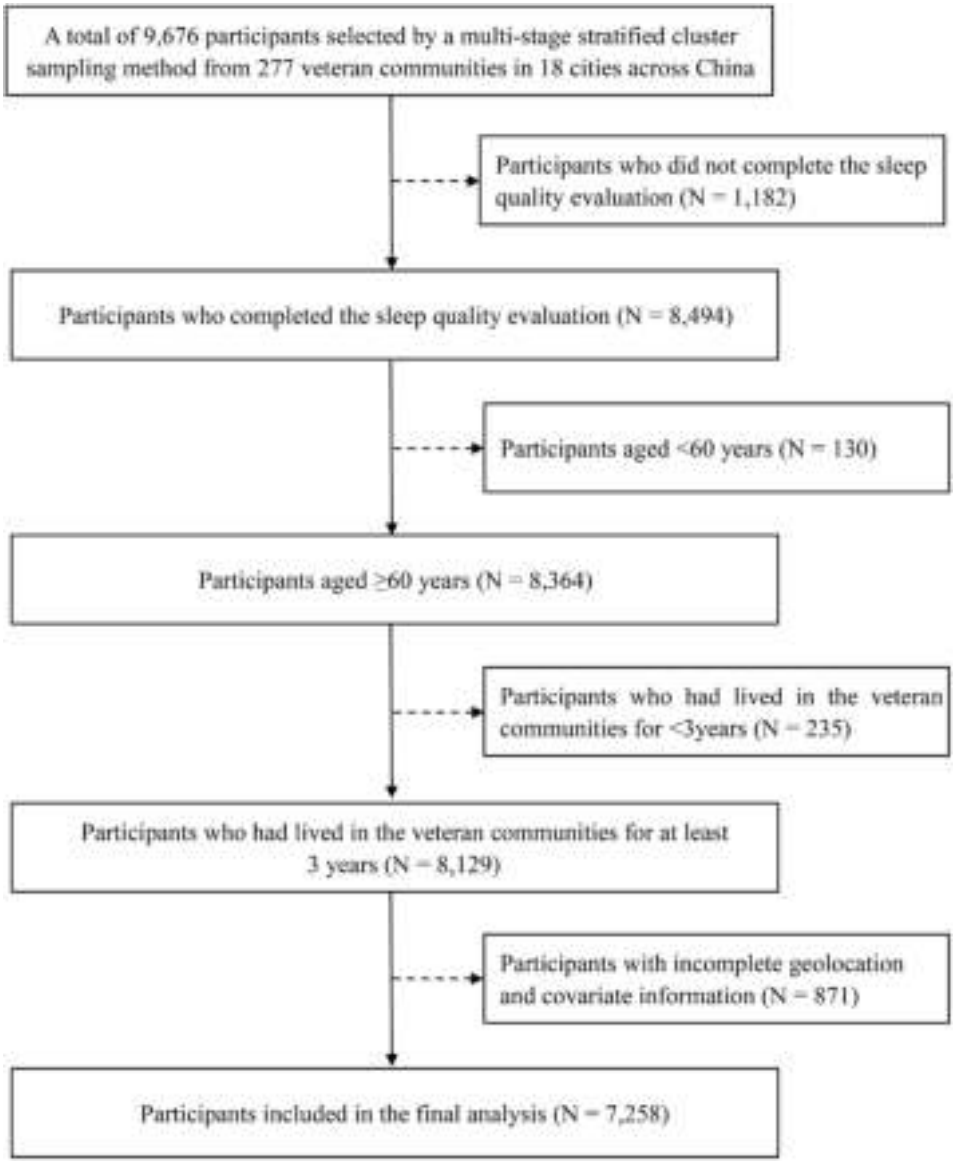

Fig. 1. The flow chart of study participants selection.

1. Introduction

Sleep disorders are a group of medical conditions that disturb the amount, timing, and quality of sleep, which could result in daytime sleepiness and impairment in functioning (Olejniczak and Fisch, 2003). Insomnia is the most common sleep disorder, with the global prevalence of acute insomnia of 30–35% and chronic insomnia estimated to be at least 5–10% (Mai and Buysse, 2008; Morin et al., 2015; Roth, 2007). Poor sleep health is linked with various physical and mental diseases, including obesity, cardiovascular disease, cancer, neurodegenerative disorder, and depression (Breen et al., 2014; Chan et al., 2018; Maglione et al., 2014; Samuelsson et al., 2018; Shi et al., 2018; Sigurdardottir et al., 2015; Tobaldini et al., 2019). There are numerous factors that can contribute to sleep difficulties and impact sleep quality, including physical and psychiatric comorbidities (e.g., chronic pain and depression), sociodemographic characteristics (e.g., age and sex), and behavioral factors (e.g., smoking and drinking) (Al Lawati et al., 2009; Becker, 2006; Ph et al., 2021; Whinnery et al., 2014). The prevalence of sleep disorders is higher among the elderly, and women are at a higher risk than men (Gulia and Kumar, 2018 new; Pengo et al., 2018). Apart from those better-known factors, recent evidence from animal and human studies indicated that artificial light at night (ALAN) could suppress melatonin secretion and disrupt normal circadian rhythms, leading to sleep difficulties (Blume et al., 2019; Navara and Nelson, 2007). The association between excessive outdoor ALAN and sleep health has attracted increasing public concern worldwide. For example, a study in the U.S. reported that outdoor ALAN exposure was in relation to increased likelihood of insufficient sleep duration (<6 h) [odds ratio (OR) and 95% confidence intervals (95%CI): 1.06 (1.02, 1.10)] (Ohayon and Milesi, 2016). Another in South Korea reported the association between higher outdoor ALAN in the residential area and insomnia symptoms [OR (95%CI): 1.53 (1.33, 1.78)] (Koo et al., 2016).

Accompanied by the considerable progress in global economy and urbanization, artificial light pollution has become an unprecedented environmental problem, with >80% of the world's population living under excessively bright skies faced with ALAN-related health threat (Bedrosian and Nelson, 2013; Falchi et al., 2016; Lai et al., 2020, 2021; Park et al., 2019; Sun et al., 2021). China is one of the countries leading the increasing global lighting trend, with an annual light growth of 6.48% (Hu and Zhang, 2020). Moreover, having a large elderly population of over 200 million (18.7% of the total population) (National Bureau of Statistics of China, 2022) with a prevalence of sleep

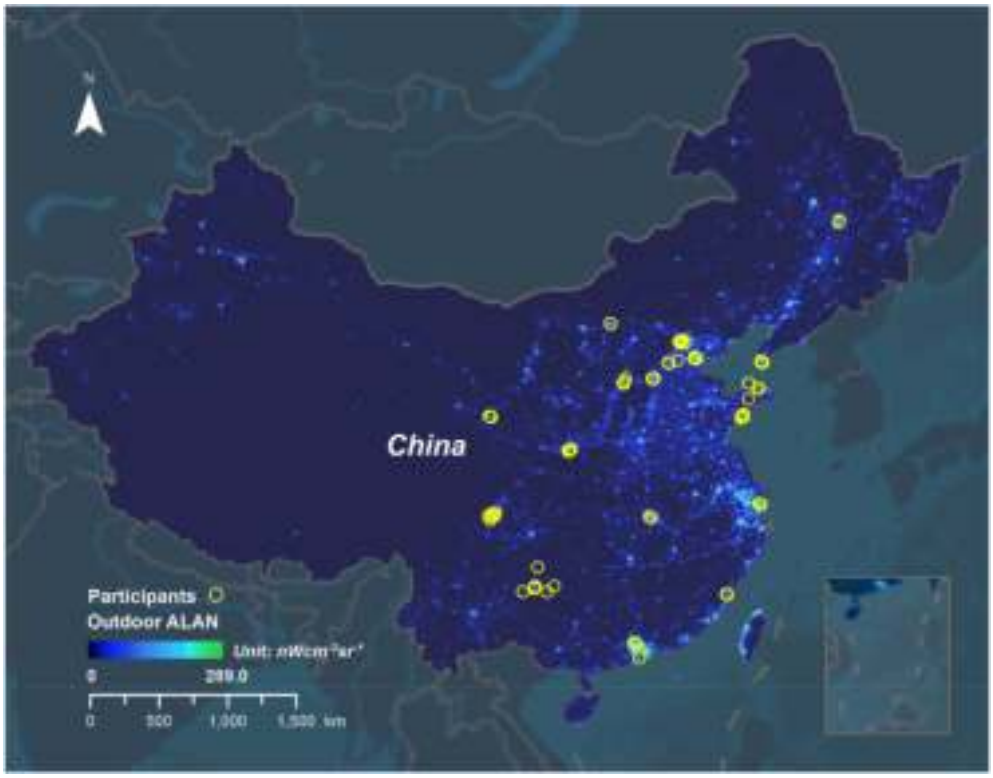

Fig. 2. A map of participants' residence and outdoor ALAN exposure in 2010.

disturbances of 35.9% (30.6%, 41.2%) (Lu et al., 2019), China bears a huge burden of sleep disorders. Owing to the accelerating trend of population ageing, this burden is expected to grow dramatically in the near future (Zhu et al., 2020). Considering the widespread exposure to outdoor ALAN and the high prevalence of sleep disorders, it is an urgent need to further evaluate the burden of poor sleep health in Chinese elderly population associated with exposure to outdoor ALAN.

Veterans are a special group of the elderly population. Due to special service experiences that may involve irregular sleep schedules, combat-related stress, physical injuries, and psychological trauma, sleep disorders and sleep-related diseases such as hypertension and depression are more prevalent among veterans than the general population (Alexander et al., 2016; Saconi et al., 2021). Veterans may be particularly vulnerable to the impacts of ALAN due to those unique stressors associated with military service and their residual effects (Babu Henry Samuel et al., 2021; Byrne et al., 2021). Thus, veterans' sleep health and the risk factors deserve more attention. A handful of studies have shown exposure to outdoor ALAN was correlated with poor sleep quality among children and adults (Koo et al., 2016; Ohayon and Milesi, 2016; Paksarian et al., 2020; Xiao et al., 2020), but it's not clear whether such association is also observed in veterans. Based on the Chinese Veteran Clinical Research platform, this study aims to examine the association between exposure to outdoor ALAN and sleep quality among Chinese veterans.

2. Material and methods

2.1. Study population

The Chinese Veteran Clinical Research (CVCR) platform was designed to study health status of Chinese veterans (established during December 2009 to December 2011), covering 277 veteran communities in 18 cities across China (Tan et al., 2014). Chinese veterans have an optimal healthcare system and detailed electronic health records, who live steadily in veteran communities after retirement (Tan et al., 2014). In China, veteran communities are residential areas or neighborhoods that are specifically designated for retired military personnel, which offer a range of amenities and facilities tailored to the unique requirements of veterans, such as healthcare services and recreational activities. While these communities are separate, veterans can still interact with the broader non-veteran population when accessing public services. Detailed description of the study design has been reported elsewhere (Tan et al., 2016, 2021). In short, a multi-stage stratified cluster sampling strategy was applied to select veterans from veteran communities in mainland China. In China, first-tier cities are typically the largest, most developed, and economically significant cities (e.g., Beijing and Shanghai). Second-tier cities in China are typically smaller than first-tier cities but still possess significant economic and development potential (e.g., Wuhan and Chengdu). Third-tier cities in China are generally smaller in size and economic importance compared to first and second-tier cities (e.g., Baoding and Tangshan). To ensure geographic coverage and representativeness, stratified sampling was first performed by economic status, location, and city size. First, we selected 6 provinces and 3 municipalities in the developed Eastern China, and 8 provinces in the less developed Central and Western China. Second, 18 cities located in those provinces and municipalities were selected, comprising 4 first-tier cities, 5 second-tier cities and 1 third-tier city in Eastern China, and 8 second-tier cities in Central and Western China. Third, all veteran communities in each selected city were included in this platform, and all eligible veterans were interviewed in each selected veteran community. The sample size was 500–1000 for cities with more veteran communities and 100–300 for those with fewer. The eligible criteria for veterans in each selected veteran community were listed as follows: (a) aged $\geq$60 years; (b) served in the army before retirement; (c) have resided in the veteran communities for at least 3 years; (d) were under the management of the CVCR platform. As a result, a total of 9676 veterans were investigated during 2009–2011 (response rate: 83.86%), covering 10.75% of all veterans in China (Fig. 1).

2.2. Outcome assessment

We evaluated participants' sleep quality using the Pittsburgh Sleep Quality Index (PSQI), a standardized questionnaire to measure subjective sleep quality in clinical practice and research (Buysse et al., 1989). It consists of 7 dimensions, subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. For example, the score of sleep latency is determined by a subjective assessment of how long it usually takes an individual to fall asleep each night during the last month and how often an individual has trouble sleeping because he/she cannot get to sleep within 30 min. The score of sleep efficiency is determined by a subjective assessment of the percentage of the hours slept against the hours spent in bed. Each component score ranges from 0 to 3, and the global score from 0 to 21. For the global score of PSQI and its dimensions, higher scores denote poorer sleep quality. We defined poor sleep quality as a PSQI global score >7 and good sleep quality as a global score ≤7 in this study. The Chinese version of PSQI with a cut-off point of 7 showed good validity and reliability among Chinese population, and has been widely used by previous studies (Ho and Fong, 2014; Liu et al., 1996; Wu et al., 2020). Among 9676 veterans recruited in the platform, 8494 took the sleep quality assessment. Participants were excluded, who aged <60 years (n = 130), had lived in the veteran communities for <3 years (n = 235), had missing geolocation data (n = 125), or had incomplete covariate information (n = 746). We eventually included 7258 participants in final analyses.

2.3. Outdoor ALAN measurement

Outdoor ALAN data were derived from the U.S. Defense Meteorological Satellite Program's Operational Linescan System (DMSP-OLS) (Database DMSP ,Accessed 27 May 2021). As an oscillating scan device performing continuous sampling of the Earth's surface, the DSMP-OLS sensor provides visual and infrared imagery with daily global coverage (Hsu et al., 2015). Our data were extracted from the DMSP Global Radiance Calibrated Nighttime Lights products, which were produced by merging data from 3 fixed gain settings (low, medium and high) and the stable lights product (Hsu et al., 2015). These composites excluded undesirable contamination from natural light sources (e.g., clouds, sunlight, moonlight, and atmospheric lightning). The data product was gridded into a 30 arcsecond grid with a spatial resolution of 1 km. We transformed the values into units of radiance, nanowatts per square centimeter per steradian ($nW/cm^2/sr$). Participants' exposure to outdoor ALAN was assigned according to geolocation of veteran communities (longitude and latitude) they resided in. We considered the 3-year average ALAN exposure as our previous works did (Chen et al., 2022; Xie et al., 2022). Based on available ALAN data for the most recent past years, we estimated the 3-year average exposure to outdoor ALAN prior to the baseline interview for veterans in each veteran community (Fig. 2).

2.4. Covariates

According to common predictors of sleep quality and previous studies on ALAN and sleep (Hu et al., 2022; Paksarian et al., 2020), we considered a broad range of covariates in the current study, including social demographic characteristics, behavioral factors and other variables linked with both ALAN and sleep (Romeo et al., 2013; Xiao et al., 2020). Specifically, the covariates included age, sex (male or female), marital status (married or others), educational years (<7, 7–12, or >12years), smoking status (current smoker, ex-smoker, or non-smoker), drinking (>2 times/week, ≤2 times/week, quit, or never), regular physical activity (yes or no), regular social activity (yes or no), and histories of hypertension, diabetes, stroke, coronary heart disease, Parkinson's disease, rheumatoid arthritis, and depression (yes or no). Since veterans' previous work may involve military installations, historical exposure to electromagnetic field (yes or no) was also included. Additionally, other covariates were considered in the sensitivity analyses, including race (Han or others), season of the investigation (spring, summer, autumn or winter), and air pollutants [particulate matter with aerodynamic diameters ≤2.5 μm ($PM_{2.5}$) and nitrogen dioxide (NO_2)].

Data on demographic characteristics and behavioral factors were collected by a structured questionnaire. Data on histories of diseases were extracted from veterans' health records of disease diagnosis and treatment from the CVCR platform. Specifically, Parkinson's disease was diagnosed by clinical specialists according to the British bank standards, and depression was assessed by the Center for Epidemiological Survey Depression (CES-D) Scale (Tan et al., 2014). Participants with a CES-D score ≥16 were deemed to meet criteria for depression (Radloff, 1977). Concentrations of air pollutants ($PM_{2.5}$ and NO_2) during 2006–2011 across China were estimated using a satellite-based random forests model, which has been previously reported (Chen et al., 2018; Zhan et al., 2018). Participants' 3-year average exposure to each air pollutant prior to the baseline interview was estimated according to their geolocation information.

2.5. Statistical analyses

We developed mixed-effects logistic regression models to evaluate the association of outdoor ALAN exposure with poor sleep quality. Given that the ALAN-sleep quality association may differ among participants across different regions, we included the city of each veteran community in the model as a random-effect term. To check the potential nonlinear exposure-response association of ALAN with poor sleep quality, ALAN was fitted with the use of a natural cubic spline with 3 degrees of freedom (df). We selected the best df by identifying the lowest Akaike Information Criterion in the model (Li et al., 2016). First, a crude model was developed including ALAN and city of the veteran community as the random-effect term. Then, an adjusted model was developed by further controlling for a range of potential confounders, including age, sex, marital status, educational years, smoking status, drinking, regular physical activity, regular social activity, historical electromagnetic field exposure, histories of hypertension, diabetes, stroke, coronary heart disease, Parkinson's disease, rheumatoid arthritis, and depression. Age was incorporated in the adjusted model as a smooth term with 3 df. To determine the threshold of ALAN, we first estimated the association between ALAN and poor sleep quality by an 0.01-unit increment in ALAN using the natural cubic splines. Then we chose the ALAN level corresponding to the minimum risk of poor sleep quality as the threshold. Afterward, we plotted the exposure-response curve of the nonlinear association between ALAN and poor sleep quality against the threshold. We calculated the ORs and 95%CIs of poor sleep quality at 75th and 95th percentiles of ALAN against the threshold. The method to determine the threshold has been previously reported and widely used to check the nonlinear relationship between temperature and mortality (Chung et al., 2009; Li et al., 2016; Yu et al., 2011). We also investigated the nonlinear association of ALAN with both PSQI global score and 7 components. In addition, we conducted stratified analyses by sex, age group, educational years, smoking, drinking, regular physical activity, regular social activity, histories of hypertension, diabetes, and depression. To examine the difference between effect estimates for subgroups (e.g., male or female), the following formula was used

$$\widehat{Q}_1 - \widehat{Q}_2 \pm 1.96\sqrt{\widehat{SE_1}^2 + \widehat{SE_2}^2},$$

where $\widehat{Q}_1$ and $\widehat{Q}_2$ are the effect estimates, and $\widehat{SE_1}$ and $\widehat{SE_2}$ are the corresponding standard errors for the two subgroups (Zeka et al., 2006).

To test the robustness of our results, a series of sensitivity analyses were conducted, including additionally adjusting for race, season of the investigation, and air pollutants ($PM_{2.5}$ and NO_2), using different ALAN exposure periods (4 years and 5 years prior to the baseline interview),

Table 1
Basic characteristics of the study population.

Variables	Total	Poor sleep quality	
		Yes	No
	n (%)	n (%)	n (%)
Age (years, mean ± SD)	81.98 (4.06)	82.45 (4.00)	81.79 (4.08)
Sex			
Male	6937 (95.6)	1921 (93.9)	5016 (96.2)
Female	321 (4.4)	124 (6.1)	197 (3.8)
Marital status			
Married	6067 (83.6)	1651 (80.7)	4416 (84.7)
Other	1191 (16.4)	394 (19.3)	797 (15.3)
Educational years			
<7 years	2713 (37.4)	809 (39.6)	1904 (36.5)
7–12 years	3365 (46.4)	917 (44.8)	2448 (47.0)
>12 years	1180 (16.3)	319 (15.6)	861 (16.5)
Regular physical activity [a]			
Yes	6222 (85.7)	1646 (80.5)	4576 (87.8)
No	1036 (14.3)	399 (19.5)	637 (12.2)
Regular social activity [b]			
Yes	2773 (38.2)	691 (33.8)	2082 (39.9)
No	4485 (61.8)	1354 (66.2)	3131 (60.1)
Smoking status			
Current smoker	604 (8.3)	161 (7.9)	443 (8.5)
Ex-smoker	2396 (33.0)	733 (35.8)	1663 (31.9)
Non-smoker	4258 (58.7)	1151 (56.3)	3107 (59.6)
Drinking			
>2 times/week	527 (7.3)	122 (6.0)	405 (7.8)
≤2 times/week	1728 (23.8)	437 (21.4)	1291 (24.8)
Quit	1123 (15.5)	363 (17.8)	760 (14.6)
Never	3880 (53.5)	1123 (54.9)	2757 (52.9)
Hypertension			
Yes	4875 (67.2)	1445 (70.7)	3430 (65.8)
No	2383 (32.8)	600 (29.3)	1783 (34.2)
Diabetes			
Yes	1981 (27.3)	603 (29.5)	1378 (26.4)
No	5277 (72.7)	1442 (70.5)	3835 (73.6)
Stroke			
Yes	1450 (20.0)	491 (24.0)	959 (18.4)
No	5808 (80.0)	1554 (76.0)	4254 (81.6)
Coronary heart disease			
Yes	5048 (69.6)	1554 (76.0)	3494 (67.0)
No	2210 (30.4)	491 (24.0)	1719 (33.0)
Parkinson's disease			
Yes	203 (2.8)	90 (4.4)	113 (2.2)
No	7055 (97.2)	1955 (95.6)	5100 (97.8)
Rheumatoid arthritis			
Yes	358 (4.9)	137 (6.7)	221 (4.2)
No	6900 (95.1)	1908 (93.3)	4992 (95.8)
Depression			
Yes	502 (6.9)	342 (16.7)	160 (3.1)
No	6756 (93.1)	1703 (83.3)	5053 (96.9)
Historical electromagnetic field exposure			
Yes	489 (6.7)	156 (7.6)	333 (6.4)
No	6769 (93.3)	1889 (92.4)	4880 (93.6)
Region			
Eastern China	5132 (70.7)	1417 (69.3)	3715 (71.3)
Central China	603 (8.3)	163 (8.0)	440 (8.4)
Western China	1523 (21.0)	465 (22.7)	1058 (20.3)
Total	7258	2045	5213

[a] Regular physical activity was defined as doing physical exercise for 30 min or more daily at an intensity equal to or greater than walking.
[b] Regular social activity referred to participation in organized activities involving social contacts.

and using different random-effect terms (region and city size). We performed all statistical analyses via R software (version 4.1.2).

3. Results

Table 1 presents basic characteristics of participants included in the current study. The mean age was 82.0 years (standard deviation, SD = 4.1) and 95.6% of the participants were male. Veterans with poor sleep quality tended to be older (82.45 vs. 81.79 years) and had a higher fraction of female (6.1% vs. 3.8%). In addition, those with poor sleep quality were more probable to have pre-existing health problems, including histories of hypertension, diabetes, stroke, coronary heart disease, Parkinson's disease, rheumatoid arthritis, and depression. Table 2 summarizes participants' exposure to outdoor ALAN and PSQI global scores during the study period. Veterans with poor sleep quality had higher level of ALAN exposure (63.11 vs. 62.60 nW/cm^2/sr) than those without. The prevalence of poor sleep quality was 28.2% (n = 2045), with the mean PSQI global score of 11.42 (SD = 8.00) for participants with poor sleep quality and 3.76 (SD = 1.89) for others.

Fig. 3 shows that the association of ALAN with the prevalent poor sleep quality (PSQI global score >7) was generally a J-shaped curve, indicating the threshold effect. The threshold value was 49.20 nW/cm^2/sr for the 3-year average ALAN exposure prior to the baseline interview. For ALAN levels below the threshold, the ORs (95%CIs) were 1.19 (0.96, 1.48) and 1.02 (0.98, 1.06) at the 5th (14.58 nW/cm^2/sr) and 25th percentiles (39.05 nW/cm^2/sr) of ALAN against the threshold. Higher ALAN exposure above the threshold was positively associated with greater risk of poor sleep quality. After adjusting for potential confounders, the ORs (95%CIs) were 1.15 (0.97, 1.36) and 1.45 (1.17, 1.78) at the 75th (85.29 nW/cm^2/sr) and 95th percentiles (119.04 nW/cm^2/sr) of ALAN against the threshold. Fig. 4 shows the associations between outdoor ALAN and PSQI scores. Higher ALAN exposure was associated with an increased PSQI global score and the component scores. After controlling for potential confounders, the PSQI global score increased by 0.29 (0.02, 0.57) and 0.58 (0.23, 0.93) at the 75th and 95th percentiles of ALAN against the threshold (50.00 nW/cm^2/sr). The component scores for subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction increased by 0.09 (−0.06, 0.24), 0.00 (−0.01, 0.01), 0.07 (0.01, 0.13), 0.07 (−0.01, 0.15), 0.07 (−0.04, 0.17), 0.07 (−0.01, 0.15), and 0.10 (−0.03, 0.23) at the 75th percentile of ALAN against the threshold, respectively, and they increased by 0.07 (−0.03, 0.16), 0.04 (−0.04, 0.13), 0.08 (0.01, 0.15), 0.11 (0.01, 0.21), 0.05 (−0.02, 0.11), 0.08 (−0.01, 0.17), and 0.08 (0.00, 0.16) at the 95th percentile of ALAN against the threshold, respectively.

Table 3 shows the results of stratified analyses. Higher OR of poor sleep quality at the 75th percentile of ALAN against the threshold was observed in veterans with depression than those without [2.09 (1.16, 3.76) vs. 1.09 (0.92, 1.30)], with the p value of 0.04 for difference. No statistically significant differences were observed among subgroups of participants stratified by sex, age group, educational years, smoking, drinking, regular physical activity, regular social activity, or histories of hypertension and diabetes.

Table 4 shows the results of sensitivity analyses. The results did not change substantially after additionally controlling for race, season of the investigation or levels of exposure to air pollutants ($PM_{2.5}$ and NO_2). For example, after additionally adjusting for air pollutants ($PM_{2.5}$ and NO_2), the ORs (95%CIs) of poor sleep quality were 1.12 (0.95, 1.32) and 1.44 (1.16, 1.79) at the 75th and 95th percentiles of ALAN against the threshold. Changing exposure period or random-effect term, the results were consistent with those in the main analyses (Tables S1–2).

4. Discussion

To the best of our knowledge, this is the first study on the association between long-term exposure to outdoor ALAN and sleep quality among veterans in China, which showed nonlinear exposure-response curves with threshold effects. Higher exposure to ALAN above the threshold was significantly associated with higher risks of poor sleep quality and shorter sleep duration. Our findings provided new evidence for the adverse effects of outdoor ALAN on sleep quality among veterans.

Existing studies focusing on the association of sleep quality with outdoor ALAN are mainly from Western countries. However, inadequate research has focused on such association in low- and middle-income countries like China. Our findings generally coincided with previous

Table 2
A summary of exposure to outdoor ALAN and sleep quality.

	Mean	SD	Min	Quartiles			Max	IQR
				25%	50%	75%		
Poor sleepers								
ALAN	63.11	33.22	3.08	35.75	58.70	89.72	147.78	53.97
PSQI global score	11.42	3.00	0.00	9.00	11.00	13.00	21.00	6.00
Good sleepers								
ALAN	62.60	31.50	3.08	39.63	58.86	82.30	153.46	42.67
PSQI global score	3.76	1.89	0.00	2.00	4.00	5.00	7.00	3.00
All participants								
ALAN	62.75	31.99	3.08	39.05	58.70	85.29	153.46	50.20
PSQI global score	5.92	4.12	0.00	3.00	5.00	8.00	21.00	5.00

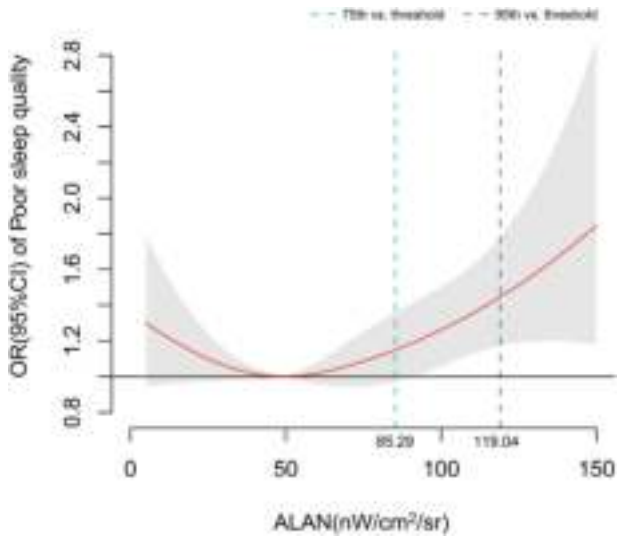

Fig. 3. The nonlinear exposure-response curve for the association between outdoor ALAN and poor sleep quality.
Note: The model was adjusted for age, sex, marital status, educational years, smoking status, drinking, regular physical activity, regular social activity, historical electromagnetic field exposure, histories of hypertension, diabetes, stroke, coronary heart disease, Parkinson's disease, rheumatoid arthritis, and depression.

studies that indicated the adverse effects of outdoor ALAN on sleep. For instance, a national study among 10,123 adolescents in the U.S. reported that increased ALAN was associated with delayed bedtime [Quartile 4 vs. Quartile 1: 29 (15, 43) minutes] and shorter sleep duration [Quartile 4 vs. Quartile 1: 11 (−19, −2) minutes] (Paksarian et al., 2020). A cross-sectional study among 8526 South Korean adults also reported higher ALAN exposure was correlated to higher risks of insufficient sleep (<6 h) [OR (95%CI): 1.22 (1.06, 1.41)] and insomnia symptoms [OR (95%CI): 1.53 (1.33, 1.78)] (Koo et al., 2016). Another conducted in China among 13,474 older adults revealed that the highest quartile of ALAN exposure was associated with a decrease of 17.04 (9.42, 24.78) minutes of sleep in comparison with the lowest quartile (Hu et al., 2022). Although the effect estimates (e.g., OR) were not exactly comparable considering the discrepancies in study population, exposure measurement, outcome assessment and covariates, all of these studies revealed the adverse impacts of outdoor ALAN on sleep.

Different from those studies, the participants of our study were veterans, a special group of the elderly population that may be more susceptible to sleep disorders and mental diseases (Raza et al., 2021; Toomey et al., 2007). For the elderly, poor sleep quality is associated with multiple chronic diseases that can result in low quality of life and heavy burdens on individuals and the whole society, including diabetes, cardiovascular disease, depression and dementia (Becker et al., 2017; Bokenberger et al., 2017; Hernández et al., 2022; Suzuki et al., 2009). Due to deployment-related factors that could chronically affect sleep quality (e.g., irregular sleep schedules, the stress of combat, physical injury and psychological trauma), sleep disorders are more prevalent in veterans than in the general population (Bramoweth and Germain, 2013). Numerous studies indicated that veterans with sleep disorders were at higher risks of comorbid mental illnesses, such as traumatic brain injury, posttraumatic stress disorder, depression, substance use disorders, and suicide (Bohnert et al., 2017; Gilbert et al., 2015; Olenick et al., 2015). Thus, our study has prompted calls for more attention on elderly people's sleep problems and their associations with outdoor ALAN. The sleep problems of other age groups associated with outdoor ALAN also need to be further explored.

Currently, the biological mechanism for the association of ALAN with sleep has not been fully known, but the available evidence has indicated several potential pathways, disruption of circadian system, immune dysregulation, and altered neurotransmission. First, the circadian clock maintains a stable 24-h day/night cycle, with the melatonin secretion of pineal gland at bedtime in reaction to it (Bedrosian and Nelson, 2017). However, exposure to ALAN can cause suppression in melatonin secretion and disrupt the circadian rhythms, which may further result in delayed sleep onset and impaired sleep quality (Stebelova et al., 2020; Touitou et al., 2017). Second, ALAN exposure can induce dysregulated inflammatory processes in the nervous system, and the chemical substances like cytokines produced during inflammation are related to disturbed sleep (Durrant et al., 2020; Ziegler et al., 2021; Zielinski and Gibbons, 2022). Last, ALAN can impact the neurotransmitter levels in brain regions concerned with the sleep-wake cycle and circadian rhythms, which may further cause sleep disorders (Castañeda et al., 2004). It is widely known that poor sleep quality is in relation to depression (Martin et al., 2010; Motivala et al., 2006; Qiao et al., 2021; Tsuno et al., 2005). In recent years, emerging evidence indicates an association of ALAN with depressive symptoms and mental disorders (Min and Min, 2018; Obayashi et al., 2013). In our stratified analyses, higher OR was observed in veterans with depression than those without. This may be due to the interaction between ALAN and depression on sleep quality.

Our study highlights the nonlinear exposure-response curve for the association between outdoor ALAN and poor sleep quality, extending previous works by pointing out threshold effects of ALAN on sleep quality. Although the OR at 5th percentile below the threshold was greater than 1 and did not appear to be very different from that at the 75th percentile, this association was not statistically significant and it may be due to small sample size at the lower end of ALAN distribution. With larger sample size, the ORs for ALAN levels below the threshold may be closer to 1. Thus, the threshold reported in our study is very informative for the establishment of the reference value of ALAN to protect population health. A growing body of studies have indicated the associations between compromised sleep quality and various diseases of the Chinese population, including hypertension, diabetes, dementia, and depression (Hu et al., 2021; Li et al., 2022; Liu et al., 2016; Lou et al., 2015; Sun et al., 2018; Wang et al., 2017). Given the ALAN-sleep quality

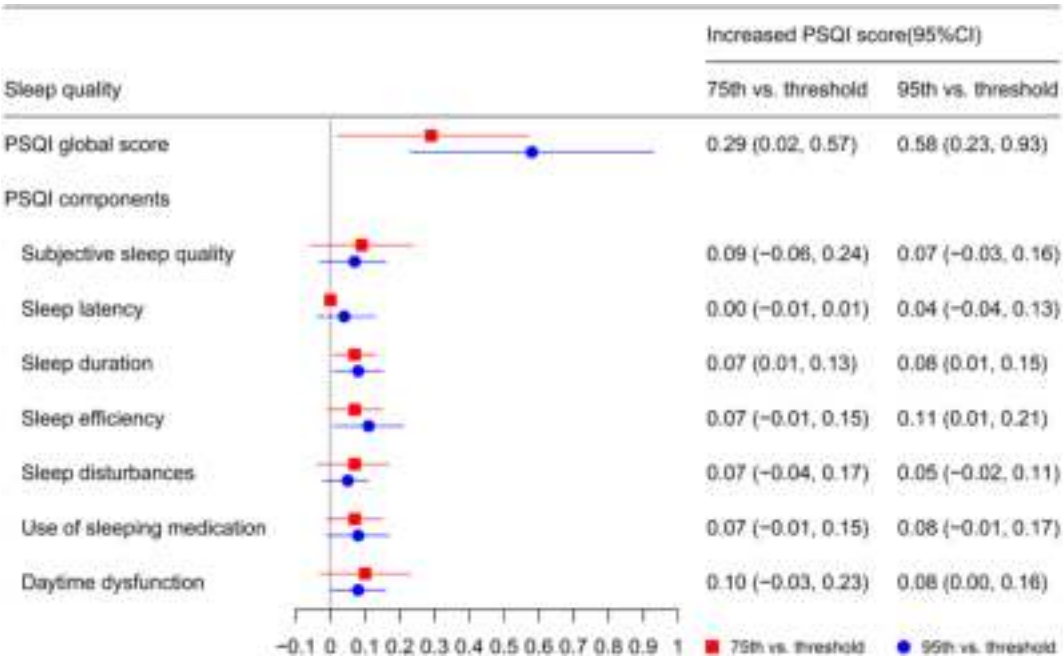

Fig. 4. Increased PSQI global score and PSQI component scores at 75th and 95th percentiles of outdoor ALAN against the threshold.
Note: The model was adjusted for age, sex, marital status, educational years, smoking status, drinking, regular physical activity, regular social activity, historical electromagnetic field exposure, histories of hypertension, diabetes, stroke, coronary heart disease, Parkinson's disease, rheumatoid arthritis, and depression.

association, keeping outdoor ALAN below the threshold may help with sleep quality improvement and relieve the heavy burden of those chronic conditions in China. In addition, our study emphasizes the significance of effective outdoor ALAN management during urban planning and policy-making. To date, no country or government around the world has issued guidelines of outdoor ALAN due to limited evidence. Our study provides clues for further exploration on outdoor ALAN-induced poor sleep health and its potential role as a determinant of health impairments. Further studies are urgently needed to assist the government in making decisions regarding prevention and control of artificial light pollution.

Several limitations of this study should be acknowledged. First, satellite light images could only estimate the average level of ALAN intensity according to residential addresses and may not reflect individuals' actual level of ALAN exposure (Huss et al., 2019). Due to data unavailability, we were not able to take into consideration factors that could affect ALAN levels during sleep time, such as the use of light-blocking shades and the time participants spent outdoors in the evening (Paksarian et al., 2020). The possibility of exposure misclassification is a common challenge faced by epidemiological studies using satellite imaging data for exposure measurement (McIsaac et al., 2021). Second, sleep quality was measured on a subjective scale and we did not have individuals' detailed sleep parameters measured. It's not clear whether outdoor ALAN influenced the rapid eye movement (REM) and non-REM (NREM) sleep. Third, our study cannot provide firm causal inference for the association between outdoor ALAN exposure and poor sleep quality, because of the cross-sectional design. Future prospective studies would provide better inferences for the adverse impacts of ALAN on sleep quality. Another limitation of this study is the possibility of selection bias stemming from the exclusion of veterans who did not complete the sleep quality evaluation. However, the main characteristics such as age, sex, smoking status, drinking and histories of diseases among veterans who were excluded and those included were generally comparable (Table S3), which means it is not likely to cause selection bias.

5. Conclusions

This study revealed the adverse effects of outdoor ALAN exposure on sleep quality among Chinese veterans. Further studies should be conducted to examine the causal relationship between ALAN and sleep problems and the underlying mechanisms. Regulation and effective policy curbing outdoor ALAN may help to reduce the burden of sleep disorders, particularly in urban areas.

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Data statement

The data that support the findings of this study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Xinyi Sun: Formal analysis, Visualization, Writing-original draft, Writing-review & editing. **Jiping Tan:** Investigation, Data collection, Writing-review & editing. **Yan Chen:** Writing-review & editing. **Yuewei Liu:** Writing-review & editing. **Guang-Hui Dong:** Writing-review & editing. **Bo-Yi Yang:** Writing-review & editing. **Nan Li:** Writing-review & editing. **Luning Wang:** Writing-review & editing. **Shanshan Li:** Data curation, Methodology, Writing-review & editing. **Gongbo Chen and Yuming Guo:** Conceptualization, Methodology, Writing-review & editing, Supervision, Project administration, Funding acquisition.

Table 3
The ORs and 95%CIs of poor sleep quality at 75th and 95th percentiles of outdoor ALAN against the threshold in stratified analyses.

Stratified traits	OR (95%CI)		OR (95%CI)	
	75th vs. threshold	p value[a]	95th vs. threshold	p value[a]
Sex				
Male	1.16 (0.97, 1.37)	0.21	1.44 (1.16, 1.77)	0.28
Female	0.73 (0.36, 1.48)		0.94 (0.45, 1.95)	
Age group				
<85	1.20 (0.99, 1.45)	0.35	1.41 (1.12, 1.78)	0.95
≥85	1.00 (0.73, 1.37)		1.39 (0.94, 2.07)	
Educational years				
<10	1.07 (0.88, 1.31)	0.28	1.36 (1.05, 1.76)	0.52
≥10	1.30 (0.97, 1.74)		1.56 (1.13, 2.15)	
Smoking				
Smoker	1.02 (0.60, 1.73)	0.63	1.08 (0.57, 2.04)	0.36
Non-smoker	1.17 (0.98, 1.39)		1.48 (1.19, 1.84)	
Drinking				
Drink every week	1.33 (0.97, 1.81)	0.28	1.28 (0.90, 1.83)	0.45
Quit or Never drink	1.08 (0.89, 1.32)		1.51 (1.17, 1.95)	
Regular physical activity				
Yes	1.10 (0.91, 1.32)	0.67	1.32 (1.06, 1.65)	0.75
No	1.20 (0.83, 1.74)		1.45 (0.85, 2.47)	
Regular social activity				
Yes	1.12 (0.85, 1.48)	0.98	1.37 (0.99, 1.90)	0.88
No	1.13 (0.92, 1.37)		1.42 (1.09, 1.84)	
Hypertension				
Yes	1.16 (0.96, 1.41)	0.81	1.28 (1.02, 1.62)	0.20
No	1.11 (0.82, 1.50)		1.72 (1.17, 2.54)	
Diabetes				
Yes	1.09 (0.82, 1.46)	0.77	1.28 (0.91, 1.80)	0.60
No	1.15 (0.94, 1.39)		1.43 (1.12, 1.83)	
Depression				
Yes	2.09 (1.16, 3.76)	**0.04**	1.80 (0.95, 3.41)	0.43
No	1.09 (0.92, 1.30)		1.37 (1.10, 1.70)	

Note: The model was adjusted for age, sex, marital status, educational years, smoking status, drinking, regular physical activity, regular social activity, historical electromagnetic field exposure, histories of hypertension, diabetes, stroke, coronary heart disease, Parkinson's disease, rheumatoid arthritis, and depression.

[a] P values for differences between effect estimates for the subgroups. $P < 0.05$ was statistically significant and marked bold.

Ethics approval and consent to participate

This study has got approval from the Chinese People's Liberation Army General Hospital Ethical Committee, and informed consent has been obtained from all participants.

Consent for publication

All authors have reviewed the final submitted manuscript and have consented to publication.

Table 4
The ORs and 95%CIs of poor sleep quality at 75th and 95th percentiles of outdoor ALAN against the threshold additionally adjusted for other confounders.

Model	threshold	75th	95th	OR (95%CI)	
				75th vs. threshold	95th vs. threshold
Model 1	49.13	85.29	119.04	1.15 (0.97, 1.36)	1.45 (1.17, 1.78)
Model 2	49.66	85.29	119.04	1.15 (0.98, 1.36)	1.43 (1.16, 1.77)
Model 3	52.36	85.29	119.04	1.12 (0.95, 1.32)	1.44 (1.16, 1.79)

Note: Model 1 was additionally adjusted for race; Model 2 was additionally adjusted for season of the investigation; Model 3 was additionally adjusted for air pollutants ($PM_{2.5}$ and NO_2).

Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2023.114218.

References

Al Lawati, N.M., Patel, S.R., Ayas, N.T., 2009. Epidemiology, risk factors, and consequences of obstructive sleep apnea and short sleep duration. Prog. Cardiovasc. Dis. 51, 285–293.
Alexander, M., Ray, M.A., Hébert, J.R., Youngstedt, S.D., Zhang, H., Steck, S.E., et al., 2016. The national veteran sleep disorder study: descriptive epidemiology and secular trends, 2000-2010. Sleep 39, 1399–1410.
Ph, D.A., Rhee, J.U., Haynes, P., Chakravorty, S., Patterson, F., Killgore, W.D.S., et al., 2021. Smoke at night and sleep worse? The associations between cigarette smoking with insomnia severity and sleep duration. Sleep Health 7, 177–182.
Babu Henry Samuel, I., Breneman, C.B., Chun, T., Hamedi, A., Murphy, R., Barrett, J.P., 2021. Compounding effects of traumatic brain injury, military status, and other factors on Pittsburgh sleep quality index: a meta-analysis. Mil. Med. 187, e589–e597.
Becker, P.M., 2006. Insomnia: prevalence, impact, pathogenesis, differential diagnosis, and evaluation. Psychiatr. Clin. 29, 855–870 (abstract vii).
Becker, N.B., Jesus, S.N., João, K., Viseu, J.N., Martins, R.I.S., 2017. Depression and sleep quality in older adults: a meta-analysis. Psychol. Health Med. 22, 889–895.
Bedrosian, T.A., Nelson, R.J., 2013. Influence of the modern light environment on mood. Mol. Psychiatr. 18, 751–757.
Bedrosian, T.A., Nelson, R.J., 2017. Timing of light exposure affects mood and brain circuits. Transl. Psychiatry 7, e1017.
Blume, C., Garbazza, C., Spitschan, M., 2019. Effects of light on human circadian rhythms, sleep and mood. Somnologie 23, 147–156.
Bohnert, K.M., Ilgen, M.A., Louzon, S., McCarthy, J.F., Katz, I.R., 2017. Substance use disorders and the risk of suicide mortality among men and women in the US Veterans Health Administration. Addiction 112, 1193–1201.
Bokenberger, K., Ström, P., Dahl Aslan, A.K., Johansson, A.L., Gatz, M., Pedersen, N.L., et al., 2017. Association between sleep characteristics and incident dementia accounting for baseline cognitive status: a prospective population-based study. J Gerontol A Biol Sci Med Sci 72, 134–139.
Bramoweth, A.D., Germain, A., 2013. Deployment-related insomnia in military personnel and veterans. Curr. Psychiatr. Rep. 15, 401.
Breen, D.P., Vuono, R., Nawarathna, U., Fisher, K., Shneerson, J.M., Reddy, A.B., et al., 2014. Sleep and circadian rhythm regulation in early Parkinson disease. JAMA Neurol. 71, 589–595.
Buysse, D.J., Reynolds, C.F., Monk, T.H., Berman, S.R., Kupfer, D.J., 1989. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatr. Res. 28, 193–213.
Byrne, S.P., McCarthy, E., DeViva, J.C., Southwick, S.M., Pietrzak, R.H., 2021. Prevalence, risk correlates, and health comorbidities of insomnia in US military veterans: results from the 2019-2020 National Health and Resilience in Veterans Study. J. Clin. Sleep Med. 17, 1267–1277.
Castañeda, T.R., de Prado, B.M., Prieto, D., Mora, F., 2004. Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light. J. Pineal Res. 36, 177–185.
Chan, W.S., Levsen, M.P., McCrae, C.S., 2018. A meta-analysis of associations between obesity and insomnia diagnosis and symptoms. Sleep Med. Rev. 40, 170–182.

Chen, G., Li, S., Knibbs, L.D., Hamm, N.A.S., Cao, W., Li, T., et al., 2018. A machine learning method to estimate PM2.5 concentrations across China with remote sensing, meteorological and land use information. Sci. Total Environ. 636, 52–60.
Chen, Y., Tan, J., Liu, Y., Dong, G.H., Yang, B.Y., Li, N., et al., 2022. Long-term exposure to outdoor light at night and mild cognitive impairment: a nationwide study in Chinese veterans. Sci. Total Environ. 847, 157441.
Chung, J.Y., Honda, Y., Hong, Y.C., Pan, X.C., Guo, Y.L., Kim, H., 2009. Ambient temperature and mortality: an international study in four capital cities of East Asia. Sci. Total Environ. 408, 390–396.
Database DMSP, Accessed 27 May 2021.
Durrant, J., Green, M.P., Jones, T.M., 2020. Dim artificial light at night reduces the cellular immune response of the black field cricket, Teleogryllus commodus. Insect Sci. 27, 571–582.
Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C.C., Elvidge, C.D., Baugh, K., et al., 2016. The new world atlas of artificial night sky brightness. Sci. Adv. 2, e1600377.
Gilbert, K.S., Kark, S.M., Gehrman, P., Bogdanova, Y., 2015. Sleep disturbances, TBI and PTSD: implications for treatment and recovery. Clin. Psychol. Rev. 40, 195–212.
Gulia, K.K., Kumar, V.M., 2018. Sleep disorders in the elderly: a growing challenge. Psychogeriatrics 18, 155–165.
Hernández, B., Scarlett, S., Moriarty, F., Romero-Ortuno, R., Kenny, R.A., Reilly, R., 2022. Investigation of the role of sleep and physical activity for chronic disease prevalence and incidence in older Irish adults. BMC Publ. Health 22, 1711.
Ho, R.T., Fong, T.C., 2014. Factor structure of the Chinese version of the Pittsburgh sleep quality index in breast cancer patients. Sleep Med. 15, 565–569.
Hsu, F.-C., Baugh, K.E., Ghosh, T., Zhizhin, M., Elvidge, C.D., 2015. DMSP-OLS radiance calibrated Nighttime lights time series with intercalibration. Rem. Sens. 7, 1855–1876.
Hu, Y., Zhang, Y., 2020. Global Nighttime light change from 1992 to 2017: brighter and more uniform. Sustainability 12, 4905.
Hu, C.P., Zhao, Y.H., Zhao, X.D., Zhu, B.G., Qin, H.Y., 2021. Correlation between sleep characteristics and cognitive decline in the elderly people: a cross-sectional study in China. Int. J. Clin. Pract. 75, e14225.
Hu, K., Li, W., Zhang, Y., Chen, H., Bai, C., Yang, Z., et al., 2022. Association between outdoor artificial light at night and sleep duration among older adults in China: a cross-sectional study. Environ. Res., 113343
Huss, A., van Wel, L., Bogaards, L., Vrijkotte, T., Wolf, L., Hoek, G., et al., 2019. Shedding some light in the dark-A comparison of personal measurements with satellite-based estimates of exposure to light at night among children in The Netherlands. Environ. Health Perspect. 127, 67001.
Koo, Y.S., Song, J.Y., Joo, E.Y., Lee, H.J., Lee, E., Lee, S.K., et al., 2016. Outdoor artificial light at night, obesity, and sleep health: cross-sectional analysis in the KoGES study. Chronobiol. Int. 33, 301–314.
Lai, K.Y., Sarkar, C., Ni, M.Y., Gallacher, J., Webster, C., 2020. Exposure to light at night (LAN) and risk of obesity: a systematic review and meta-analysis of observational studies. Environ. Res. 187, 109637.
Lai, K.Y., Sarkar, C., Ni, M.Y., Cheung, L.W.T., Gallacher, J., Webster, C., 2021. Exposure to light at night (LAN) and risk of breast cancer: a systematic review and meta-analysis. Sci. Total Environ. 762, 143159.
Li, S., Guo, Y., Williams, G., 2016. Acute impact of hourly ambient air pollution on preterm birth. Environ. Health Perspect. 124, 1623–1629.
Li, M., Wang, N., Dupre, M.E., 2022. Association between the self-reported duration and quality of sleep and cognitive function among middle-aged and older adults in China. J. Affect. Disord. 304, 20–27.
Liu, X., Tang, M., Hu, L., 1996. Reliability and validity of the Pittsburgh sleep quality index. Chinese journal of psychiatry 29, 103–107.
Liu, R.Q., Qian, Z., Trevathan, E., Chang, J.J., Zelicoff, A., Hao, Y.T., et al., 2016. Poor sleep quality associated with high risk of hypertension and elevated blood pressure in China: results from a large population-based study. Hypertens. Res. 39, 54–59.
Lou, P., Zhang, P., Zhang, L., Chen, P., Chang, G., Zhang, N., et al., 2015. Effects of sleep duration and sleep quality on prevalence of type 2 diabetes mellitus: a 5-year follow-up study in China. Diabetes Res. Clin. Pract. 109, 178–184.
Lu, L., Wang, S.B., Rao, W., Zhang, Q., Ungvari, G.S., Ng, C.H., et al., 2019. The prevalence of sleep disturbances and sleep quality in older Chinese adults: a comprehensive meta-analysis. Behav. Sleep Med. 17, 683–697.
Maglione, J.E., Ancoli-Israel, S., Peters, K.W., Paudel, M.L., Yaffe, K., Ensrud, K.E., et al., 2014. Subjective and objective sleep disturbance and longitudinal risk of depression in a cohort of older women. Sleep 37, 1179–1187.
Mai, E., Buysse, D.J., 2008. Insomnia: prevalence, impact, pathogenesis, differential diagnosis, and evaluation. Sleep Med Clin 3, 167–174.
Martin, J.L., Fiorentino, L., Jouldjian, S., Josephson, K.R., Alessi, C.A., 2010. Sleep quality in residents of assisted living facilities: effect on quality of life, functional status, and depression. J. Am. Geriatr. Soc. 58, 829–836.
McIsaac, M.A., Sanders, E., Kuester, T., Aronson, K.J., Kyba, C.C.M., 2021. The impact of image resolution on power, bias, and confounding: a simulation study of ambient light at night exposure. Environ Epidemiol 5, e145.
Min, J.Y., Min, K.B., 2018. Outdoor light at night and the prevalence of depressive symptoms and suicidal behaviors: a cross-sectional study in a nationally representative sample of Korean adults. J. Affect. Disord. 227, 199–205.
Morin, C.M., Drake, C.L., Harvey, A.G., Krystal, A.D., Manber, R., Riemann, D., et al., 2015. Insomnia disorder. Nat. Rev. Dis. Prim. 1, 15026.
Motivala, S.J., Levin, M.J., Oxman, M.N., Irwin, M.R., 2006. Impairments in health functioning and sleep quality in older adults with a history of depression. J. Am. Geriatr. Soc. 54, 1184–1191.
National Bureau of Statistics of China, 2022.
Navara, K.J., Nelson, R.J., 2007. The dark side of light at night: physiological, epidemiological, and ecological consequences. J. Pineal Res. 43, 215–224.
Obayashi, K., Saeki, K., Iwamoto, J., Ikada, Y., Kurumatani, N., 2013. Exposure to light at night and risk of depression in the elderly. J. Affect. Disord. 151, 331–336.
Ohayon, M.M., Milesi, C., 2016. Artificial outdoor Nighttime lights associate with altered sleep behavior in the American general population. Sleep 39, 1311–1320.
Olejniczak, P.W., Fisch, B.J., 2003. Sleep disorders. Med. Clin. 87, 803–833.
Olenick, M., Flowers, M., Diaz, V.J., 2015. US veterans and their unique issues: enhancing health care professional awareness. Adv. Med. Educ. Pract. 6, 635–639.
Paksarian, D., Rudolph, K.E., Stapp, E.K., Dunster, G.P., He, J., Mennitt, D., et al., 2020. Association of outdoor artificial light at night with mental disorders and sleep patterns among US adolescents. JAMA Psychiatr. 77, 1266–1275.
Park, Y.M., White, A.J., Jackson, C.L., Weinberg, C.R., Sandler, D.P., 2019. Association of exposure to artificial light at night while sleeping with risk of obesity in women. JAMA Intern. Med. 179, 1061–1071.
Pengo, M.F., Won, C.H., Bourjeily, G., 2018. Sleep in women across the life span. Chest 154, 196–206.
Qiao, C., Zhang, H., Song, Q., Wang, X., Wang, X., Yao, Y., 2021. Sleep disturbances are associated with depressive symptoms in a Chinese population: the rugao longevity and aging cohort. Front. Psychiatr. 12, 731371.
Radloff, L.S., 1977. The CES-D scale: a self-report depression scale for research in the general population. Appl. Psychol. Meas. 1, 385–401.
Raza, Z., Hussain, S.F., Ftouni, S., Spitz, G., Caplin, N., Foster, R.G., et al., 2021. Dementia in military and veteran populations: a review of risk factors-traumatic brain injury, post-traumatic stress disorder, deployment, and sleep. Mil Med Res 8, 55.
Romeo, S., Viaggi, C., Di Camillo, D., Willis, A.W., Lozzi, L., Rocchi, C., et al., 2013. Bright light exposure reduces TH-positive dopamine neurons: implications of light pollution in Parkinson's disease epidemiology. Sci. Rep. 3, 1395.
Roth, T., 2007. Insomnia: definition, prevalence, etiology, and consequences. J. Clin. Sleep Med. 3, S7–S10.
Saconi, B., Polomano, R.C., Compton, P.C., McPhillips, M.V., Kuna, S.T., Sawyer, A.M., 2021. The influence of sleep disturbances and sleep disorders on pain outcomes among veterans: a systematic scoping review. Sleep Med. Rev. 56, 101411.
Samuelsson, L.B., Bovbjerg, D.H., Roecklein, K.A., Hall, M.H., 2018. Sleep and circadian disruption and incident breast cancer risk: an evidence-based and theoretical review. Neurosci. Biobehav. Rev. 84, 35–48.
Shi, L., Chen, S.J., Ma, M.Y., Bao, Y.P., Han, Y., Wang, Y.M., et al., 2018. Sleep disturbances increase the risk of dementia: a systematic review and meta-analysis. Sleep Med. Rev. 40, 4–16.
Sigurdardottir, L.G., Markt, S.C., Rider, J.R., Haneuse, S., Fall, K., Schernhammer, E.S., et al., 2015. Urinary melatonin levels, sleep disruption, and risk of prostate cancer in elderly men. Eur. Urol. 67, 191–194.
Stebelova, K., Roska, J., Zeman, M., 2020. Impact of dim light at night on urinary 6-sulphatoxymelatonin concentrations and sleep in healthy humans. Int. J. Mol. Sci. 21.
Sun, X., Zheng, B., Lv, J., Guo, Y., Bian, Z., Yang, L., et al., 2018. Sleep behavior and depression: findings from the China Kadoorie Biobank of 0.5 million Chinese adults. J. Affect. Disord. 229, 120–124.
Sun, S., Cao, W., Ge, Y., Ran, J., Sun, F., Zeng, Q., et al., 2021. Outdoor light at night and risk of coronary heart disease among older adults: a prospective cohort study. Eur. Heart J. 42, 822–830.
Suzuki, E., Yorifuji, T., Ueshima, K., Takao, S., Sugiyama, M., Ohta, T., et al., 2009. Sleep duration, sleep quality and cardiovascular disease mortality among the elderly: a population-based cohort study. Prev. Med. 49, 135–141.
Tan, J., Li, N., Gao, J., Guo, Y., Hu, W., Yang, J., et al., 2014. Construction of the Chinese Veteran Clinical Research (CVCR) platform for the assessment of non-communicable diseases. Chin. Med. J. 127, 448–456.
Tan, J.P., Li, N., Cui, B., Wang, L.N., Zhao, Y.M., Zhang, B.H., et al., 2016. Characteristics of participants' and caregivers' influence on non-response in a cross-sectional study of dementia in an older population. Arch. Gerontol. Geriatr. 62, 143–151.
Tan, J., Li, N., Wang, X., Chen, G., Yan, L., Wang, L., et al., 2021. Associations of particulate matter with dementia and mild cognitive impairment in China: a multicenter cross-sectional study. Innovation 2, 100147.
Tobaldini, E., Fiorelli, E.M., Solbiati, M., Costantino, G., Nobili, L., Montano, N., 2019. Short sleep duration and cardiometabolic risk: from pathophysiology to clinical evidence. Nat. Rev. Cardiol. 16, 213–224.
Toomey, R., Kang, H.K., Karlinsky, J., Baker, D.G., Vasterling, J.J., Alpern, R., et al., 2007. Mental health of US Gulf War veterans 10 years after the war. Br. J. Psychiatry 190, 385–393.
Touitou, Y., Reinberg, A., Touitou, D., 2017. Association between light at night, melatonin secretion, sleep deprivation, and the internal clock: health impacts and mechanisms of circadian disruption. Life Sci. 173.
Tsuno, N., Besset, A., Ritchie, K., 2005. Sleep and depression. J. Clin. Psychiatry 66, 1254–1269.
Wang, H., Leng, J., Li, W., Wang, L., Zhang, C., Li, W., et al., 2017. Sleep duration and quality, and risk of gestational diabetes mellitus in pregnant Chinese women. Diabet. Med. 34, 44–50.
Whinnery, J., Jackson, N., Rattanaumpawan, P., Grandner, M.A., 2014. Short and long sleep duration associated with race/ethnicity, sociodemographics, and socioeconomic position. Sleep 37, 601–611.
Wu, W., Jiang, Y., Wang, N., Zhu, M., Liu, X., Jiang, F., et al., 2020. Sleep quality of Shanghai residents: population-based cross-sectional study. Qual. Life Res. 29, 1055–1064.
Xiao, Q., Gee, G., Jones, R.R., Jia, P., James, P., Hale, L., 2020. Cross-sectional association between outdoor artificial light at night and sleep duration in middle-to-older aged adults: the NIH-AARP Diet and Health Study. Environ. Res. 180, 108823.

Xie, Y., Jin, Z., Huang, H., Li, S., Dong, G., Liu, Y., et al., 2022. Outdoor light at night and autism spectrum disorder in Shanghai, China: a matched case-control study. Sci. Total Environ. 811, 152340.
Yu, W., Hu, W., Mengersen, K., Guo, Y., Pan, X., Connell, D., et al., 2011. Time course of temperature effects on cardiovascular mortality in Brisbane, Australia. Heart 97, 1089–1093.
Zeka, A., Zanobetti, A., Schwartz, J., 2006. Individual-level modifiers of the effects of particulate matter on daily mortality. Am. J. Epidemiol. 163, 849–859.
Zhan, Y., Luo, Y., Deng, X., Zhang, K., Zhang, M., Grieneisen, M.L., et al., 2018. Satellite-based estimates of daily NO(2) exposure in China using hybrid random forest and spatiotemporal kriging model. Environ. Sci. Technol. 52, 4180–4189.
Zhu, X., Hu, Z., Nie, Y., Zhu, T., Chiwanda Kaminga, A., Yu, Y., et al., 2020. The prevalence of poor sleep quality and associated risk factors among Chinese elderly adults in nursing homes: a cross-sectional study. PLoS One 15, e0232834.
Ziegler, A.K., Watson, H., Hegemann, A., Meitern, R., Canoine, V., Nilsson, J., et al., 2021. Exposure to artificial light at night alters innate immune response in wild great tit nestlings. J. Exp. Biol. 224.
Zielinski, M.R., Gibbons, A.J., 2022. Neuroinflammation, sleep, and circadian rhythms. Front. Cell. Infect. Microbiol. 12, 853096.

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Who benefits from green spaces? Surrounding greenness and incidence of cardiovascular disease in a population-based electronic medical records cohort in Madrid

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ABSTRACT

The objective was to study the association between surrounding greenness and the incidence of cardiovascular diseases (CVD) with a four years follow-up in almost half a million high CVD-risk women and men, as well as its differential effect by area-level deprivation in Madrid. We analyzed 2015–2018 primary healthcare electronic medical records for 437,513 high CVD risk individuals representing more than 95% of the population of that age range residing in Madrid. The outcome variable was any cardiovascular event. We measured surrounding residence greenness at 200 m, 300 m, 500 m, and 1000 m through the Normalized Difference Vegetation Index (NDVI). We assessed socioeconomic deprivation through a census-based deprivation index. We estimated the 4-year relative risk of CVD by an increase in 0.1 units of NDVI and then stratified the models by quintiles of deprivation (Q5 the most deprived).

We found that for every increase in 0.1 units of NDVI at 1000 m there was a 16% decrease in CVD risk (RR = 0.84 95% CI 0.75–0.94). CVD risk for the remaining distance exposures (at 200 m, 300 m, and 500 m) were none statistically significant. In general, the protective effect of green spaces was present in medium-deprivation areas and males, but the associations were inconsistent across deprivation levels. This study highlights the relevance of evaluating the interaction between physical and social urban components to further understand possible population prevention approaches for cardiovascular diseases. Future studies should focus on the mechanisms of context-specific interactions between social inequalities and green spaces' effects on health.

1. Background

Cardiovascular diseases (CVD) are the leading cause of death in developing and developed nations (Beaglehole and Bonita, 2008; Nichols et al., 2013), and the social, medical, and economic burden of CVD is likely to increase over the next decades worldwide (Beaglehole and Bonita, 2008). Using data from 2016 reported by the European Society of Cardiology, there were 54255 and 64078 annual deaths in Spain by CVD in men and women, respectively (Benjamin et al., 2019). The costs of CVD, both direct and indirect, as well as those of their prevention, represent and will continue to pose a challenge to public health, the economy, and world politics in the coming years (Smith, 2012). In fact, the World Heart Federation estimates that the global costs of CVD will increase from 863 to 1044 billion between 2010 and 2030

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(World Heart Federation, 2020).

In most countries, CVD prevention strategies focus on the risk at the individual level. However, in the last decades, there has been an increased interest in population prevention strategies targeting contextual factors and social determinants of health (Franco et al., 2013; Powell-Wiley et al., 2022; Rose, 1985). Cities and neighborhoods present unique opportunities to study population prevention approaches, as they are highly dense, and characterized by substantial man-made components and frequent social interactions (Franco et al., 2015; Ribeiro et al., 2022).

For instance, there is mounting evidence that green spaces are beneficial for the health of urban residents (Fong et al., 2018; James et al., 2015). Different theories suggest that green spaces improve and promote health through different pathways, such as reducing harm (mitigating exposures to heat, noise, and air pollution), relieving mental and physiologic stress, and promoting healthy behaviors such as physical activity (Markevych et al., 2017; Nieuwenhuijsen et al., 2017).

Previous research has explored the association of residential green spaces with cardiovascular health (Chow et al., 2009; James et al., 2015; Liu et al., 2022). There is evidence that a high amount of green spaces is associated with a decrease in cardiovascular mortality (Bauwelinck et al., 2021; Gascon et al., 2016), a lower hazard of CVD events (Tamosiunas et al., 2014), or a decrease in the prevalence and incidence of cardiovascular risk factors such as obesity (Astell-Burt et al., 2014) and high blood pressure (Bijnens et al., 2017). In fact, a recent meta-analysis showed that a 0.1 increase in the Normalized Difference Vegetation Index (NDVI) was associated with 2–13% lower odds of CVD mortality (OR: 0.97, 95% CI: 0.96–0.99) and heart disease mortality (OR: 0.98, 95% CI: 0.96–1.00), stroke incidence (OR: 0.97, 95% CI: 0.96–0.99), and coronary heart disease incidence (OR: 0.87, 95% CI: 0.83–0.91) (Liu et al., 2022).

However, it is less clear who benefits from green spaces and whether green spaces might narrow or widen inequities in CVD by neighborhood or individual socioeconomic position. One recent systematic review found that, for the most part, the effect of green spaces on CVD was stronger for high-SES populations (Liu et al., 2022), while a previous systematic review found that green spaces health-related effects (including CVD and other health outcomes such as birth weight or obesity) tended to be higher in low-SES populations (Rigolon et al., 2021).

There are potential explanations for these differences such as differential measurement and definitions of green spaces (Klompmaker et al., 2018) or socioeconomic position, potential selection bias in samples, interaction with age or gender, differences in health effects or the local context such as other urban and health policies, and the history of segregation in the cities. Indeed, new research is warranted to develop approaches for assessing CVD inequities (Bhatnagar, 2017; Powell-Wiley et al., 2022). In order to try to encompass some of these limitations, the objective was to study the association between the availability of green spaces and the incidence of cardiovascular diseases with a four years follow-up in almost half a million high CVD risk population in Madrid, Spain, as well as its differential effect by area-level deprivation.

2. Material and methods

2.1. Study design and setting

This is a retrospective cohort using data from Primary Health Care Electronic Medical Records (EMR) in Madrid, Spain, between 2015 and 2018. This study was conducted in the city of Madrid, within the Heart Healthy Hoods project (www.hhhproject.es) (Bilal et al., 2016). In 2015, Madrid had a population of 3.2 M residents and was divided into 21 districts and 128 neighborhoods. Within each neighborhood, there were small geographical administrative units of ~1,500 people, called census sections (N = 2,415), which we set as the ecological unit of analysis.

2.2. Study population

The Heart Healthy Hoods retrospective closed cohort was based on real-world data from primary care including baseline information from 1,384,503 (40–75 years old) residents, representing more than 95% of the population of that age range residing in the municipality of Madrid in 2015. The inclusion criteria included individuals that were (Bilal et al., 2016): (a) registered at one of the 128 primary health care centers in the Municipality of Madrid; (b) who lived in the Municipality of Madrid; (c) aged 40–75 years; (d) those free of cardiovascular disease (CVD) at baseline.

From this original sample, we excluded those with missing data on residential information (n = 43,477) and those who moved during the study period (n = 185,082). Then, we selected those at high risk of developing CVD, meaning those that were either: a) over 65 years old, or b) diagnosed with dyslipidemia, diabetes, or hypertension as defined by the International Classification of Primary Care (ICPC-2) (Verbeke et al., 2006) leaving a final analytical sample of 437,513 individuals. A comparison between the initial and the analytical sample is presented in Supplementary file 1.

2.3. Outcome variables

The outcome variable was the incident cardiovascular event, including both cardiac ischemic events and cerebrovascular events. Diagnoses were collected from annual data extraction from the Electronic Medical Records for each participant (passive follow-up) (Williams, 2021). For cardiac ischemic events, we used the International Classification of Primary Care codes k74, k75, and k76, and for cerebrovascular events, ICPC codes k89, k90, and k91.

2.4. Exposure variable: greenness

We measured residential outdoor surrounding greenness by obtaining the normalized difference vegetation index (NDVI), an indicator of green spaces and greenness based on land surface reflectance of visible (red) and near-infrared parts of the spectrum (Weier and Herring, 2000). It ranges between −1 and 1 with higher numbers indicating more photosynthetically active greenness. We generated our NDVI map using the image obtained on 2015 by the Landsat 8 OLI (Operational Land Imager) and TIRS (Thermal Infrared Sensor) at 30 m × 30 m resolution. Surrounding greenness was abstracted as the average of NDVI in buffers of 200 m, 300 m, 500 m, and 1000 m around the centroid of the census section of residence of each participant. We decided to take the centroid of the census section as the specific address for each individual was not available. As sensitivity analysis, we also used the mean NDVI of the census section (Supplementary files 2 and 3).

2.5. Area-level deprivation

We used a socioeconomic deprivation index at the census-section level using data from the 2011 Spanish census (Cebrecos et al., 2018; Duque et al., 2021). Area-level deprivation in Madrid has been previously associated with health outcomes, such as higher mortality risk or, more recently, COVID-19 incidence (Borrell et al., 2010; Gullón et al., 2022; Rodríguez-Fonseca et al., 2013). This index includes six indicators in four dimensions that were selected by a principal component analysis: occupation (manual workers and occasional salaried workers), unemployment, education (incomplete compulsory education, both in the general population and in the population aged 16–29), and lack of internet access. The index was constructed by extracting the first component of the principal components analysis, standardized to a mean of 0 and a standard deviation of 1. Higher values of this index mean higher socioeconomic deprivation (or low socioeconomic status). A detailed explanation of how the index was constructed is described elsewhere (Duque et al., 2021). We compared the area-level deprivation

index with two additional area-level socioeconomic indicators available for the city of Madrid: (1) mean household income at the census section level for 2015, available at the Spanish Ministry of Finances, and (2) a socioeconomic status index that was calculated for all census sections in Madrid in 2014 (Gullón et al., 2017). We found a high negative correlation (0.8) between the area-level deprivation index and both indicators (supplementary file 4).

3. Covariates

We included demographics including age, sex, country of origin, and census-section walkability. We measured census-section walkability using a composite index of four indicators (Residential Density, Population Density, Retail Destinations, and Street Connectivity); higher values of this index represent higher census-section walkability; similar walkability indexes have been associated with lower cardiometabolic risk (Chandrabose et al., 2019). Details of the index are explained elsewhere (Gullón et al., 2017).

3.1. Statistical analyses

Descriptive analysis used baseline individual- and neighborhood-level variables. We estimated the relative risk of the 4-year risk by an increase in 0.1 units of NDVI using multilevel Poisson regression models that included a random intercept at the census-section level to account for the non-independency of the independent variables at the census-section level. Thus, our dependent variable in these models was the presence of a CVD event in the follow-up, and the main independent variables were the NDVI at different buffers. The observation started at the Heart Healthy Hoods retrospective cohort baseline (2015) and included CVD events - cardiac ischemic or cerebrovascular events - through December 31, 2018. Individuals were censored at the time of their event, death, or the end of their follow-up. We first tried to perform Cox survival models, but the models did not meet the proportional hazards assumption so we decided on the Poisson models.

First, we computed unadjusted models (Model 0). Then (Model 1) models were adjusted by sex, age, country of origin, and neighborhood walkability. After this, we adjusted by area-level deprivation (Model 2). We then stratified Model 1 by quintiles of deprivation (being Q5 the most deprived) to study the differential effect of the association between green spaces and cardiovascular diseases. The effect of each buffer was estimated in a separate model. To test for statistical interaction between NDVI and deprivation, we performed a nested likelihood ratio test. All models are presented overall and stratified by men and women. Analyses were conducted in R version 4.1.3.

3.2. Ethics

This study was carried out under the umbrella of the Heart Healthy Hoods study and following the Declaration of Helsinki guidelines. The study received IRB approval from the Ethics Research Committee of the Madrid Health Care System on May 12th, 2015, and the Ethics Research Committee of Hospital la Princesa on September 12th, 2022.

4. Results

The analytical sample comprised 437,513 individuals. Of this, 54.1% were female and 93.4% were born in Spain. Regarding cardiovascular risk factors, 81.7%, 20.6%, and 46.2% had high cholesterol, diabetes, and hypertension, respectively – with prevalence rates being slightly higher among individuals living in the most deprived census sections (Table 1). Mean NDVI values were higher in the medium-deprived (Q2, Q3) areas, and walkability increased while deprivation increased in the descriptive analysis. Supplementary file 1 shows the comparison between the initial and the analytical population, where the analytical sample had higher age and prevalence of risk factors due to the selection criteria, but no differences in exposure variables. Fig. 1 shows the spatial distribution of neighborhood deprivation and the NDVI. Neighobrhood deprivation follows a general North-to-South distribution, where the census sections with a highest deprivation index are located in the south of Madrid. NDVI values were higher in the North-West area of Madrid, with some spots in the city center corresponding to the major parks of the city.

Table 2 shows the results of the association between the availability of surrounding greenness and the 4-year incidence of cardiovascular disease. In the unadjusted models (model 0) we found that a greater availability of surrounding greenness was associated with an increased 4-year risk of cardiovascular disease; for instance, an increase in 0.1 units of NDVI in the 200 m buffer was associated with a 10% increase in the 4-year risk of cardiovascular disease (95% CI 1.04–1.17). After adjusting by sex, age, country of origin, and neighborhood walkability, we found no significant association between greenness and 4-year incidence of cardiovascular disease except for males in the longest buffer, where we found a protective effect of surrounding greenness on cardiovascular disease (RR = 0.85; 95% CI 0.76–0.96). In model 2 (adjusted also by area-level deprivation) we found some protection both in the overall and males in the longest buffers (1000 m); a 0.1 increase in the mean NDVI in the 1000 m buffer was associated with a 9% decrease in the risk of 4-year cardiovascular disease (95% CI 0.83–0.98).

Overall, in the stratified models by area-level deprivation (Fig. 2), we found a significant interaction between NDVI and area-level deprivation for all buffers (p-values of the likelihood ratio test were 0.013, 0.011, 0.014 and 0.008 respectively for 200 m, 300 m, 500 m, and 1000 m).

Table 1
Individual characteristics of the sample (N = 437,513).

Individual characteristics[a]	Total		Deprivation quintiles									
			Q1 (least deprived)		Q2		Q3		Q4		Q5 (most deprived)	
	N	%	N	%	N	%	N	%	N	%	N	%
Sex (female)	236645	54.10	65550	54.70	44920	53.90	45627	54.80	45120	53.70	35428	52.90
Country of birth (Spain)	408665	93.40	113298	94.50	78021	93.60	77953	93.60	77751	92.50	61642	92.10
Age (years), mean SD	60.73	9.66	61.29	9.37	60.89	9.75	61.24	9.75	60.21	9.82	59.51	9.93
High Cholesterol	357336	81.70	99049	82.60	67293	80.70	68209	81.90	67844	80.80	54941	82.10
Diabetes	90078	20.60	20555	17.10	16682	20.00	17518	21.00	19444	23.10	15879	23.70
Hypertension	202157	46.20	54011	45.00	38211	45.80	39920	48.00	39386	46.90	30629	45.70
NDVI 200m, mean SD	0.096	0.037	0.096	0.042	0.091	0.039	0.106	0.034	0.095	0.030	0.093	0.032
NDVI 300m, mean SD	0.099	0.034	0.098	0.038	0.094	0.037	0.109	0.031	0.098	0.029	0.097	0.030
NDVI 500m, mean SD	0.102	0.031	0.100	0.038	0.097	0.033	0.114	0.028	0.102	0.026	0.101	0.025
NDVI 1000m, mean SD	0.108	0.027	0.104	0.029	0.102	0.031	0.120	0.025	0.109	0.019	0.104	0.017
Walkability Index, mean SD	−0.002	2.792	−0.437	3.010	0.300	2.887	−0.046	2.688	0.173	2.504	0.242	2.630

SD: standard deviation.
[a] Reported as N and % unless specified.

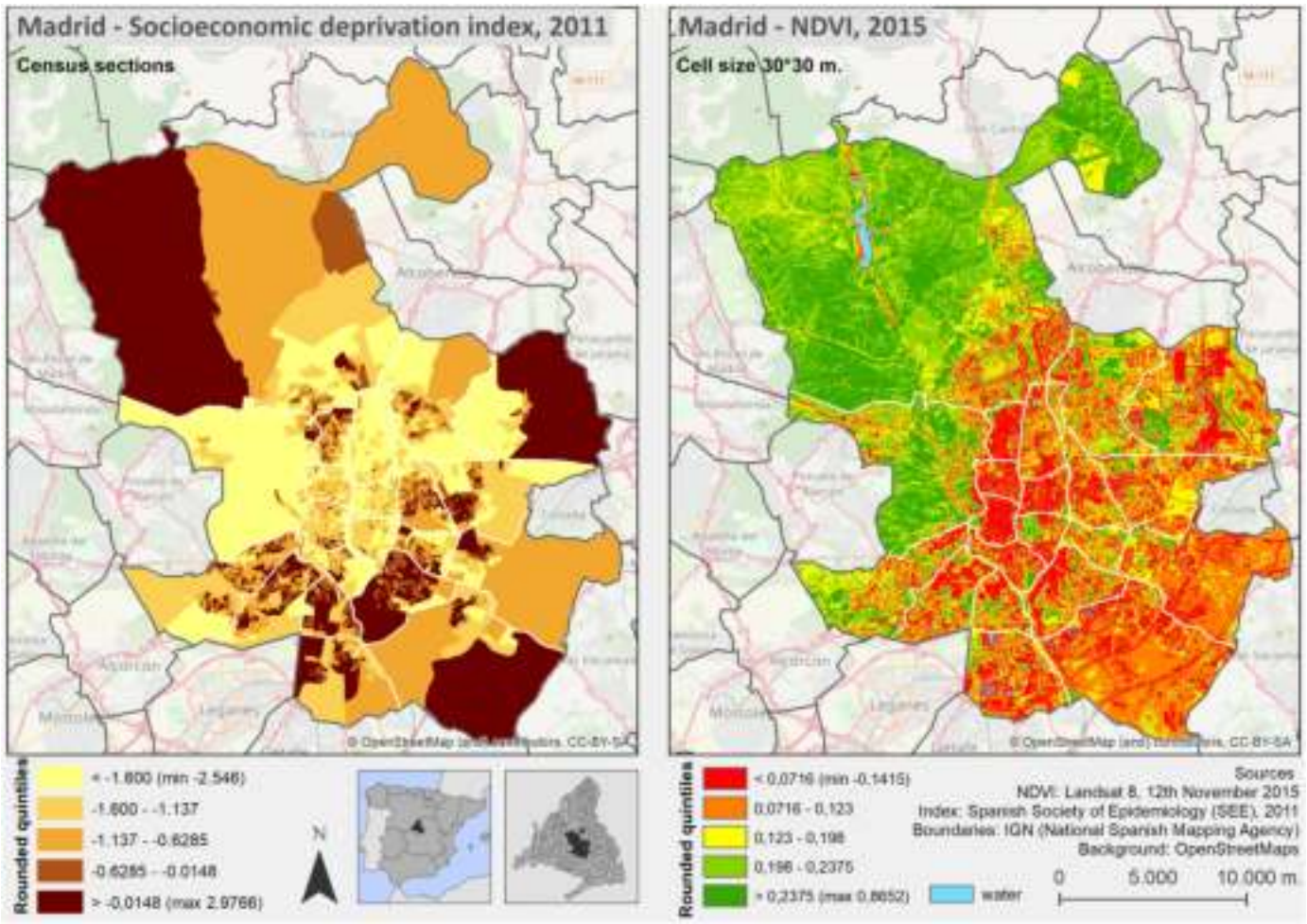

Fig. 1. Spatial distribution of the deprivation index (left panel) and the Normalized Difference Vegetation Index (right panel) in the city of Madrid.

Table 2
Association between the availability of surrounding greenness and 4-year incidence of cardiovascular disease (Madrid, 2015–2018).

Overall	Model 0		Model 1		Model 2	
	RR	95% CI	RR	95% CI	RR	95% CI
200 m	**1.11**	**1.04–1.17**	1.00	0.93–1.07	0.99	0.92–1.06
300 m	**1.10**	**1.04–1.17**	0.97	0.90–1.05	0.96	0.89–1.04
500 m	**1.11**	**1.04–1.19**	0.97	0.89–1.05	0.95	0.88–1.03
1000 m	1.07	0.99–1.16	0.92	0.85–1.01	**0.91**	**0.83–0.98**
Females	RR	95% CI	RR	95% CI	RR	95% CI
200 m	1.07	0.98–1.17	0.99	0.88–1.11	0.98	0.87–1.10
300 m	1.07	0.97–1.18	0.99	0.88–1.11	0.97	0.86–1.10
500 m	1.11	0.99–1.23	1.02	0.90–1.16	1.00	0.88–1.14
1000 m	**1.15**	**1.01–1.30**	1.04	0.91–1.19	1.02	0.89–1.17
Males	RR	95% CI	RR	95% CI	RR	95% CI
200 m	**1.12**	**1.04–1.21**	1.00	0.91–1.10	0.99	0.90–1.09
300 m	**1.11**	**1.02–1.20**	0.97	0.87–1.07	0.95	0.86–1.05
500 m	1.09	0.99–1.19	0.93	0.84–1.04	0.92	0.83–1.02
1000 m	1.01	0.91–1.12	**0.85**	**0.76–0.96**	**0.84**	**0.75–0.94**

There was an association between increased availability of greenness at 300 m, 500 m, and 1000 m and a reduction of CVD incidence for those living in the Q3 of area-level deprivation (RR = 0.80, RR = 0.76, and RR = 0.73 respectively). In males, we observed a similar pattern, where the largest buffers (300 m, 500 m, and 1000 m) had a protective effect only in medium-deprived areas (Q2 and Q3 in the case of males). In females, associations were weaker, and there was only a significant protective effect in the 1000 m for Q3 of deprivation (RR = 0.71; 95% CI 0.52–0.98). In the sensitivity analysis using census section mean NDVI as greenness exposure, we found no statistically significant associations (Supplementary file 2); in the stratified models, we found an increased risk of CVD for Q4 of deprivation (Supplementary file 3).

5. Discussion

This study showed a weak association between the availability of surrounding greenness and a 4-year risk reduction in cardiovascular disease in a population-based retrospective cohort of high-risk CVD individuals in Madrid. We found that the associations were inconsistent across different buffer sizes and deprivation levels. In general, the association tended to be stronger in larger buffers for both male participants and those living in medium-deprivation areas.

Previous longitudinal studies have found an association between the availability of green spaces or surrounding greenness and a reduction of CVD risk (Gascon et al., 2016; Liu et al., 2022), both in terms of CVD mortality (Klompmaker et al., 2021) and CVD incidence (Astell-Burt et al., 2021; Dalton and Jones, 2020), even in a similar population of high-risk individuals (Astell-Burt et al., 2021). In fact, a recent meta-analysis found that a 0.1 increase in NDVI was significantly associated with 2–3% lower odds of stroke incidence or prevalence (OR: 0.98, 95% CI: 0.96–0.99) (Liu et al., 2022), similar with our findings of 4-year incidence of CVD in the larger buffers. Potential mechanisms include (Liu et al., 2022; Markevych et al., 2017; Nieuwenhuijsen et al., 2017): (a) mitigating harmful environmental exposure to heat, noise, or

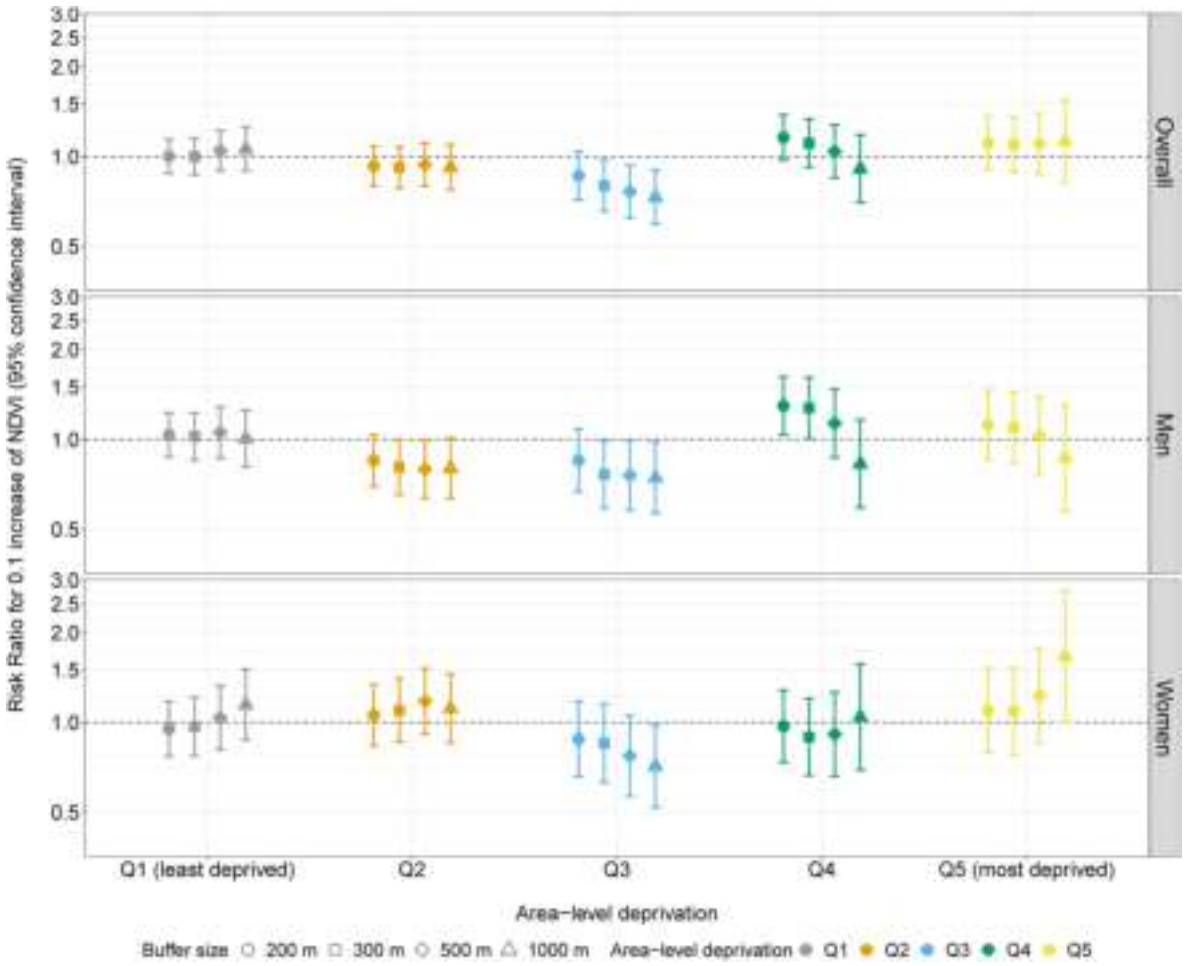

Fig. 2. Association between the availability of surrounding greenness and 4-year incidence of cardiovascular disease stratified by area-level deprivation.

air pollution; (b) promoting physical activity; (c) increasing social capital and social cohesion; and (d) reducing physiological stress. Despite previous studies that have explored these potential mechanisms through mediation analysis (Orioli et al., 2019), additional research is necessary to clarify these potential mechanisms.

Interestingly, we found that our crude models showed an inverse association between greenness and 4-year incidence of CVD (higher greenness associated with a higher risk of CVD); however, after adjusting by potential confounders in Models 1 and 2, we found that higher NDVI was associated with a decrease in 4-year incidence of CVD; suggesting a strong confusion of our potential confounders, especially after adjusting by neighborhood walkability. Indeed, neighborhood walkability is inversely associated with green spaces (Shuvo et al., 2021) and it is a known determinant of cardiovascular disease and its risk factors (Chandrabose et al., 2019).

Our association was stronger as the size of the buffer increased. Indeed, in the full models (without the stratification by area-level deprivation) the association was only present in the 1000 m buffers. Yet, previous studies have found that, in general, the protective effect of surrounding greenness on cardiovascular disease tends to be higher with smaller buffers (up to 500 m) (Liu et al., 2022). Other studies did not find significant differences when using a range of buffer sizes (Fong et al., 2018). A possible explanation might be that different causal pathways are more important for each buffer size. For example, smaller buffer sizes might be more relevant for visual access and stress reduction (Larkin and Hystad, 2019) or for reducing exposure to noise and air pollution (Markevych et al., 2017). On the other hand, a systematic review found that the association between green spaces and objectively-measured physical activity was stronger for larger buffers (Bancroft et al., 2015). This fact opens research questions on how in different contexts there might be more relevant pathways than in other contexts. Another potential explanation is that we used the centroid of the census section as the starting point for the buffers, so smaller buffers could be more biased than larger buffers as smaller buffers (especially in the bigger census sections where residents might not live around the centroid) might not represent the current exposure to residential greenness and move our associations toward the null; on the contrary, larger buffers starting at the centroid of the census section might be more likely to encompass individual exposure.

We found that the associations were stronger for men. Some other studies have found that the protective effect of green spaces on CVD is higher for men than women (Crouse et al., 2017; Richardson and Mitchell, 2010); however, results are inconsistent in this matter (Bolte et al., 2019), as it might depend on specific CVD outcomes (Fernández Núñez et al., 2022). A potential explanation is that daily behaviors and gender roles such as caring responsibilities, gender violence, and safety change the exposure time and use of green spaces (Cohen et al., 2019). For example, a study in Madrid using systematic social observation showed that the proportion of men and women visiting parks (as one of the most frequent forms of green spaces in cities) was similar; however, men were more frequently observed performing physical activity of all intensities than women (Fontán-Vela et al., 2021a). An alternative explanation is that associations in men are easier to find as they have a higher base risk for CVD (Gullón et al., 2020) and that women suffer from underdiagnosis of CVD outcomes (Vogel et al., 2021).

We also found that participants living in medium deprivation areas

were the most benefited from surrounding greenness. Two recent systematic reviews have found mixed results on how individual-level or area-level SES might modify the association between green spaces and cardiovascular disease (Liu et al., 2022; Rigolon et al., 2021). The distribution of green spaces, the use of these spaces, the vulnerability of participants in the study, or the differences in study design might explain these differences between studies and contexts.

In Madrid, as shown in our descriptive analysis, it seems that the distribution of green spaces follows an inverse U-shape, and medium-deprivation individuals might be more exposed to green spaces due to higher accessibility, which can partially explain our results; however, this would suggest that the effects of greenness on CVD is not linear, which is not likely based on previous studies (Pereira et al., 2012). However, the use of green spaces in Madrid (at least, the use of parks (Fontán-Vela et al., 2021b)) is lower in the most deprived areas (Fontán-Vela et al., 2021a). Residents of these neighborhoods identified several barriers to green space use, such as maintenance and area perception, work constraints and time available, insecurity and crime, and the availability of organized activities (Fontán-Vela et al., 2021a). Research has found that the quality and maintenance of green spaces might add interesting information for understanding the use of green spaces by different socioeconomic profiles (Hoffimann et al., 2017), a matter of environmental injustice (Anguelovski et al., 2022).

Future research should explore how the different mechanisms (harm reduction, physical activity, etc) affect health inequalities, how green spaces might benefit the most-needed or the privileged residents, and identify the mechanisms behind the inequalities.

We are aware that this study has some limitations. First, we did not assess or measure the mechanisms behind the association through a mediation analysis, which could help us to understand why we have mixing results depending on the size of the buffer. Previous research has suggested some mechanisms, but more research in this field is needed. Second, the use of census section centroids to create the starting point of the buffers instead of individual residential addresses might lead to a misclassification bias in the exposure, especially for smaller buffers and larger census sections where residents might not live around the centroid. However, as we tested different buffers, we were able to do sensitivity analysis. Third, we did not have records of the past residence of the participants, and CVD is the result of multiple social and physical exposures throughout life (Powell-Wiley et al., 2022). Forthly, we were not able to include lifestyle data (smoking, alcohol) which could be confounders as being associated with deprivation and CVD (Huxley and Woodward, 2011; Ronksley et al., 2011; Stimpson et al., 2007); however, lifestyle data is not routinely collected in the Electronic Medical Records, and including them could induce a selection bias. Fifthly, area-level deprivation was available for 2011 with a significant difference to the other exposure data; however, there was a high correlation between the area-level deprivation index and other socioeconomic indicators available for 2014 and 2015 (supplementary file 4). Lastly, we have a follow-up of 4-years, which might not be enough to claim causality from a temporal perspective; however, on the one hand, including high-risk individuals potentiates the possibility of finding some results, and, on the other hand, this study is an improvement compared with most cross-sectional analyses (Fong et al., 2018). In addition, it opens the possibility of future studies using Electronic Medical Records in Madrid. Indeed, the use of Electronic Medical Records with mostly universal coverage opens the possibility of using real-world applications less susceptible to selection bias (Bilal et al., 2016).

6. Conclusions

In conclusion, by using Electronic Medical Records of a high-risk population in Madrid, we observed that those living in areas with greater availability (at 1000 m) of surrounding greenness had a reduced risk of 4-year incidence of cardiovascular disease. These associations were stronger for men and for those living in medium-deprivation areas. However, the inconsistency of the results across buffers and deprivation levels requires further research on the mechanisms and limitations.

This study highlighted the relevance of evaluating the interaction between relevant physical and social urban components as surrounding greenness and social inequailities to further understand population prevention approaches for cardiovascular diseases (Franco et al., 2015). Future studies should focus on the understanding of complex mechanisms of context-specific interactions between social inequalities and green spaces' effects on cardiovascular health.

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Author contributions

PG: Conceptualization, Methodology, Formal analysis, Writing-Original Draft, Funding acquisition. MFV: Conceptualization, Writing - Review & Editing, Validation. JD: Data Curation, Writing - Review & Editing. MN: Writing - Review & Editing, Methodology. DRR: Writing - Review & Editing, Methodology. FE: Software, Writing - Review & Editing, Visualization. MF: Writing - Review & Editing, Supervision, Funding acquisition.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijheh.2023.114221.

References

Anguelovski, I., Brand, A.L., Ranganathan, M., Hyra, D., 2022. Decolonizing the green city: from environmental privilege to emancipatory green justice. Environ. Justice 15, 1–11. https://doi.org/10.1089/env.2021.0014.

Astell-Burt, T., Feng, X., Kolt, G.S., 2014. Greener neighborhoods, slimmer people evidence from 246 920 Australians. Int. J. Obes. 38, 156–159. https://doi.org/10.1038/ijo.2013.64.

Astell-Burt, T., Navakatikyan, M.A., Walsan, R., Davis, W., Figtree, G., Arnolda, L., Feng, X., 2021. Green space and cardiovascular health in people with type 2 diabetes. Health Place 69, 102554. https://doi.org/10.1016/j.healthplace.2021.102554.

Bancroft, C., Joshi, S., Rundle, A., Hutson, M., Chong, C., Weiss, C.C., Genkinger, J., Neckerman, K., Lovasi, G., 2015. Association of proximity and density of parks and objectively measured physical activity in the United States: a systematic review. Soc. Sci. Med. 138, 22–30. https://doi.org/10.1016/j.socscimed.2015.05.034.

Bauwelinck, M., Casas, L., Nawrot, T.S., Nemery, B., Trabelsi, S., Thomas, I., Aerts, R., Lefebvre, W., Vanpoucke, C., Van Nieuwenhuyse, A., Deboosere, P., Vandenheede, H., 2021. Residing in urban areas with higher green space is associated with lower mortality risk: a census-based cohort study with ten years of follow-up. Environ. Int. 148, 106365 https://doi.org/10.1016/j.envint.2020.106365.

Beaglehole, R., Bonita, R., 2008. Global public health: a scorecard. Lancet 372, 1988–1996. https://doi.org/10.1016/S0140-6736(08)61558-5.

Benjamin, E.J., Muntner, P., Alonso, A., Bittencourt, M.S., Callaway, C.W., Carson, A.P., Chamberlain, A.M., Chang, A.R., Cheng, S., Das, S.R., Delling, F.N., Djousse, L., Elkind, M.S.V., Ferguson, J.F., Fornage, M., Jordan, L.C., Khan, S.S., Kissela, B.M., Knutson, K.L., Kwan, T.W., Lackland, D.T., Lewis, T.T., Lichtman, J.H., Longenecker, C.T., Loop, M.S., Lutsey, P.L., Martin, S.S., Matsushita, K., Moran, A.E., Mussolino, M.E., O'Flaherty, M., Pandey, A., Perak, A.M., Rosamond, W.D., Roth, G. A., Sampson, U.K.A., Satou, G.M., Schroeder, E.B., Shah, S.H., Spartano, N.L., Stokes, A., Tirschwell, D.L., Tsao, C.W., Turakhia, M.P., VanWagner, L.B., Wilkins, J. T., Wong, S.S., Virani, S.S., 2019. Heart disease and stroke statistics—2019 update: a report from the American heart association. Circulation 139, e209. https://doi.org/10.1161/CIR.0000000000000659.

Bhatnagar, A., 2017. Environmental determinants of cardiovascular disease. Circ. Res. 121, 162–180. https://doi.org/10.1161/CIRCRESAHA.117.306458.

Bijnens, E.M., Nawrot, T.S., Loos, R.J., Gielen, M., Vlietinck, R., Derom, C., Zeegers, M.P., 2017. Blood pressure in young adulthood and residential greenness in the early-life environment of twins. Environ. Heal. 16, 53. https://doi.org/10.1186/s12940-017-0266-9.

Bilal, U., Díez, J., Alfayate, S., Gullón, P., Del Cura, I., Escobar, F., Sandín, M., Franco, M., HHH Research Group, 2016. Population cardiovascular health and urban environments: the Heart Healthy Hoods exploratory study in Madrid, Spain. BMC Med. Res. Methodol. 16, 104. https://doi.org/10.1186/s12874-016-0213-4.

Bolte, G., Nanninga, S., Dandolo, L., 2019. Sex/gender differences in the association between residential green space and self-rated health—a sex/gender-focused systematic review. Int. J. Environ. Res. Publ. Health 16, 4818. https://doi.org/10.3390/ijerph16234818.

Borrell, C., Marí-Dell'Olmo, M., Serral, G., Martínez-Beneito, M., Gotsens, M., 2010. Inequalities in mortality in small areas of eleven Spanish cities (the multicenter MEDEA project). Health Place 16, 703–711. https://doi.org/10.1016/j.healthplace.2010.03.002.

Chandrabose, M., Rachele, J.N., Gunn, L., Kavanagh, A., Owen, N., Turrell, G., Giles-Corti, B., Sugiyama, T., 2019. Built environment and cardio-metabolic health: systematic review and meta-analysis of longitudinal studies. Obes. Rev. 20, 41–54. https://doi.org/10.1111/obr.12759.

Cebrecos, A., Domínguez-Berjón, M.F., Duque, I., Franco, M., Escobar, F., 2018. Geographic and statistic stability of deprivation aggregated measures at different spatial units in health research. Appl. Geogr. 95, 9–18. https://doi.org/10.1016/j.apgeog.2018.04.001.

Chow, C.K., Lock, K., Teo, K., Subramanian, S., McKee, M., Yusuf, S., 2009. Environmental and societal influences acting on cardiovascular risk factors and disease at a population level: a review. Int. J. Epidemiol. 38, 1580–1594. https://doi.org/10.1093/ije/dyn258.

Cohen, D.A., Han, B., Park, S., Williamson, S., Derose, K.P., 2019. Park use and park-based physical activity in low-income neighborhoods. J. Aging Phys. Activ 27, 334–342. https://doi.org/10.1123/japa.2018-0032.

Crouse, D.L., Pinault, L., Balram, A., Hystad, P., Peters, P.A., Chen, H., van Donkelaar, A., Martin, R.V., Ménard, R., Robichaud, A., Villeneuve, P.J., 2017. Urban greenness and mortality in Canada's largest cities: a national cohort study. Lancet Planet. Health 1, e289–e297. https://doi.org/10.1016/S2542-5196(17)30118-3.

Dalton, A.M., Jones, A.P., 2020. Residential neighbourhood greenspace is associated with reduced risk of cardiovascular disease: a prospective cohort study. PLoS One 15, e0226524. https://doi.org/10.1371/journal.pone.0226524.

Duque, I., Domínguez-Berjón, M.F., Cebrecos, A., Prieto-Salceda, M.D., Esnaola, S., Calvo Sánchez, M., Marí-Dell'Olmo, M., 2021. Índice de privación en España por sección censal en 2011. Gac. Sanit. 35, 113–122. https://doi.org/10.1016/j.gaceta.2019.10.008.

Fernández Núñez, M.-B., Campos Suzman, L., Maneja, R., Bach, A., Marquet, O., Anguelovski, I., Knobel, P., 2022. The differences by sex and gender in the relationship between urban greenness and cardiometabolic health: a systematic review. J. Urban Health 99, 1054–1067. https://doi.org/10.1007/s11524-022-00685-9.

Fong, K.C., Hart, J.E., James, P., 2018. A review of epidemiologic studies on greenness and health: updated literature through 2017. Curr. Environ. Heal. Reports 39, 11–28. https://doi.org/10.1007/s40572-018-0179-y.

Fontán-Vela, M., Rivera-Navarro, J., Gullón, P., Díez, J., Anguelovski, I., Franco, M., 2021a. Active use and perceptions of parks as urban assets for physical activity: a mixed-methods study. Health Place 71, 102660. https://doi.org/10.1016/j.healthplace.2021.102660.

Fontán-Vela, M., Valiente, R., Franco, M., Gullón, P., 2021b. An integrated approach to create a spatial database of parks for urban health research. Gac. Sanit. 7–9. https://doi.org/10.1016/j.gaceta.2021.07.005.

Franco, M., Bilal, U., Diez-Roux, A.V., 2015. Preventing non-communicable diseases through structural changes in urban environments. J. Epidemiol. Community Health 69, 509–511. https://doi.org/10.1136/jech-2014-203865.

Franco, M., Bilal, U., Guallar, E., Sanz, G., Gómez, A.F., Fuster, V., Cooper, R., 2013. Systematic review of three decades of Spanish cardiovascular epidemiology: improving translation for a future of prevention. Eur. J. Prev. Cardiol. 20, 565–576. https://doi.org/10.1177/2047487312455314.

Gascon, M., Triguero-Mas, M., Martínez, D., Dadvand, P., Rojas-Rueda, D., Plasència, A., Nieuwenhuijsen, M.J., 2016. Residential green spaces and mortality: a systematic review. Environ. Int. 86, 60–67. https://doi.org/10.1016/j.envint.2015.10.013.

Gullón, P., Bilal, U., Cebrecos, A., Badland, H.M., Galán, I., Franco, M., 2017. Intersection of neighborhood dynamics and socioeconomic status in small-area walkability: the Heart Healthy Hoods project. Int. J. Health Geogr. 16, 21. https://doi.org/10.1186/s12942-017-0095-7.

Gullón, P., Cuesta-Lozano, D., Cuevas-Castillo, C., Fontán-Vela, M., Franco, M., 2022. Temporal trends in within-city inequities in COVID-19 incidence rate by area-level deprivation in Madrid, Spain. Health Place 76, 102830. https://doi.org/10.1016/j.healthplace.2022.102830.

Gullón, P., Díez, J., Cainzos-Achirica, M., Franco, M., Bilal, U., 2020. Social inequities in cardiovascular risk factors in women and men by autonomous regions in Spain. Gac. Sanit. https://doi.org/10.1016/j.gaceta.2020.04.014.

Hoffimann, E., Barros, H., Ribeiro, A.I., 2017. Socioeconomic inequalities in green space quality and Accessibility—evidence from a Southern European city. Int. J. Environ. Res. Publ. Health 14. https://doi.org/10.3390/ijerph14080916.

Huxley, R.R., Woodward, M., 2011. Cigarette smoking as a risk factor for coronary heart disease in women compared with men: a systematic review and meta-analysis of prospective cohort studies. Lancet 378, 1297–1305. https://doi.org/10.1016/S0140-6736(11)60781-2.

James, P., Banay, R.F., Hart, J.E., Laden, F., 2015. A review of the health benefits of greenness. Curr. Epidemiol. Reports 2, 218. https://doi.org/10.1007/s40471-015-0044-6, 218.

Klompmaker, J.O., Hoek, G., Bloemsma, L.D., Gehring, U., Strak, M., Wijga, A.H., van den Brink, C., Brunekreef, B., Lebret, E., Janssen, N.A.H., 2018. Green space definition affects associations of green space with overweight and physical activity. Environ. Res. 160, 531–540. https://doi.org/10.1016/j.envres.2017.10.027.

Klompmaker, J.O., Janssen, N.A.H., Bloemsma, L.D., Marra, M., Lebret, E., Gehring, U., Hoek, G., 2021. Effects of exposure to surrounding green, air pollution and traffic noise with non-accidental and cause-specific mortality in the Dutch national cohort. Environ. Heal. 20, 82. https://doi.org/10.1186/s12940-021-00769-0.

Larkin, A., Hystad, P., 2019. Evaluating street view exposure measures of visible green space for health research. J. Expo. Sci. Environ. Epidemiol. 29, 447–456. https://doi.org/10.1038/s41370-018-0017-1.

Liu, X.-X., Ma, X.-L., Huang, W.-Z., Luo, Y.-N., He, C.-J., Zhong, X.-M., Dadvand, P., Browning, M.H.E.M., Li, L., Zou, X.-G., Dong, G.-H., Yang, B.-Y., 2022. Green space and cardiovascular disease: a systematic review with meta-analysis. Environ. Pollut. 301, 118990 https://doi.org/10.1016/j.envpol.2022.118990.

Markevych, I., Schoierer, J., Hartig, T., Chudnovsky, A., Hystad, P., Dzhambov, A.M., de Vries, S., Triguero-Mas, M., Brauer, M., Nieuwenhuijsen, M.J., Lupp, G., Richardson, E.A., Astell-Burt, T., Dimitrova, D., Feng, X., Sadeh, M., Standl, M., Heinrich, J., Fuertes, E., 2017. Exploring pathways linking greenspace to health: theoretical and methodological guidance. Environ. Res. 158, 301–317. https://doi.org/10.1016/j.envres.2017.06.028.

Nichols, M., Townsend, N., Scarborough, P., Rayner, M., 2013. Trends in age-specific coronary heart disease mortality in the European Union over three decades: 1980-2009. Eur. Heart J. 34, 3017–3027. https://doi.org/10.1093/eurheartj/eht159.

Nieuwenhuijsen, M.J., Khreis, H., Triguero-Mas, M., Gascon, M., Dadvand, P., 2017. Fifty shades of green. Epidemiology 28, 63–71. https://doi.org/10.1097/EDE.0000000000000549.

Orioli, R., Antonucci, C., Scortichini, M., Cerza, F., Marando, F., Ancona, C., Manes, F., Davoli, M., Michelozzi, P., Forastiere, F., Cesaroni, G., 2019. Exposure to residential greenness as a predictor of cause-specific mortality and stroke incidence in the rome longitudinal study. Environ. Health Perspect. 127, 027002 https://doi.org/10.1289/EHP2854.

Pereira, G., Foster, S., Martin, K., et al., 2012. The association between neighborhood greenness and cardiovascular disease: an observational study. BMC Publ. Health 12, 466. https://doi.org/10.1186/1471-2458-12-466.

Powell-Wiley, T.M., Baumer, Y., Baah, F.O., Baez, A.S., Farmer, N., Mahlobo, C.T., Pita, M.A., Pothuraju, K.A., Tamura, K., Wallen, G.R., 2022. Social determinants of cardiovascular disease. Circ. Res. 130, 782–799. https://doi.org/10.1161/CIRCRESAHA.121.319811.

Ribeiro, A.I., Gullón, P., Murray, E.T., 2022. Neighbourhood influences on population health: time to unpack the black box. Publ. Health Rev. 43 https://doi.org/10.3389/phrs.2022.1605053.

Richardson, E.A., Mitchell, R., 2010. Gender differences in relationships between urban green space and health in the United Kingdom. Soc. Sci. Med. 71, 568–575. https://doi.org/10.1016/j.socscimed.2010.04.015.

Rigolon, A., Browning, M.H.E.M., McAnirlin, O., Yoon, H., Violet), 2021. Green space and health equity: a systematic review on the potential of green space to reduce health disparities. Int. J. Environ. Res. Publ. Health 18, 2563. https://doi.org/10.3390/ijerph18052563.

Rodríguez-Fonseca, M., Palència, L., Marí-Dell'Olmo, M., Gandarillas, A., Domínguez-Berjón, M.F., Gotsens, M., Rodríguez-Sanz, M., Borrell, C., 2013. Evolution of socio-economic inequalities in mortality in small geographical areas of the two largest cities in Spain (Barcelona and Madrid). Publ. Health 127, 916–921. https://doi.org/10.1016/j.puhe.2013.07.001, 1996–2007.

Ronksley, P.E., Brien, S.E., Turner, B.J., Mukamal, K.J., Ghali, W.A., 2011. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. BMJ 342, d671. https://doi.org/10.1136/bmj.d671 d671.

Rose, G., 1985. Sick individuals and sick populations. Int. J. Epidemiol. 14, 32–38. https://doi.org/10.1093/ije/14.1.32.

Shuvo, F.K., Mazumdar, S., Labib, S.M., 2021. Walkability and greenness do not walk together: investigating associations between greenness and walkability in a large metropolitan city context. Int. J. Environ. Res. Publ. Health 18 (9), 4429. https://doi.org/10.3390/ijerph18094429. Apr 21.

Smith, R., 2012. Why a macroeconomic perspective is critical to the prevention of noncommunicable disease. Science 80 337, 1501–1503. https://doi.org/10.1126/science.1222569.

Stimpson, J.P., Ju, H., Raji, M.A., Eschbach, K., 2007. Neighborhood deprivation and health risk behaviors in NHANES III. Am. J. Health Behav. 31, 215–222. https://doi.org/10.5993/AJHB.31.2.10.

Tamosiunas, A., Grazuleviciene, R., Luksiene, D., Dedele, A., Reklaitiene, R., Baceviciene, M., Vencloviene, J., Bernotiene, G., Radisauskas, R., Malinauskiene, V., Milinaviciene, E., Bobak, M., Peasey, A., Nieuwenhuijsen, M.J., 2014. Accessibility and use of urban green spaces, and cardiovascular health: findings from a Kaunas cohort study. Environ. Heal. A Glob. Access Sci. Source 13, 1–11. https://doi.org/10.1186/1476-069X-13-20.

Verbeke, M., Schrans, D., Deroose, S., De Maeseneer, J., 2006. The international classification of primary care (ICPC-2): an essential tool in the EPR of the GP. Stud. Health Technol. Inf. 124, 809–814.

Vogel, B., Acevedo, M., Appelman, Y., Bairey Merz, C.N., Chieffo, A., Figtree, G.A., Guerrero, M., Kunadian, V., Lam, C.S.P., Maas, A.H.E.M., Mihailidou, A.S., Olszanecka, A., Poole, J.E., Saldarriaga, C., Saw, J., Zühlke, L., Mehran, R., 2021. The Lancet women and cardiovascular disease Commission: reducing the global

burden by 2030. Lancet 397, 2385–2438. https://doi.org/10.1016/S0140-6736(21)00684-X.
Weier, J., Herring, D., 2000. Measuring vegetation (NDVI & EVI) [WWW Document]. URL. https://earthobservatory.nasa.gov/features/MeasuringVegetation.
Williams, B.A., 2021. Constructing epidemiologic cohorts from electronic health record data. Int. J. Environ. Res. Publ. Health 18. https://doi.org/10.3390/ijerph182413193.
World Heart Federation, 2020. The Costs of CVD.