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Indoor environmental quality in households of families with infant twins under 1 year of age living in Porto

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ABSTRACT

Exposure to air pollution in early years can exacerbate the risk of noncommunicable diseases throughout childhood and the entire life course. This study aimed to assess temperature, relative humidity (RH), carbon dioxide (CO_2) and monoxide (CO), particulate matter ($PM_{2.5}$, PM_{10}), ultrafine particles, nitrogen dioxide (NO_2), ozone (O_3), formaldehyde, acetaldehyde and volatile organic compounds (VOC) levels in the two rooms where infant twins spend more time at home (30 dwellings, Northern Portugal). Findings showed that, in general, the worst indoor environmental quality (IEQ) settings were found in bedrooms. In fact, although most of the bedrooms surveyed presented adequate comfort conditions in terms of temperature and RH, several children are sleeping in a bedroom with improper ventilation and/or with a significant degree of air pollution. In particular, mean concentrations higher than recommended limits were found for CO_2, $PM_{2.5}$, PM_{10} and total VOC. Additionally, terpenes and decamethylcyclopentasiloxane were identified as main components of emissions from indoor sources. Overall, findings revealed that factors related to behaviors of the occupants, namely related to a conscientious use of cleaning products, tobacco and other consumer products (air-fresheners, incenses/candles and insecticides) and promotion of ventilation are essential for the improvement of air quality in households and for the promotion of children's health.

1. Introduction

Humans are exposed to a wide and complex spectrum of substances in their surrounding environment, many of which may adversely affect their health and well-being. According to the World Health Organization (WHO) nearly two thirds of the 12.6 million deaths caused by the environment each year are due to noncommunicable diseases (NCDs) and 23% of global deaths could be prevented through healthier environments (WHO, 2016a). Evidence shows that newborns and infants are particularly at risk for the consequences of exposure to air pollution due to their immature respiratory and immune systems which make them more vulnerable to toxic damages, and higher daily inhalation rate, mouth breathing and hand-to-mouth behavior, characteristics that significantly potentiate the intake of air pollutants (Ferguson and Solo-Gabriele, 2016; Gouveia et al., 2018; WHO, 2005; Zhang and Zhu, 2012). In fact, exposure to air pollution in early childhood has been linked to an increased risk of premature mortality (Yorifuji et al., 2016), and of development of NCDs, including respiratory and cardiovascular diseases, and cancer, in childhood, but also throughout the entire life span (Breton et al., 2016; Kuiper et al., 2018; Landrigan et al., 2019; Spycher et al., 2015; WHO, 2005). Most of the NCDs are very heterogeneous conditions likely to result from a combination of the diverse genetic and environmental factors, rather than a single exposure (Bønnelykke and Ober, 2016). Given the high percentage of time that children spend indoors, particularly in homes, during the early years exposure to inadequate environmental conditions in the households are expected to significantly affect children's health. This justifies the increasing global concern to promote healthy indoor environmental quality (IEQ) in homes, as a major opportunity for promotion of public health and well-being (WHO, 2017).

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Several studies have reported evidence on the impact of unhealthy indoor air conditions – mostly in terms of comfort, ventilation conditions and proximity to outdoor pollution sources – and levels of pollutants in homes on children's health (Behrens et al., 2005; Dannemiller et al., 2016; Jaakkola et al., 2004; Li et al., 2019; Mikeš et al., 2019; Stamatelopoulou et al., 2020; Tong et al., 2012; Zhuge et al., 2018). Recently, the VELUX Group commissioned by RAND Europe, 2019 published the Healthy Homes Barometer 2019 edition with results based on EU datasets provided by European Union Statistics on Income and Living Conditions (EU-SILC) and Eurostat. This report states that, based on four primary indicators for assessing living conditions – dampness, darkness, cold and excess noise – one in 3 European children live in unhealthy homes (Gehrt et al., 2019; RAND Europe, 2019). In particular, Portugal was labeled as the worst of the 28 EU countries recording a rate of one in two children living in an unhealthy home, while Finland performed the best, with one in five (Gehrt et al., 2019; RAND Europe, 2019). In the last decade, initiatives to assess indoor environment conditions of homes of children have been conducted in Portugal in order to identify indoor air quality (IAQ)-related health risk factors and opportunities for risk mitigation (Faria et al., 2020; Gabriel et al., 2020; Madureira et al., 2016a). In particular, our group conducted a case-control study to compare the living conditions of asthmatic school-age children (n = 38) to those of non-asthmatic school-age children (n = 30) in the cold season of 2012/2013. Although this study did not provide strong evidence of causative factors for asthmatic condition, a relevant percentage of homes were found to have poor IAQ (Madureira et al., 2016b).

An effective intervention to reduce IAQ-related health risk needs to start from the definition of accurate and comprehensive means for the assessment and identification of the critical substances and their sources, to: i) determine the existence of association of indoor environmental conditions with health effects in occupants; ii) building up to policies and/or preventive measures based on source control and ventilation conditions improvement; and iii) demonstrate improvements of IAQ interventions on health (Carrer et al., 2018; Oliveira Fernandes et al., 2009). With this in mind, in the last two years a comprehensive evaluation of indoor conditions in homes of Portuguese families recruited for the study was conducted in two phases. The first phase included the collection of data in public maternity wards. The initial results of the implementation of an 'IAQ home' questionnaire to 309 families with newborns showed that the main concerns linked to early life exposures at home are likely to be related to emissions from use of household solid fuels, indoor tobacco, household cleaning products (HCP), fragranced consumer products (e.g. air fresheners, incense and candles), moisture-related pathologies, new furniture, recent remodeling/painting works and nearby traffic-related pollution sources (Gabriel et al., 2020). This activity allowed to properly inform and recruit families and to define a priority set of parameters to be assessed during home-visits. These occurred at a later stage when children reached the age of 5–9 months in the second phase of the study. The main aim of the work presented here was to describe the indoor air conditions and pollutant levels in households of families with infant twins under 1 year of age living in Porto Metropolitan Area (PMA), in order to identify opportunities for promotion of childhood health.

2. Methods

2.1. Study design and residential buildings location

This study was developed as part of the BiTWin cohort – a birth cohort study developed in Porto as part of a broader investigation on the impact of long-term and short-term environmental exposures on children's health, integrated in a pilot European Exposure and Health Examination Survey of children including singletons and twins, built within the HEALS – *Health and Environment-wide Associations based on Large population Surveys* – project (http://www.heals-eu.eu/) activities. The first phase started in 2017 with the recruitment of families at the four public maternity hospitals in PMA. Data on potential air pollutant sources in the homes of 309 families was collected at the time of birth using a standardized and user-friendly checklist (Gabriel et al., 2020). The second phase involved home-visits for building survey and comprehensive IAQ assessment, thirty homes of families with twins living in PMA were audited in this second phase. The socioeconomic and demographic characteristics of the 30 participant families are presented in Table 1. At the time of the home-visits, the average age of children was 6.8 months. The location of dwellings of the participant families is presented in Fig. S1 in the Supplementary Material.

The IAQ audit was conducted from July 2018 and June 2019 and families were informed that the assessments should take place during normal activities and under representative occupancy, use, cleaning and ventilation conditions of the house. Trained personnel visited each home in 3 pre-defined times/days (Fig. S2 in the Supplementary Material) to conduct a walkthrough inspection and building survey, installation of equipment for continuous monitoring and sampling tubes, and collection of any relevant complementary information. For each home, two different indoor locations were selected to be simultaneously investigated. The children's bedroom was the mandatory indoor location for executing the air sampling and monitoring work. The second preferential indoor location (2nd room) was the room where the parents reported that infants spend more time at home, after bedroom.

2.2. Checklist and building survey

An extensive electronic checklist was developed to assist in the collection of data during the comprehensive building survey as part of

Table 1
Demographic and socioeconomic characteristics of the 30 participant families.

Characteristic	*n* (%)	n_{missing} (%)
Age		
Mother (years-old)		1 (3)
18-25	2 (7)	
26-35	16 (55)	
36-45	11 (38)	
Father (years-old)		3 (10)
18-25	0 (0)	
26-35	16 (59)	
36-45	11 (41)	
Infant Twins (months-old)		0 (0)
5-6	14 (47)	
7-9	16 (53)	
Educational level		
Mother		1 (3)
Low (≤high school level education)	11 (38)	
Medium (bachelor's degree or equivalent)	11 (38)	
High (≥master's degree)	7 (24)	
Father		2 (7)
Low (≤high school level education)	17 (61)	
Medium (bachelor's degree or equivalent)	6 (21)	
High (≥master's degree)	5 (18)	
Social class (self-perception)		10 (33)
Low	1 (5)	
Medium low	9 (45)	
Medium high	10 (50)	
Monthly income (€)		4 (13)
≤500	1 (4)	
501-1000	4 (15)	
1001-1500	5 (19)	
1501-2000	5 (19)	
2001-2500	8 (31)	
2501-3000	0 (0)	
>3000	3 (12)	
House ownership		1 (3)
Yes	19 (66)	
No	10 (34)	

n (%) refers to the total number of respondent families and respective percentage in the valid cases.

Table 2
Characteristics of the 30 homes surveyed.

	1st phase			2nd phase	
	n (%)	n_{missing} (%)	Mean (SD)	*n* (%)	Mean (SD)
Building/dwelling (N = 30)					
Period of construction		1 (3)			
Before 1950	1 (3)			1 (3)	
1950–1980	4 (14)			4 (13)	
1980–2010	18 (62)			19 (63)	
After 2010	6 (21)			6 (20)	
Energy supply systems		0 (0)			
Electricity	30 (100)			30 (100)	
LPG, natural gas	22 (73)			26 (87)	
Solar energy	4 (13)			2 (7)	
Charcoal/wood	3 (10)			3 (10)	
Heating, ventilation/acclimatization devices		0 (0)			
Portable heating appliances	21 (70)			22 (73)	
Air conditioners	3 (10)			3 (10)	
Space radiators	11 (37)			11 (37)	
Central heating	9 (30)			8 (27)	
Humidifiers/dehumidifiers	4 (13)			4 (13)	
Combustion devices	13 (43)			15 (50)	
Fireplace, brazier	6 (20)			8 (27)	
Heating stove	2 (7)			3 (10)	
Gas stove/gas water heater	3 (10)			1 (3)	
Portable gas heater	3 (10)			4 (13)	
Fan	10 (33)			12 (40)	
Pedestal/towel fan	4 (13)			6 (20)	
Fan heater	5 (17)			6 (20)	
Air freshener diffusers and others	18 (95)	11 (37)		19 (63)	
Air fresheners	11 (58)			15 (50)	
Incense/candles	12 (63)			7 (23)	
Automatic aerosol insecticides	2 (11)			6 (20)	
Indoors pets	19 (63)	0 (0)		17 (57)	
Dog	10 (33)			8 (27)	
Cat	8 (27)			8 (27)	
Other	2 (7)			2 (7)	
Plants inside the house	18 (60)	0 (0)		19 (63)	
Current practice to smoke indoors	3 (10)	0 (0)		3 (10)	
Cigar/cigarettes	3 (10)			3 (10)	
Cleaning products					
Bleach or detergent with bleach	23 (85)	3 (10)		23 (77)	
Spray	3 (11)			2 (7)	
Liquid	21 (78)			22 (73)	
Frequency (times per week)	–		1.8 (1.8)	–	1.9 (2.1)
Detergent with ammonia	13 (59)	8 (27)		7 (23)	
Liquid	13 (59)			7 (23)	
Frequency (times per week)	–		1.9 (1.9)	–	1.3 (0.8)
Other detergent/cleaning products	22 (88)	5 (17)		29 (97)	
Spray	4 (16)			3 (10)	
Liquid	20 (80)			27 (90)	
Frequency (times per week)	–		2.9 (2.4)	–	1.8 (1.9)
Wax/Furniture polish	4 (21)	11 (37)		6 (20)	
Liquid	4 (21)			6 (20)	
Frequency (times per week)	–		1.0 (0.0)	–	1.2 (0.4)
Opening windows		1 (3)			
During the house cleaning procedure	29 (100)			30 (100)	
Signs of indoor pathologies		0 (0)			
Physical	6 (20)			4 (13)	
Moisture-related	6 (20)			1 (3)	
Surrounding outdoor sources at distance up to 100 m		3 (10)			
Traffic-related	16 (59)			29 (97)	
Busy road	6 (22)			6 (20)	
Highway	2 (7)			1 (3)	
Car parking	4 (15)			26 (87)	
Gas stations	1 (4)			1 (3)	
Industrial	3 (11)			4 (13)	
Chimneys, smoke stacks	1 (4)			1 (3)	
Construction work	1 (4)			3 (10)	
Agriculture	7 (26)			12 (40)	

(*continued on next page*)

Table 2 (*continued*)

	1st phase			2nd phase	
	n (%)	n_{missing} (%)	Mean (SD)	*n* (%)	Mean (SD)
Animal husbandry	4 (15)			2 (7)	
Cultivated fields	3 (11)			5 (17)	
Commercial	12 (44)			15 (50)	
Laundry	1 (4)			1 (3)	
Coffee bar	9 (33)			7 (23)	
Green/Forested area	11 (41)			11 (37)	
Others	3 (11)			5 (17)	

n (%) refers to the total number of respondent families and respective percentage in the valid cases.
LPG, liquefied petroleum gas; SD, standard deviation.
1st phase is relative to the results from checklist filled out by the parents at the maternity wards (at the birth); 2nd phase is relative to information collected by the researcher during the field work (5–9 months old).
Only the characteristics that exist in at least one building/dwelling are presented.

the audits. Similar to the user-friendly short checklist used in the first phase of the work, the extended checklist is available on-line at http://heals.inegi.up.pt/. The extensive checklist is a more exhaustive tool that requires some degree of expertise. The checklist is composed of 3 sections structured to ensure a uniform collection of information at the levels of: i) the building and surroundings; ii) dwelling; and iii) room level. The tool includes a large set of questions related to the specific topics such as: outdoor environment characteristics surrounding the building, building construction characteristics, heating and ventilation systems and conditions, past and present visible problems (e.g. moisture signals in the walls) and evident building-originated indoor pollution sources. Specific information about indoor spaces (twins' bedroom and a 2nd room with high occupancy of the infants such as living room) and their use were object of special attention. The information on the prevalence of the main characteristics of all the buildings and indoor spaces studied is summarized in Tables 2 and 3.

2.3. *Continuous monitoring of a set of chemical, physical and comfort air parameters*

A comprehensive set of comfort and environmental parameters were monitored minute-by-minute during a 22 to 24hr-period. Temperature, relative humidity (RH), carbon dioxide (CO_2) and monoxide (CO) levels were measured using IAQ-CALC monitors (model 7545, TSI, Inc., MN, USA), with an accuracy of ±0.6 °C to ±1.6 °C for temperature, ±3.0% to ±3.15% for RH, ±50 ppm to ±93 ppm for CO_2 and ±3 ppm for CO. Airborne particulate matter ($PM_{2.5}$ and PM_{10}) concentrations were obtained by DustTrak DRX aerosol monitors (model 8533, TSI, Inc., MN, USA) with a range of operation from 0.001 to 150 mg/m^3. Levels of ultrafine particles (UFP) were determined using P-Trak portable condensation particle counters (model 8525, TSI, Inc., MN, USA) which are able to count airborne particles sizing from 0.02 to 1 μm at a concentration range from 0 to 5 × 10^5 particles/cm^3. To minimize the impact of instrument drift on the measurement, all DustTrak and P-Trak monitors were auto-zeroed immediately before the monitoring work conducted in each indoor space surveyed. According to the manufacturer, both equipment had an accuracy of ±5%. Measurements were simultaneously conducted indoors in two pre-selected locations: i) in the twins' bedroom, in the vicinity of the crib(s), placed at a similar height to the location of the pillow (0.5–1.0 m) and ii) in the 2nd room, in proximity to the area where children spend most of their time, positioned at a height of 0.5–0.9 m. In addition, nitrogen dioxide (NO_2) and ozone (O_3) were monitored in the bedroom as described in i) using Aeroqual (Series 500 IAQ, New Zealand) instruments with NO_2 and low O_3 sensors, respectively. The accuracy of sensors was $<\pm 38$ μg/m^3 for NO_2 and $<\pm 16$ μg/m^3 for O_3 readings. All the equipment was calibrated by the respective manufacturer within the 12 months preceding the work and was operated in accordance with the manufacturer's instructions. For equipment used for monitoring parameters' levels concomitantly in the two indoor locations, validation tests were systematically performed weekly to evaluate acquisition differences. No statistically significant differences were found between readings obtained with different monitors during the internal verifications.

2.4. *Air sampling and laboratorial analysis*

Indoor air samples were collected in the twins' bedroom to determine volatile organic compounds (VOC) and aldehydes levels. In the 2nd room, only VOC concentrations were assessed. Sampling locations were chosen based on the standard ISO 16000-1:2004 (ISO 16000-1, 2004) and whenever possible at a similar position of the monitoring equipment. Passive sampling over 5 to 7-day period was employed for collecting airborne VOC and aldehydes. For VOC, stainless steel tubes containing Tenax TA (60/80 mesh) with glass wool at one end and sorbent retaining gauze in the open end were installed in duplicate in all sampling locations. After sampling, the tubes were thermally desorbed (model STD 33.50, DANI Instruments, Italy) followed by quantification of VOC content using a capillary column (HP-5: 50 m × 0.2 mm × 0.5 μm) by gas chromatography (model 6890N, Agilent Technologies, USA) coupled to a mass spectrometer detector (model 5975C, Agilent Technologies, USA). Quantification of VOC content was done in accordance with ISO 16017-2:2003 (ISO 16017-2, 2003) and cyclodecane was injected in all tubes as the internal standard. The total VOC (TVOC) was defined as the sum of concentration of all detected substances presenting a retention time comprised between n-hexane and n-hexadecane, using the specific response factor for identified compounds and the toluene response factor in the remaining cases. The limit of detection (LOD, μg/m^3) was 0.4 for benzene, 0.6 for limonene, 0.9 for toluene, 1.0 for octane, 1.1 for ethylbenzene and tetrachloroethylene, 1.2 for 1,2,4-trimethylbenzene, 1.3 for styrene, 1.6 for 2-ethylhexanol and 1.9 for 2-phenoxyethanol. Aldehydes (formaldehyde and acetaldehyde) were collected via cartridge adsorbents filled with 2,4-dinitrophenylhydrazine (RAD165, Radiello, Italy). After sampling, the cartridges were labeled and stored at 4 °C. The analysis of the samples was performed using reverse phase high-performance liquid chromatography (HPLC) (model 1220 Infinity LC, Agilent Technologies, USA) with UV detection, according with ISO 16000-4:2011 (ISO 16000-4, 2011). Internal standards were injected in samples to identify and quantify aldehydes species and the LOD was 0.064 μg/m^3 for formaldehyde and 0.074 μg/m^3 for acetaldehyde. Both VOC and aldehydes analysis were carried out at INEGI's Indoor Air Quality Laboratory and diffusion coefficients were adjusted for the mean temperature registered during sampling. All the analyses were subjected to a validation process: calibration curves, intermediate precision, repeatability, limits of quantification and participation in round-robin tests every year. For determination of VOC, the laboratory performs quality control tests in a daily basis, through the use of control charts. Within a 95% confidence level the analytical method is linear in the range 10–5000 ng for toluene The recovery was determined

Table 3
Characteristics of the rooms surveyed.

	Infant twin's bedroom (N = 30)					Second room (N = 30)	
	1st phase			2nd phase		2nd phase	
	n (%)	n_{missing} (%)	Mean (SD)	*n* (%)	Mean (SD)	*n* (%)	Mean (SD)
Signs of pathologies		0 (0)					
Physical	2 (7)			1 (3)		2 (7)	
Moisture-related	1 (3)			1 (3)		0 (0)	
Number of occupants		0 (0)					
1	0 (0)			1 (3)		1 (3)	
2	12 (40)			11 (37)		2 (7)	
3	3 (10)			5 (17)		6 (20)	
4	14 (47)			13 (43)		13 (43)	
More than 4	1 (3)			0 (0)		8 (27)	
Other occupants of the room		0 (0)					
Mother/parents	19 (63)			18 (60)		27 (90)	
Other	1 (3)			1 (3)		10 (33)	
Floor area (m^2)	–	8 (27)	28.9 (35.7)	–	15.0 (5.8)	–	23.6 (8.7)
Ceiling height (m)	–	7 (23)	2.7 (0.4)	–	2.6 (0.2)	–	2.6 (0.1)
Interior of the room remodelled, renovated or painted in the past 12 months	15 (52)	1 (3)		9 (30)		5 (17)	
New furniture installed in the past 12 months	25 (86)	1 (3)		28 (93)		10 (33)	
Option that displays the possible physical boundary conditions of the room in the building		6 (20)					
Single-storey house							
Room with two or more outdoor facades	1 (4)			0 (0)		1 (3)	
Room with one or none outdoor facade	1 (4)			1 (3)		0 (0)	
Multi-storey house							
Room on the top floor							
Two or more outdoor facades	5 (21)			6 (20)		1 (3)	
One or none outdoor facade	4 (17)			6 (20)		4 (13)	
Room on the ground floor							
Two or more outdoor facades	0 (0)			0 (0)		2 (7)	
One or none outdoor facade	2 (8)			1 (3)		2 (7)	
Room on an intermediate floor							
Two or more outdoor facades	6 (25)			3 (10)		5 (17)	
One or none outdoor facade	5 (21)			13 (43)		15 (50)	
Fenestration/windows							
Openable windows		1 (3)					
0	0 (0)			1 (3)		0 (0)	
1	27 (93)			25 (83)		21 (70)	
2	1 (3)			3 (10)		5 (17)	
3 or more	1 (3)			1 (3)		4 (13)	
Orientation		4 (13)					
North	9 (35)		1.0 (0.0)	11 (37)	1.0 (0.0)	12 (40)	1.3 (0.6)
South	5 (19)		1.0 (0.0)	2 (7)	1.5 (0.7)	11 (37)	1.6 (1.6)
West	6 (23)		1.5 (0.7)	11 (37)	1.2 (0.4)	6 (20)	1.5 (0.5)
East	6 (23)		1.0 (0.0)	6 (20)	1.2 (0.4)	4 (13)	1.0 (0.0)
Surface walls		1 (3)					
Painted	29 (100)			30 (100)		30 (100)	
Wallpaper	0 (0)			1 (3)		1 (3)	
Stone	0 (0)			0 (0)		2 (7)	
Surface floor		1 (3)					
Tiles	3 (10)			1 (3)		7 (23)	
Plastic (Vinyl/PVC)	4 (14)			3 (10)		3 (10)	
Wood/Parquet	26 (90)			27 (90)		21 (70)	
Small carpet(s)	4 (14)			24 (80)		23 (77)	

n (%) refers to the total number of respondent families and respective percentage in the valid cases.
PVC, polyvinyl chloride; SD, standard deviation.
1st phase is relative to the results from checklist filled out by the parents at the maternity wards (at the birth); 2nd phase is relative to information collected by the researcher during the field work (5–9 months old).Only the characteristics that exist in at least one room are presented.

to be higher than 99% for VOC. Although this percentage was not determined for aldehydes, a recovery rate higher than 99% is also assumed. A calibration curve is performed every day for aldehydes. In order to eliminate the effect of possible cross-contaminations during transport and handling, field blanks were included and analyzed for all sampled parameters. In case of VOC, sampling was also carried out outdoors, normally in a window/balcony of the bedroom (or when it was not possible, outside in the same facade of the bedroom).

2.5. Statistical analysis and assumptions

Data was described by absolute and relative values, variables were described by means and standard deviation but also the range of values were presented. Regarding the VOC concentrations, values below the LOD were assumed as 0 and the detection frequency (DF), defined as the fraction of measurements above the LOD, was calculated for each quantified VOC. Indoor-to-outdoor (I/O) ratios were estimated for each measured VOC in order to investigate the relationship between indoor and outdoor levels. Statistical analysis was performed using IBM SPSS Statistics version 25. Normality of the distribution was tested by the Shapiro-Wilk test. In order to compare concentrations between locations, Wilcoxon and t-tests were applied. Differences among parameter levels in periods of occupancy and non-occupancy were also tested with Mann-Whitney U and t-tests. Difference analysis between data collected in homes audited in different seasons (cold season: 23rd September to 20th March; warm season: 21st March to 22nd September) and between measured concentrations and dichotomous variables related to information collected through checklist were studied through Mann-Whitney U tests. Spearman and Pearson methods were used to investigate the existence of significant correlations.

3. Results and discussion

3.1. General characteristics of dwellings and indoor spaces surveyed

Twenty (67%) residential buildings were surveyed during the cold season while the remaining 10 (33%) were audited during the warm season. For the second indoor space evaluated was the room in which infant twins spend more time at home after the bedroom, which was the living room for 26 (87%), the kitchen for 1 (3%), and other rooms for 3 (10%) of the households.

The information collected through the checklist (Tables 2 and 3) revealed that, in general, the information provided by the parents at the time of birth in the maternity wards (1st phase) were very concordant with the data gathered by the researchers during their evaluations in the field (2nd phase). Interestingly, according to results presented in Table 2 parents were found to be more reserved in recognizing that they live in proximity to relevant traffic-related sources. In fact, the high percentage of homes located nearby traffic-related sources is likely to be explained by the exclusive selection of families living in the metropolitan area for the study. In addition, an overestimation of the children's bedroom area reported by parents at the time of birth (28.9 m^2) was confirmed by comparison with the dimensions assessed "in situ" (15.0 m^2). This demonstrates that data on dimensions collected through questionnaires are not representative of the reality, and thus should be avoided in further surveys.

Table 4
Descriptive statistics for air parameters continuously measured indoors in the infant twin's bedroom and in the second room surveyed.

	Location	Mean (SD)	Range (Min-Max)[a]	p value
T, °C	Bedroom	20.6 (3.5)	14.0–27.0	.645
	2nd room	20.7 (3.5)	14.3–28.8	
RH, %	Bedroom	61.9 (9.6)	38.4–75.9	**<.001**
	2nd room	56.7 (8.3)	37.6–75.4	
CO_2, ppm	Bedroom	1209 (493)	607–2249	**<.001**
	2nd room	955 (336)	509–1603	
CO, mg/m^3	Bedroom	0.46 (0.41)	0.00–1.58	**<.001**
	2nd room	0.10 (0.23)	0.00–1.08	
NO_2, $\mu g/m^3$	Bedroom	75.1 (32.7)	40.9–147.6	n.a.
	2nd room	n.m.	n.m.	
O_3, $\mu g/m^3$	Bedroom	5.9 (7.5)[b]	0.0–32.0[b]	n.a.
	2nd room	n.m.	n.m.	
$PM_{2.5}$, $\mu g/m^3$	Bedroom	43.8 (28.9)	15.0–125.9	1.000
	2nd room	43.0 (29.3)	11.2–126.2	
PM_{10}, $\mu g/m^3$	Bedroom	46.8 (29.8)	17.7–132.9	1.000
	2nd room	46.4 (30.3)	13.2–135.1	
UFP, pt/cm^3	Bedroom	12,549 (10,865)[c]	2994–50887[c]	.080
	2nd room	14,337 (11,463)	3356–54083	

CO, carbon monoxide; CO_2, carbon dioxide; Max, maximum; Min, minimum; n.a., not applicable; n.m., not measured; NO_2, nitrogen dioxide; O_3, ozone; PM, particulate matter; RH, relative humidity; SD, standard deviation; T, temperature; UFP, ultrafine particles.
N, corresponds to the total number of households with valid data used in statistics.

[a] Correspond to the range of values for the 22hr-mean (except UFP: 5 to 15hr-mean) obtained for each room.

[b] Data from 6 bedrooms were lost due technical problems.

[c] Data from 1 bedroom were lost due technical problems.

General characteristics of the sample of the 30 dwellings surveyed in this work are also found to be similar to those reported by the whole sample (n = 309) of families recruited in the first phase (Gabriel et al., 2020), with slight deviations. In particular, for some of the identified main concerns linked to early life exposures in homes, including for the use of household solid fuels (2nd $phase_{n=30}$: 10% vs 1st $phase_{n=309}$: 19%), indoor tobacco (10% vs 9%), HCP with bleach (77% vs 78%), ammonia (23% vs 29%) and wax (20% vs 22%) and air fresheners (50% vs 47%), and for the existence of moisture-related pathologies (3% vs 7%). However, situations of different prevalence of answers among the results obtained here and those reported by the 309 families were also observed, namely the lower frequency of use of incense and candles (2nd $phase_{n=30}$: 23% vs 1st $phase_{n=309}$: 61%), and remodeling/painting (30% vs 51%), in contrast with the more common installation of new furniture (93% vs 75%). The results collected through checklist also show that most of the mothers (90%) were also living in the same house during the pregnancy, indicating that for most infants the collected data can also be, at least to some extent, representative of "in utero" exposures.

From the sampling and monitoring work conducted in the households of the 30 participant families, it was observed that comfort parameters and pollutant levels measured indoors presented a wide range of values as described in detail in the following sections.

3.2. Comfort and ventilation conditions

Adequate temperature, humidity and control of dampness are commonly cited as being important to the well-being and health of occupants. In this work, mean temperatures and RH percentages varied widely between dwellings surveyed (Table 4). The WHO recommends temperatures of 21 °C in the living rooms and 18 °C in other occupied rooms to achieve an adequate standard of warmth (WHO Regional Office for Europe, 2007). For the homes audited in the cold season, the observed mean daily indoor temperature (19.4 °C for both bedrooms and living rooms) was significantly lower than those verified in the warm period (bedroom: 23.2 °C, $U = 32.0$, $z = -3.0$; $p = .002$; living room: 23.3 °C, $U = 29.0$, $z = -3.1$; $p = .001$). The daily mean temperatures in the bedroom for the occupied period were higher than 25 °C in

Table 5
Descriptive statistics for air parameters continuously measured indoors in the infant twin's bedrooms for the occupancy and non-occupancy periods.

	Period	Mean (SD)	Range (Min-Max)	p value
T, °C	Occupancy	20.7 (3.5)	13.8–27.0	.806
	Non-occupancy	20.5 (3.6)	14.2–27.0	
RH, %	Occupancy	63.0 (9.2)	40.6–78.3	.253
	Non-occupancy	60.1 (10.1)	35.9–74.4	
CO_2, ppm	Occupancy	1414 (611)	630–3096	**.000**
	Non-occupancy	919 (420)	462–1932	
CO, mg/m^3	Occupancy	0.55 (0.51)	0.00–1.70	**.038**
	Non-occupancy	0.30 (0.41)	0.00–1.71	
NO_2, $\mu g/m^3$	Occupancy	70.3 (32.9)	39.7–146.3	**.035**
	Non-occupancy	81.5 (34.9)	46.1–165.1	
$PM_{2.5}$, $\mu g/m^3$	Occupancy	44.7 (28.4)[a]	14.5–109.7[a]	.784
	Non-occupancy	43.0 (31.1)	15.3–138.3	
PM_{10}, $\mu g/m^3$	Occupancy	47.1 (28.6)[a]	16.4–110.3[a]	.919
	Non-occupancy	46.5 (32.3)	17.9–146.6	
UFP, pt/cm^3	Occupancy	10,249 (8588)[a]	1622–40,401[a]	**.042**
	Non-occupancy	13,583 (10,218)[b]	3904–53870[b]	

CO, carbon monoxide; CO_2, carbon dioxide; Max, maximum; Min, minimum; NO_2, nitrogen dioxide; PM, particulate matter; RH, relative humidity; SD, standard deviation; T, temperature; UFP, ultrafine particles.
N, corresponds to the total number of households with valid data used in statistics.

[a] Data presented are related to 29 bedrooms.

[b] Data presented are related to 28 bedrooms.

13% (warm season: 30%; cold season: 5%), between 20 and 25 °C in 37% (warm season: 50%; cold season: 30%), between 18 and 20 °C in 30% (warm season: 20%; cold season: 35%), between 18–14 in 17% (warm season: 0%; cold season: 25%) and below 14 °C in 3% (warm season: 0%; cold season: 5%) of the surveyed households. The measured temperatures for dwellings surveyed in the cold season are notably higher than those obtained for a study that measured indoor temperatures of 141 residential buildings in the Northern Portugal during the winter of 2013/2014, which reported mean daily indoor temperatures during the occupied period of 14.9 °C for the bedrooms and 16.6 °C for the living rooms (Magalhães et al., 2016). This discrepancy is likely to result from a combination of several factors, including age and type of construction of surveyed buildings, trend for a less rigorous winter in recent years and an increased concern of parents to heat indoor space for the well-being of their babies. In addition, another previous study comparing the living conditions of 38 asthmatic school-age children to those of 30 non-asthmatic school-age children living in Porto showed that median indoor temperature was significantly lower in the bedrooms of the asthmatic children (16.7 vs 17.7 °C; $p = .045$) (Madureira et al., 2016b). In the present study, 4 bedrooms of infants presented a median temperature equal or lower than 16.7 °C.

As shown in the Tables 4 and 5, the mean indoor RH percentages were found to be within the acceptable ranges. Moreover, indoor RH levels were shown to be correlated to the year of construction of the building (bedroom: $r_s = -0.46$, $p = .010$; 2nd room: $r_s = -0.42$, $p = .022$), with more recent buildings presenting significantly lower RH levels. Additionally, although the results obtained in the first survey at the maternity wards (Gabriel et al., 2020) showed that presence of dampness was significantly linked to the age of the building, this correlation cannot be ascertained in the second phase, since signs of dampness were only observed for one household (Table 2).

For CO_2, which was measured as an indicator of occupancy and of ventilation conditions, significantly higher levels were found in bedrooms compared with those measured in the 2nd rooms ($z = 4.1$, $p < .001$). Based on the national limit defined for CO_2 (1250 ppm (Portaria n.º 353-A/2013 de 4 de dezembro, 2013)) and using the obtained 22hr-mean values, 37% of the bedrooms and 23% of the studied 2nd rooms may present inadequate levels of ventilation and/or have very high levels of occupancy (Fig. 1). Eight bedrooms (27%) demonstrated a very high air stuffiness (mean concentrations of $CO_2 > 1700$ ppm) and this scenario was even worse when only night occupancy periods are considered, as shown in Table 5. In fact, for periods when infants are sleeping in the room the CO_2 level exceeded 1250 ppm in a half of the bedrooms assessed. In the previous case control study (Madureira et al., 2016b), 61% of the bedrooms of school-age children displayed median CO_2 concentration above 1000 ppm and about 4% reported CO_2 concentration exceeding 2000 ppm. In this work, 50 and 13% of the infant twins' bedrooms presented median CO_2 levels that exceeded 1000 and 2000 ppm, respectively. The respective percentages of the bedrooms exceeding these limit values was found to be even higher (70 and 27%, respectively), if only CO_2 concentrations obtained for occupancy period are considered. In fact, these findings suggest that an important percentage of children at different ages living in Porto are sleeping in bedrooms with very high air stuffiness probably due to inadequate levels of ventilation. Because it is naturally expected that families with twins have their infants sleeping in a room with a greater occupancy, the CO_2 levels found in this study are probably not fully representative of those verified in bedrooms of singleton infants. In this context, a non-statistically significant negative correlation ($r_s = -0.34$, $p = .070$) was found between the mean CO_2 concentrations and volume of air (m^3) per person sleeping in the room. In fact, it was also observed that 60% of the infant twins shared the bedroom with their progenitor(s) (mother or both parents), 37% of infant twins had a bedroom only for themselves, and only 3% of twins sleep in different rooms (Table 3).

It is of major importance to promote awareness among families, in order to guarantee proper ventilation conditions to keep CO_2 levels below 1000 ppm, even in small size bedrooms with high occupancy. Adequate levels of ventilation of the indoor spaces are particularly important to remove air pollutants introduced indoors that in addition to the products of occupants' metabolism (mainly CO_2 concentrations), include toxic chemicals and aerosols resultant from building materials, consumer products and/or occupant activities.

3.3. Particulate matter ($PM_{2.5}$, PM_{10} and UFP) concentrations in the 30 households

The concentration of airborne particles assessed in this work showed a considerable fluctuation across the 30 households surveyed as shown in Fig. 2. Although no specific standards are defined for residential buildings, Portuguese legislation for public buildings (Portaria n.o 353-A/2013 de 4 de dezembro, 2013) has established recommended limits for 8hr-exposure in accordance with WHO guidelines (WHO, 2006) of 25 and 50 μg/m^3 for airborne $PM_{2.5}$ and PM_{10}, respectively. Considering the 22hr-mean concentrations, 23% of the bedrooms ($n = 7$) exceeded both these levels (Fig. 2). If the recorded peak 8hr-exposure is considered, 60% ($n = 18$) and 23% ($n = 7$) of the homes exceeded the recommended guidelines for 8hr-exposure of 25 μg $PM_{2.5}$/m^3 and 50 μg PM_{10}/m^3, respectively.

PM concentrations measured in the bedrooms were strongly correlated with those found in the 2nd rooms ($PM_{2.5}$: $r_s = 0.85$, $p < .001$; PM_{10}: $r_s = 0.86$, $p < .001$). In this regards, a very recent study also conducted in Porto found PM levels very similar to those obtained in this study but reported $PM_{2.5}$ and PM_{10} concentrations in bedrooms of newborns ($n = 16$) (46 and 49 μg/m^3, respectively) that were about 1.2 times lower than those measured in living room ($n = 47$) (Madureira et al., 2020). Here, a very similar concentration was obtained for bedrooms ($n = 30$, $PM_{2.5}$: 43.8 μg/m^3; PM_{10}: 46.8 μg/m^3) and the 2nd room ($n = 30$, 87% living rooms, $PM_{2.5}$: 43.0 μg/m^3; PM_{10}: 46.4 μg/m^3). This

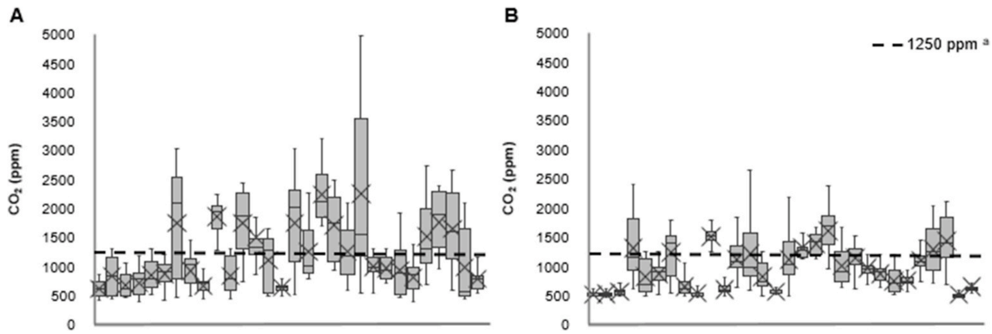

Fig. 1. Box-plot representing carbon dioxide (CO_2) levels in each twins' bedroom (A) and second room (B). The bottom and the top of the boxes represent 25th and 75th percentiles. The band near the middle of the box and the X represent the median and the mean values, respectively. The ends of the whiskers indicate 10th and 90th percentiles. [a] National recommended limit (Portaria n.o 353-A/2013 de 4 de dezembro, 2013).

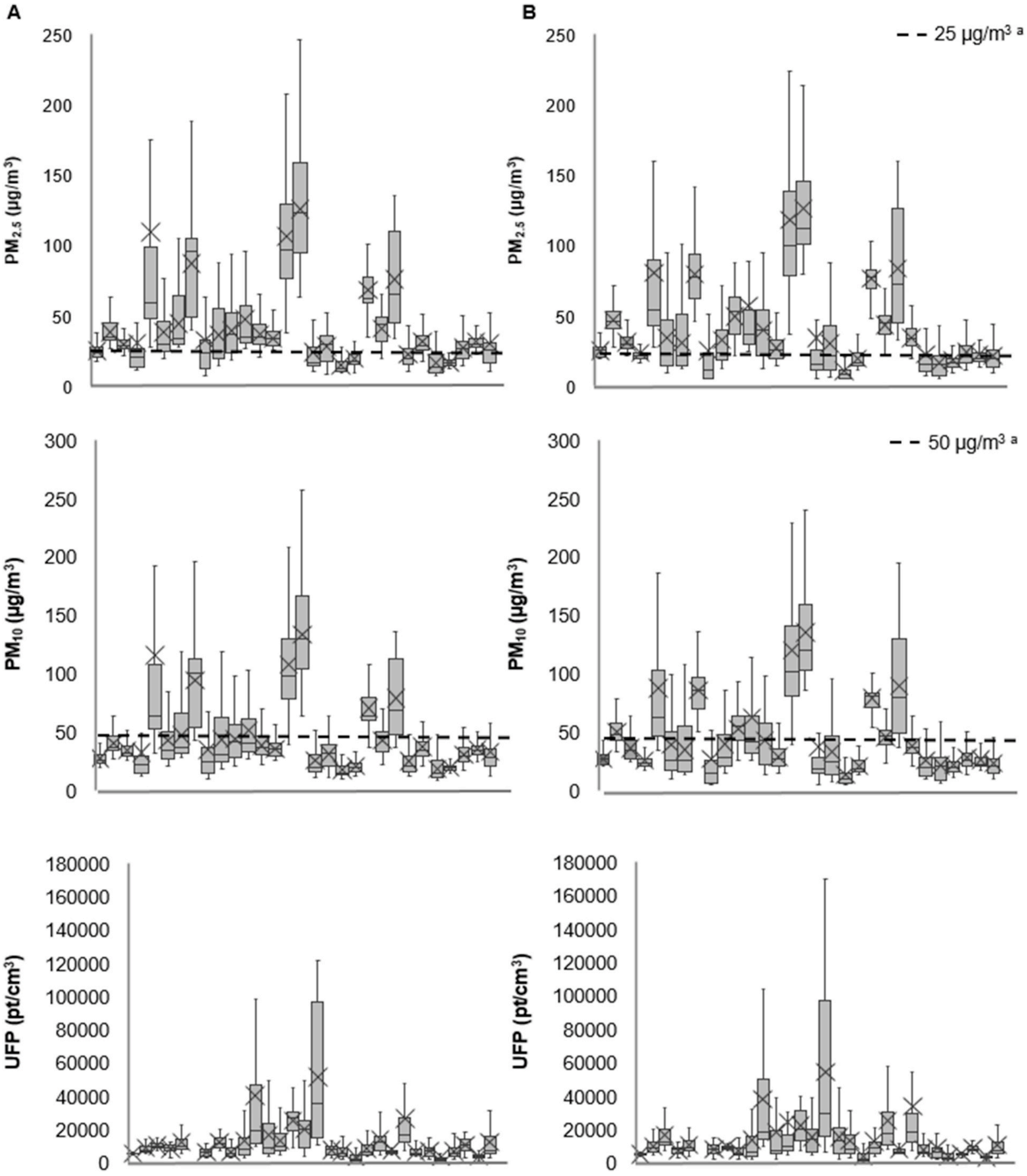

Fig. 2. Box-plot representing particulate matter ($PM_{2.5}$ and PM_{10}) and ultrafine particles (UFP) levels in twins' bedroom (A) and second room (B). The bottom and the top of the boxes represent 25th and 75th percentiles. The band near the middle of the box and the X represent the median and the mean values, respectively. The ends of the whiskers indicate 10th and 90th percentiles. ^{a} WHO and national recommended limit values (Portaria n.o 353-A/2013 de 4 de dezembro, 2013; WHO, 2006).

is likely to result from the improved uniformity of sample size from concurrent measurements conducted in our study. Focusing on data obtained for the bedrooms, PM levels found in this study were about 2-fold lower than mass concentrations reported for 68 bedrooms of school-age children surveyed in the cold season of 2012/2013 (Madureira et al., 2016a). Even if only the data collected from households surveyed in cold season are considered, a similar result is also obtained (1.7-fold lower). In fact, in accordance with most of existing research on PM characterization in households, statistically significant higher PM levels were found in homes audited during the cold season ($PM_{2.5}$, bedrooms: $U = 45.0$, $z = -2.4$, $p = .015$ and 2nd rooms: $U = 33.0$, $z = -2.9$, $p = .002$; PM_{10}, bedrooms: $U = 47.0$, $z = -2.3$, $p = .019$ and 2nd rooms: $U = 35.0$, $z = -2.9$, $p = .003$). Comparing data collected in living rooms, a survey recently conducted in Lisbon (Faria et al., 2020) found that PM concentrations in living rooms for both size fractions where on average approximately 3-fold lower than those reported in this study.

For UFP, it was found that the distributions of the indoor counts largely varied among the 30 dwellings, with mean values that ranged from 2994 to 50,887 pt/cm^3 in bedrooms and from 3356 to 54,083 pt/cm^3 in the 2nd rooms (Table 4, Fig. 2). On average, measured UFP number concentrations were similar to those found in homes of newborns (Madureira et al., 2020), and slightly higher than those reported in primary schools (Cavaleiro Rufo et al., 2016), indoor swimming pools (Gabriel et al., 2019) and fitness centers (Slezakova et al., 2018) also located in Porto. Similar to what was reported above for PM, a significant correlation was found between number concentrations in bedrooms and in the 2nd room of the house ($r_s = 0.89$, $p < .001$). Likewise, a seasonal trend of significantly increased levels in dwellings surveyed in the cold season was only detected for UFP levels assessed in the 2nd rooms ($U = 38.0$, $z = -2.6$, $p = .008$).

The statistical analysis to investigate the existence of association of PM levels and data on putative indoor sources collected through the checklist, showed a significant outcome only for the use of automatic aerosol products for domestic insect control. Statistically significant higher levels of PM_{10} and UFP in bedrooms ($U = 28.0$, $z = -2.3$, $p = .021$; $U = 16.0$, $z = -2.9$, $p = .003$, respectively) and of $PM_{2.5}$, PM_{10} and UFP in 2nd rooms ($U = 28.0$, $z = -2.3$, $p = .021$; $U = 25.0$, $z = -2.4$, $p = .013$; $U = 17.0$, $z = -2.8$, $p = .003$, respectively) were recorded in households where automatic aerosol insecticide products were used (6 out of 30 homes). A non-significant trend of increased levels of both size fractions of PM and UFP was also observed for homes of families that reported regular use of incense or candles, existence of dogs, indoor plants and smoking indoors.

In addition, homes located on the ground floor presented higher levels of $PM_{2.5}$ and PM_{10} (bedrooms: $U = 4.0$, $z = -2.0$, $p = .041$; 2nd rooms: $U = 5.0$, $z = -3.2$, $p < .001$) than those located in upper floors. This result suggests that indoor PM levels are greatly influenced by outdoor pollution sources at street level (e.g. traffic-related sources). Indeed, although the real contribution from outdoor sources cannot be accurately estimated due to the lack of measurements of PM in the outdoor environment, the high PM concentration found indoors is very likely to have a great contribution from the reported proximity (<100 m) of 97% of the houses to declared traffic-related pollution sources. The investigation of statistically significant associations with data related to the surrounding outdoor environment shows significant higher number concentrations of UFP detected in households located in proximity to agriculture-related sources (bedrooms: $U = 54.0$, $z = 2.1$, $p = .034$; 2nd rooms: $U = 51.0$, $z = 2.3$, $p = .024$). Noteworthy, this is in disagreement with previous research that provided evidence of reduced indoor UFP levels in rural areas (Cavaleiro Rufo et al., 2016). This finding can be influenced by the fact of most of households located near agriculture-related sources (11 out of 12) have also declared traffic-related sources in the surroundings, due to their urban context. Nevertheless, in contrast to the findings for PM, for UFP no differences were observed between rooms located in ground floor and those in upper floors. UFP fraction seems to be, in fact, less impacted by local pollutant sources at ground level than PM. This is likely to be explained by the possible complex origin of UFP (both primary, directly from sources such as road traffic and secondary, formed from chemical reactions in the atmosphere). In addition, because of their very small size, UFP typically can easily move away from their source of emission and undergo rapid dynamic transformations (Lewis et al., 2018), making it difficult to establish primary sources of UFP.

The WHO has reported that 92% of the world's population lives in areas with an ambient PM exceeding the WHO guidelines, which is associated with 4.2 million annual premature deaths worldwide (WHO, 2016b). Thus, further studies with both concurrent measurements of indoor and outdoor PM (and also UFP) levels would be needed to improve the source analysis and identify the predominant source of particles (outdoor infiltrations vs indoor sources and activities).

3.4. Volatile organic compounds detected in the air

Concurrent sampling of airborne VOC indoors and outdoors showed that indoor TVOC concentrations found in infant bedrooms and 2nd rooms significantly exceeded outdoor levels (bedrooms: $z = -4.7$, $p < .001$; 2nd rooms: $z = -4.6$, $p < .001$). Thus, the indoor sources seem to represent the major contribution for the total load of chemical substances in the living environment. Furthermore, as described above, while the PM levels were found to be 2-fold lower than that obtained in bedrooms of school-age children, for TVOC the scenario was the opposite with mean TVOC concentrations being 2-fold higher (Table 6) than the obtained in the audits of 2012/2013 (Madureira et al., 2016a). This observation suggests that the pattern of the air pollution in Portuguese homes is likely to be different among different population ages and/or that it is changing over the time due to differences in building construction features, consumer trends or occupant behavior.

Indoor TVOC concentrations higher than guideline values are commonly regarded as a surrogate of total chemical load in the indoor environment and of inadequate ventilation conditions. In this study, indoor TVOC concentrations exceeding the national recommended limit (<600 μg TVOC/m^3 (Portaria n.o 353-A/2013 de 4 de dezembro, 2013)) were found in 3 homes (Fig. 3A). Since a poor association between TVOC concentrations and detriments on health is typically documented (Mølhave et al., 1997; Rumchev et al., 2007), concentrations of TVOC found in this work are of unclear significance in terms of risk to health of occupants. Identification and quantification of the individual substances existing in the environmental mixture are known to be essential for the accurate assessment of chemical exposure (Fromme et al., 2019).

The pattern and relative concentrations for the individual substances identified in this study were highly heterogeneous among the studied households. The individual VOC that were detected in at least 10% of the samples collected indoors (DF above 10%) are listed in Table 6**.** At these conditions, a total of 30 compounds including aromatic hydrocarbons (benzene, toluene, ethylbenzene, m/o/p-xylenes, 1,2,4-trimethylbenzene, naphthalene, and styrene), aliphatic hydrocarbons (2,2,4,4,6,8,8-heptamethylnonane, n-decane, nonane, octane, tetradecane, and undecane), chlorinated hydrocarbons (tetrachloroethylene), alcohols (2-ethylhexanol, butanol), carboxylic acids (acetic acid), aromatic ketones (acetophenone), esters (benzyl acetate, butyl acetate, ethyl acetate), siloxanes (decamethylcyclopentasiloxane (D5)) aldehydes (benzaldehyde, decanal, nonanal), glycol ethers (1-methoxy-2-propanol, 2-phenoxyethanol) and terpenes (3-carene, limonene, α/β-pinene) were identified. From the previous, mean I/O concentration ratios below the unity were obtained for acetophenone, benzaldehyde and benzene thus indicating that these compounds are primarily originated from outdoor sources. 2-ethylhexanol, 2-phenoxyethanol, decanal, ethylbenzene, m/o/p-xylenes, nonanal, octane, tetrachloroethylene and toluene were detected at mean I/O concentration ratios from 1 to 10, having their putative nature attributed to both outdoor and indoor sources. In addition, 1,2,4-trimethylbenzene, 1-methoxy-2-propanol, 2,2,4,4,6,8,8-heptamethylnonane, 3-carene, acetic acid, benzyl acetate, butanol, butyl acetate, D5, decane, ethyl acetate, limonene, naphthalene, nonane, styrene, tetradecane, undecane and α/β-pinenes were the VOC identified has having primarily or exclusively indoor sources (I/O concentration ratios >10). Most of the previous are of toxicological concern and are reported as being common components of machine wash liquids/detergents, HCP, personal care products (PCP), automotive care products, paints and coating or adhesives, fragrances and air fresheners (European Chemicals Agency, n.d.; United States Environmental Protection Agency, n.d.). Among the substances identified as having primarily indoor sources, limonene and α/β-pinenes were the most prevalent VOC, quantified in about 80% in the total indoor air samples. The high reported rate of use of air fresheners and HCP is likely to have an important contribution in terms of exposure to these potentially hazardous terpenes. In fact, the use of air fresheners can be a major source of exposure as these are possible sources of over 100 different chemicals, such as terpenes, terpenoids, ethanol, formaldehyde, benzene, toluene, xylene and phthalates and indoor oxidants such as O_3, hydroxyl radicals, and nitrate radicals (Steinemann, 2017). Exposure to

Table 6
Volatile organic compounds concentrations measured in air samples collected in the 30 dwellings surveyed.

	Location	Mean (SD)[a]	Max[a]	n (DF)[b]	I/O ratio[c]
1,2,4-Trimethylbenzene	Bedroom	1.9 (3.4)	12.7	10 (33.3)	19.0
	2nd room	2.2 (4.9)	24.4	9 (31.0)	22.0
	Outdoor	0.1 (0.8)	4.1	1 (3.4)	–
1-Methoxy-2-propanol	Bedroom	2.6 (8.5)	41.6	4 (13.3)	>10[d]
	2nd room	2.7 (7.7)	31.8	4 (13.8)	>10[d]
	Outdoor	n.d.	n.d.	n.d.	–
2,2,4,4,6,8,8-Heptamethylnonane	Bedroom	7.9 (32.3)	175.3	5 (16.6)	26.3
	2nd room	2.0 (5.4)	22.1	6 (20.7)	6.7
	Outdoor	0.3 (1.4)	7.5	1 (3.4)	–
2-Ethylhexanol	Bedroom	1.5 (2.8)	9.7	8 (26.6)	3.0
	2nd room	1.2 (2.8)	11.2	6 (20.7)	2.4
	Outdoor	0.5 (2.0)	11.2	2 (6.9)	–
2-Phenoxyethanol	Bedroom	1.6 (4.6)	19.6	4 (13.3)	4.0
	2nd room	0.5 (2.1)	9.8	2 (6.9)	1.3
	Outdoor	0.4 (1.9)	10.2	1 (3.4)	–
3-Carene	Bedroom	2.1 (5.0)	23.7	7 (23.3)	>10[d]
	2nd room	1.2 (4.7)	25.0	5 (17.2)	>10[d]
	Outdoor	n.d.	n.d.	n.d.	–
Acetic acid	Bedroom	1.7 (9.3)	51.1	1 (3.3)	>10[d]
	2nd room	6.2 (17.6)	74.8	4 (13.8)	>10[d]
	Outdoor	n.d.	n.d.	n.d.	–
Acetophenone	Bedroom	1.7 (2.8)	11.1	12 (40.0)	0.6
	2nd room	1.6 (1.9)	5.1	13 (44.8)	0.5
	Outdoor	3.0 (4.4)	21.7	16 (55.2)	–
Benzaldehyde	Bedroom	5.0 (4.7)	17.9	24 (80.0)	0.7
	2nd room	4.8 (3.5)	14.6	25 (86.2)	0.7
	Outdoor	7.3 (6.9)	32.3	25 (86.2)	–
Benzene	Bedroom	1.2 (1.1)	3.3	18 (60.0)	0.7
	2nd room	1.3 (1.3)	6.0	18 (62.1)	0.8
	Outdoor	1.7 (1.4)	5.0	22 (75.9)	–
Benzyl acetate	Bedroom	0.7 (1.9)	7.3	4 (13.3)	>10[d]
	2nd room	0.6 (1.7)	5.4	4 (13.8)	>10[d]
	Outdoor	n.d.	n.d.	n.d.	–
Butanol	Bedroom	1.7 (5.4)	28.5	5 (16.6)	>10[d]
	2nd room	1.6 (4.1)	19.9	6 (20.7)	>10[d]
	Outdoor	n.d.	n.d.	n.d.	–
Butyl acetate	Bedroom	2.7 (8.5)	37.5	4 (13.3)	>10[d]
	2nd room	3.4 (9.6)	36.3	4 (13.8)	>10[d]
	Outdoor	n.d.	n.d.	n.d.	–
Decamethylcyclopentasiloxane	Bedroom	81.5 (160.1)	736.2	11 (36.6)	18.5
	2nd room	47.1 (100.7)	486.8	10 (34.5)	10.7
	Outdoor	4.4 (23.6)	127.3	1 (3.4)	–
Decanal	Bedroom	1.4 (2.0)	6.7	11 (36.6)	2.3
	2nd room	1.6 (2.0)	5.5	12 (41.4)	2.7
	Outdoor	0.6 (1.5)	5.3	5 (17.2)	–
Decane	Bedroom	2.3 (5.5)	26.4	7 (23.3)	11.5
	2nd room	2.7 (6.4)	28.9	8 (27.6)	13.5
	Outdoor	0.2 (0.9)	5.0	1 (3.4)	–
Ethyl acetate	Bedroom	8.3 (21.5)	98.8	9 (30.0)	83.0
	2nd room	9.2 (26.4)	130.2	10 (34.5)	92.0
	Outdoor	0.1 (0.6)	2.9	2 (6.9)	–
Ethylbenzene	Bedroom	1.9 (3.2)	13.9	11 (36.6)	9.5
	2nd room	1.9 (3.6)	14.3	9 (31.0)	9.5
	Outdoor	0.2 (0.7)	3.5	3 (10.3)	–
Limonene	Bedroom	15.7 (19.1)	94.4	25 (83.3)	17.4
	2nd room	21.5 (28.5)	106.7	24 (82.8)	23.9
	Outdoor	0.9 (3.9)	21.1	3 (10.3)	–
m/o/p-Xylenes	Bedroom	15.6 (18.2)	92.0	27 (90.0)	4.2
	2nd room	15.7 (20.6)	81.0	27 (93.1)	4.2
	Outdoor	3.7 (4.4)	21.0	22 (75.9)	–
Naphthalene	Bedroom	0.1 (0.4)	1.6	3 (10.0)	>10[d]
	2nd room	0.2 (0.5)	1.9	4 (13.8)	>10[d]
	Outdoor	n.d.	n.d.	n.d.	–
Nonanal	Bedroom	3.6 (3.8)	10.3	16 (53.3)	6.0
	2nd room	3.4 (3.5)	12.0	16 (55.2)	5.7
	Outdoor	0.6 (1.5)	6.8	5 (17.2)	–
Nonane	Bedroom	0.5 (1.7)	8.0	3 (10.0)	>10[d]
	2nd room	0.8 (2.6)	10.3	3 (10.3)	>10[d]
	Outdoor	n.d.	n.d.	n.d.	–
Octane	Bedroom	0.5 (2.1)	9.3	2 (6.6)	2.5
	2nd room	1.7 (4.9)	24.1	5 (17.2)	8.5
	Outdoor	0.2 (1.3)	6.9	1 (3.4)	–
Styrene	Bedroom	1.2 (4.3)	22.7	5 (16.6)	12.0
	2nd room	0.8 (2.7)	13.6	4 (13.8)	8.0

(*continued on next page*)

Table 6 (*continued*)

	Location	Mean (SD)[a]	Max[a]	n (DF)[b]	I/O ratio[c]
	Outdoor	0.1 (0.2)	0.8	3 (10.3)	–
Tetrachloroethylene	Bedroom	0.6 (1.7)	7.9	4 (13.3)	6.0
	2nd room	0.1 (0.6)	2.8	2 (6.9)	1.0
	Outdoor	0.1 (0.5)	2.8	2 (6.9)	–
Tetradecane	Bedroom	0.1 (0.8)	4.3	1 (3.3)	>10[d]
	2nd room	0.4 (1.1)	3.9	3 (10.3)	>10[d]
	Outdoor	n.d.	n.d.	n.d.	–
Toluene	Bedroom	16.6 (16.5)	67.1	29 (96.6)	3.5
	2nd room	14.8 (23.0)	110.7	28 (96.6)	3.1
	Outdoor	4.7 (4.3)	21.7	26 (89.7)	–
Undecane	Bedroom	1.2 (4.3)	22.5	3 (10.0)	>10[d]
	2nd room	1.6 (4.3)	17.1	4 (13.8)	>10[d]
	Outdoor	n.d.	n.d.	n.d.	–
α/β-Pinenes	Bedroom	10.1 (11.5)	52.2	23 (76.7)	16.8
	2nd room	7.3 (7.5)	31.8	23 (79.3)	12.2
	Outdoor	0.6 (2.8)	15.1	2 (6.9)	–
TVOC	Bedroom	297.8 (217.5)	59.2–978.3	30 (100)	4.9
	2nd room	249.1 (151.5)	46.9–604.1	29 (100)	4.1
	Outdoor	60.6 (50.9)	15.7–278.4	29 (100)	–

DF, detection frequency; Max, maximum; n.d., not detected; SD, standard deviation; TVOC, total volatile organic compounds.

[a] Values are given in μg/m^3.

[b] n corresponds to the number of samples with concentrations above of the limit of detection (LOD) and DF to the detection frequency, in percentage, considering the total number of samples. LOD values (μg/m^3): 0.4 for benzene, 0.6 for limonene, 0.9 for toluene, 1.0 for octane, 1.1 for ethylbenzene and tetrachloroethylene, 1.2 for 1,2,4-trimethylbenzene, 1.3 for styrene, 1.6 for 2-ethylhexanol and 1.9 for 2-phenoxyethanol.

[c] I/O ratios of individual VOC were calculated using the mean concentration obtained in each sampling location.

[d] Substances exclusively detected indoors were considered as having a percentage of detection higher than 10.

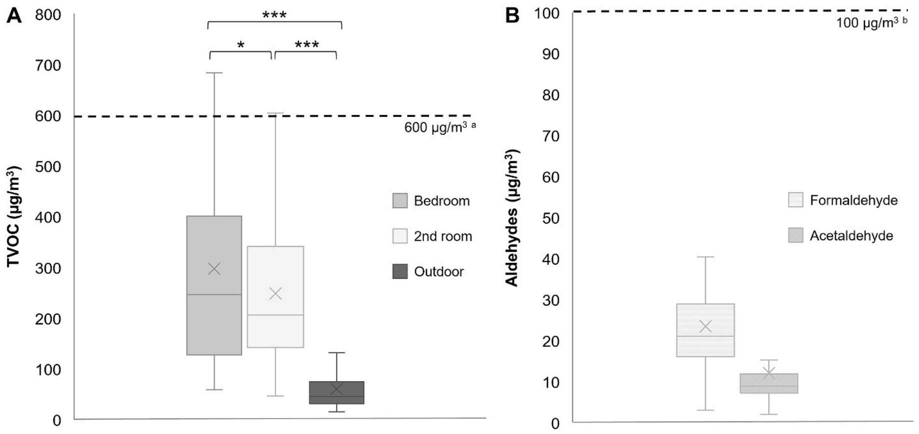

Fig. 3. Box-plot representing airborne total volatile organic compounds (TVOC) (A) and aldehydes (B) concentrations. The bottom and the top of the boxes represent 25th and 75th percentiles. The band near the middle of the box and the X represent the median and the mean values, respectively. The ends of the whiskers indicate 10th and 90th percentiles. Significant differences were determined by Wilcoxon tests (*p < .05, **p < .01, ***p < .001). [a] National recommended limit (Portaria n.o 353-A/2013 de 4 de dezembro, 2013). [b] WHO and national guidelines for formaldehyde Portaria n.o 353-A/2013 de 4 de dezembro, 2013; WHO, 2010).

fragranced products, including air-fresheners, PCP and HCP, has been recently associated to a wide spectrum of health problems including respiratory constraints, mucosal symptoms, headaches, dermatological-related effects, asthma attacks and neurological problems (Steinemann, 2019). Compared to the results obtained from the study conducted in 2012/2013, in which the prevalence of use of air-fresheners was of about 25% in homes of families with school age children, the percentage found here (50%) suggests a trend for the increased use of this kind of consumer products. The regular use of automatic aerosol insecticide products also reported for 20% of the households surveyed was shown to significantly impact concentrations of ethylbenzene in bedrooms ($U = 37.0$, $z = -2.1$; $p = .022$).

D5, 2,2,4,4,6,8,8-heptamethylnonane, 2-phenoxyethanol, styrene, tetrachloroethylene and α/β-pinenes were the substances identified as having their origin from sources present in the bedroom microenvironment due to the respective I/O that was higher, in at least one unity, in the bedroom than that assessed in the 2nd room. The three first substances are typically released to the environment mainly from cosmetics and PCP; styrene and α/β-pinenes can be emitted from washing and cleaning products, paints and coating or adhesives, toys, fragrances and air fresheners; and tetrachloroethylene has been described as a common component of dry cleaning agents, but also of treated fabric and toys (European Chemicals Agency, n.d.; United States Environmental Protection Agency, n.d.).

The cyclic siloxane D5 was the VOC identified that recorded the higher maximum indoor concentration. This substance was recently assessed to be persistent, bioaccumulative and toxic (PBT) and, then, classified a new Substance of Very High Concern (SVHCs) by the European Union (EU) under the REACH regulation (European Chemicals Agency, 2018). D5 was detected in 37% of the bedrooms in concentrations that varied from 37 to 736 μg/m^3 (average in homes with indoor D5 concentration > LOD: 222 μg/m^3). These concentrations are very similar to the mean values found in homes located in Barcelona (23–293 μg/m^3) (Companioni-Damas et al., 2014), Italy (38–170 μg/m^3), and UK (45–150 μg/m^3) (Pieri et al., 2013). Nevertheless, the airborne D5 concentrations obtained in this study were expressively higher than those reported in some of existing reports for residential buildings, namely from studies conducted in USA (Albany, New York) (18–812 ng/m^3) (Tran and Kannan, 2015), Vietnam (not detected to 600 ng/m^3) (Tran et al., 2017) and Sweden (300–2300 ng/m^3) (Sha et al., 2018). The differences in the exposure to D5 can result from different consumption patterns observed between different regions. The German

Environment Agency has defined a total guide value for indoor exposure to cyclic dimethylsiloxanes (sum of D3-D6 concentrations) of 4000 $\mu g/m^3$ and a precautionary guideline value of 400 $\mu g/m^3$ (Umweltbundesamt, 2019). Based on D5 concentrations assessed, the German precautionary limit value was exceeded in the indoor air of bedroom and 2nd room of one household (H13). No additional cyclic volatile methylsiloxanes were detected. Linear volatile methylsiloxanes, including dodecamethylpentasiloxane (L5, bedroom: 10.9 $\mu g/m^3$; 2nd room: 5.3 $\mu g/m^3$), tetradecamethylhexasiloxane (L6, bedroom: 29.8 $\mu g/m^3$; 2nd room: 14.6 $\mu g/m^3$) and hexadecamethylheptasiloxane (L7, bedroom: 16.3 $\mu g/m^3$; 2nd rom: 9.8 $\mu g/m^3$) were detected only in one household (H30), for which no quantifiable levels of D5 were obtained.

Results from the first phase of this work – recruitment at the maternity wards – showed that 75% of the bedrooms of newborn children (n = 446) underwent renovations, including introduction of new furniture and remodeling or painting works, carried out during the pregnancy (Gabriel et al., 2020). Considering the higher emission rates reported for recently manufactured materials in the recently refurbished rooms (Huang et al., 2017) the indoor formaldehyde was highlighted as an important IAQ issue in early years, and thus, it was included in the priority set of parameters to assess in infant bedrooms. According to the information collected for the 30 bedrooms that were comprehensively surveyed, the percentage of recent refurnishing (last 12 months) was even higher (93%) than the obtained during recruitment. Despite this, formaldehyde was detected (mean ± SD: 23.5 ± 12.3 $\mu g/m^3$) in all samples at lower concentration than the guidelines values established by both the WHO and national guidelines (Portaria n.o 353-A/2013 de 4 de dezembro, 2013; WHO, 2010) of 100 $\mu g/m^3$ (Fig. 3B). Indoor formaldehyde concentrations are consistent with those reported in households of other European countries (Brown et al., 2015; Rovira et al., 2016). Nevertheless, seven bedrooms exceeded the guideline value of 30 $\mu g/m^3$ proposed for the prevention of irritant effects (Kotzias et al., 2005). In fact, at the measured levels, formaldehyde can indeed constitute a relevant risk for occupants' health (Rovira et al., 2016). Formaldehyde levels appeared to be significantly associated to higher indoor temperatures ($r_s = 0.47$, $p = .009$). Indoor acetaldehyde levels sampled in the bedroom ranged from 2 to 75 $\mu g/m^3$ (mean ± SD: 12.1 ± 12.8 $\mu g/m^3$) and were significantly and negatively correlated with the building construction date ($r_s = -0.40$, $p = .028$). A similar, but non-statistically significant, trend was found for formaldehyde levels, with more recent buildings presenting lower indoor concentrations. Regarding indoor sources, higher levels of both aldehydes were observed in bedrooms of households where incense/candles are used (formaldehyde: 27.0 vs 22.4 $\mu g/m^3$; acetaldehyde: 20.9 vs 9.4 $\mu g/m^3$).

3.5. Indoor concentrations of CO, NO_2 and O_3

For CO, levels expressively lower than the respective WHO guideline for 24hr-exposure (7 mg/m^3 (WHO, 2010)) were observed for both indoor locations (Fig. S3, Supplementary Material). Nevertheless, significantly higher CO concentrations were found in the 2nd room of houses for which indoor smoking (n = 3) is reported (mean CO concentrations 0.5 vs 0.1 mg/m^3; $U = 5.0$, $z = 2.5$, $p = .004$). Although no significant differences were noticed for CO concentrations measured in the bedrooms and of the limited number of families who reported indoor smoking (3 out of 30) these results suggests that smoking indoors can significantly increase the levels of tobacco smoke-related pollutants in the living environment, and should be thus completely avoided in homes with vulnerable people.

In addition, no significant differences were found for concentrations of CO, of NO_2 and O_3 between homes audited in different seasons. Mean concentrations of NO_2 and O_3, only assessed in the infant bedrooms, were below the limit values (200 $\mu g/m^3$ 1-hr mean (WHO, 2010) and 100 $\mu g/m^3$ 8-hr mean for ambient air (WHO, 2018), respectively) in all households. Nevertheless, mean NO_2 concentrations measured in all bedrooms were equal or higher than the annual limit values for the protection of human health (40 $\mu g/m^3$ (WHO, 2010)). No significant correlations were found for any indoor characteristics, in particular information on existence of combustion-based items, collected by checklist. This, along with the fact that NO_2 is regarded as a major component and common proxy of traffic-related air pollution (Achakulwisut et al., 2019), suggests that indoor levels are strongly impacted by outdoor levels. Although outdoor measurements were not carried out, this can be partially supported by the observed significantly higher NO_2 concentrations found in the bedroom in periods of non-occupancy than occupied periods ($U = 307.5$, $z = -2.1$, $p = .035$). In fact, non-occupancy periods typically include the characteristic peak NO_2 levels in the outdoor environment, which coincide with morning and afternoon rush hours (Farraj et al., 2016). In line with this, a study recently conducted by our group showed that ambient NO_2 levels were expressively high in the Northern Portugal (Gabriel et al., 2019). In particular, from analysis of this previous study's data sets it can be observed that for outdoor sampling conducted in PMA (n = 14) between January and July 2018 presented an average diurnal NO_2 concentration of 149.4 $\mu g/m^3$ (data not shown).

Concerning airborne O_3, very low concentration were found in infant bedrooms. If the comparison with our recent internal records for O_3 concentrations measured in outdoor locations in the PMA (January to July 2018, 64.8 $\mu g/m^3$, data not shown) is established, it can be determined that an 11-fold lower concentration is found in bedrooms. This discrepancy suggests the existence of considerable sinks (as building surfaces and decorations) (Shen and Gao, 2018) for indoor O_3 and/or of abundant indoor chemistry through reactions between ozone and unsaturated compounds in the indoor atmosphere (Weschler and Carslaw, 2018). In this regards, statistically significant lower indoor concentrations were found in households where families reported the use of liquid detergents/cleaning agents other than bleach or ammonia based products more frequently than once per/week along with the use of air fresheners ($U = 0.0$, $z = -2.3$, $p = .004$). As referred above, many HCP, air fresheners and aromatizers contain terpenoids and related compounds that volatilize during the product application/diffusion (Singer et al., 2006). Some of these compounds, mostly limonene and α-pinene, react quickly with O_3 (ozone/terpene reactions) to form secondary pollutants, such as formaldehyde and acetaldehyde, hydroxyl (OH) radical, fine and ultrafine particles (Nazaroff and Weschler, 2004). This is likely to explain the reduced levels of O_3 in households using a high variety of HCP and fragranced products.

3.6. Study limitations

As far as we know this is the largest study to comprehensively assess IEQ covering the concurrent and harmonized assessment of a subset of physical-chemical parameters in two indoor environments in which infant twins spend most of the time at home. It is expected that the results presented in this work are representative of the indoor air conditions of the studied households. As some significant associations were established between the characteristics of the dwellings and pollutant levels, the findings presented here could be also, at least in some extent, extrapolated to the generality households of families with infants in the region. Nevertheless, some degree of uncertainty related to characteristics of the executed study design need to be properly disclosed and carefully taken into consideration. The limited duration of the monitoring and sampling work devoted to studying the air quality in each home is very likely to introduce some bias in the representativeness of the reported concentrations for the households conditions verified in the whole year. In fact, since a seasonal trend for some of the parameters assessed was observed in homes surveyed in different seasons, and in agreement to previous research, two auditing campaigns in different seasons would be preferential for a better and more realistic evaluation of exposure taking into consideration seasonal variations. $PM_{2.5}$, PM_{10}, UFP, O_3 and NO_2 were only assessed for one day of study in each house due to: i) difficulties associated with the high volume of equipment located inside the bedroom (Fig. S2, Supplementary Material); and ii) the use of equipment and aerosol monitors that generate an unpleasant noise. This is very likely to introduce some degree of uncertainty in the results namely those from statistical analysis with parameters that were assessed during a greater sampling time (e.g. VOC and aldehydes). Measurement of O_3 and NO_2 was only conducted in the bedrooms due to lack of equipment availability for simultaneous assessment in both indoor spaces. Some parameters as $PM_{2.5}$, PM_{10}, O_3 and NO_2 were assessed through methodologies that are different of the recommended reference methods. Thus, the concentrations reported here for $PM_{2.5}$, PM_{10}, O_3 and NO_2 should be regarded as approximate values and considerations resultant from comparison of the assessed levels with internationally recognized limit values need to be interpreted with caution. Regarding the 2nd room in which infants spend more time, after the bedroom, results for IEQ taking into consideration the differential typologies of the rooms assessed (living room, kitchen, other room) were not fully addressed since the situations in which the room was not the living room were very scarce (13%) and results of these situations were not differentiating. In fact, for the only case in which the 2nd room audited was the kitchen levels of pollutants measured ($PM_{2.5}$: 18.8 μg/m^3, PM_{10}: 22.0 μg/m^3, UFP: 5513 pt/cm^3; TVOC: 368.3 μg/m^3) were, on average, in the typical range obtained for the overall sample of 2nd rooms. The ease of installation of samplers for VOC in a window or a balcony alongside with the lack of a sheltered location for the installation of the remaining continuous monitoring equipment were the reasons for VOC being the only parameter to be consistently assessed both indoors and outdoors for all households. It is recognized that this lack of data for the remaining array of IEQ indicators significantly limits the ascertainment of the real contribution of ambient air pollution in the overall household IEQ. Moreover, because concentrations lower than LOD were treated as zero for calculations, an eventual underestimation of the amount of some substances cannot be excluded.

4. Conclusion

This research shows that the IEQ homes can reflect in some extent the patterns and types of emissions that take place in the outdoor neighboring areas (such as traffic-related pollution) mainly in terms of PM, NO_2 and some specific VOC concentrations. However, emissions occurring indoors from construction materials, HCP, PCP and other consumer products and activities indoors (e.g. tobacco smoke) were shown to be of major concern for the correct management of chemical pollution in households. In addition, the results reported in this study call special attention to the fact that existing regulation tend to focus in controlling emissions from building materials, especially on concentrations of formaldehyde, benzene, and toluene, with less attention being paid to potentially hazardous airborne substances that can be emitted from the use of PCP, HCP and other fragranced products which are known to have a substantial contributor to air pollution in domestic environments (Yeoman et al., 2020). Findings presented in this work support the theory that the public is largely unaware of the potential adverse and often serious health effects related to exposures resulting from the use of fragranced consumer products. Since rooms with poor ventilation promote conditions for accumulation of pollutants emitted inside the household, families should be informed that in the case of small rooms with poor ventilation it is even more important to avoid declared pollutant sources and manage ventilation in the rest of the household properly. A list of recommendations and best practices (Fig. S4, Supplementary Material) was produced based on the main findings of this work to empower participant families to actively manage their own exposures at home.

According to the environmental data collected, it is very likely that the home's environment may influence the maturation of the immune and respiratory systems of the infants, possibly disturbing and reflected in the further development of NCDs. Further studies aiming at the follow-up of the indoor conditions to which the participant twins are exposed at home along with clinical assessments are being planned for next years. This will allow to ascertain eventual changing patterns of indoor air pollution as well as identifying health-relevant indoor exposures throughout the childhood.

Overall, this work provides important insights to establish evidence-based educational/awareness campaigns to promote public health by creating healthy households for children.

Author statement

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Declaration of competing interest

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

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Appendix A. Supplementary data

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