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Determinants of paediatric asthma: a three-level approach

Inês Ribeiro Paciência

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Determinants of paediatric asthma: a three-level approach

Tese de Candidatura ao grau de Doutor em Ciências Biomédicas, submetida ao Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto

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No artigo "*A systematic review of evidence and implications of spatial and seasonal variations of volatile organic compounds in indoor human environments*" fui responsável pela revisão da literatura, seleção dos artigos incluídos na revisão sistemática e elaboração do artigo. Na publicação "*Exposure to indoor endocrine disrupting chemicals and childhood asthma and obesity*" estive envolvida e fui responsável pela conceptualização e interpretação do estudo, análise estatística dos dados e na redação do artigo. Da mesma forma, no artigo "*School environment is associated with lung function and autonomic nervous system activity in children: a cross-sectional study*" estive envolvida e fui responsável pela conceptualização e interpretação de resultados, mapeamento da utilização do espaço e caminhabilidade em redor das escolas avaliadas, análise estatística e elaboração do artigo. No artigo "*School neighbourhood impacts children obesity and body composition*" fui responsável pela análise dos resultados, análise estatística, mapeamento da utilização do espaço em redor das escolas, interpretação dos resultados e elaboração do artigo. Por último, no artigo "*Swimming pool training environment may drive skin and gut dysbiosis in elite swimmers*" estive envolvida e fui responsável pela recolha e análise laboratorial das amostras, análise estatística, interpretação dos resultados e elaboração do artigo.

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"Those who pass by us, do not go alone, and do not leave us alone; they leave a bit of themselves, and take a little of us."

Antoine de Saint-Exupéry

Contents

List of scientific papers

This thesis is based on the following papers, which are referred to in the text by their numerals:

I. Paciência I., Madureira J., Rufo JC., Moreira A., Fernandes EO.. A systematic review of evidence and implications of spatial and seasonal variations of volatile organic compounds in indoor human environments. Journal of Toxicology and Environmental Health Part B 19(2) 2015. DOI: 10.1080/10937404.2015.1134371

II. Paciência I, Cavaleiro Rufo J, Silva D, Martins C, Mendes F, Farraia M, Delgado L, Fernandes EO, Padrão P, Moreira P, Severo M, Barros H, Moreira A. Exposure to indoor endocrine disrupting chemicals and childhood asthma and obesity. Allergy. 2019. doi:10.1111/all.13740.

III. Paciência I, Cavaleiro Rufo J, Silva D, Martins C, Mendes F, Rama T, Rodolfo A, Madureira J, Delgado L, Fernandes EO, Padrão P, Moreira P, Severo M, Pina MF, Teixeira JP, Barros H, Ruokolainen L, Haahtela T, Moreira A. School environment is associated with lung function and autonomic nervous system activity in children: a cross-sectional study. Scientific Reports. 2019;9(1):15156. doi:10.1038/s41598-019-51659-y.

IV. Paciência I, Cavaleiro Rufo J, Mendes F, Farraia M, Cunha P, Silva D, Delgado L, Padrão P, Moreira P, Moreira A. School neighbourhood impacts children obesity and body composition. *Under review in BMC Public Health*

V. Paciência I, Pereira P, Aho V, Cavaleiro Rufo, Silva D, Martins C, Mendes F, Rama T, Rodolfo A, Leão L, Delgado L, Padrão P, Moreira P, Barros H, Haahtela T, Paulin L, Auvinen P, Moreira A. Swimming pool training environment may drive skin and gut dysbiosis in elite swimmers.

Abstract

During the 20th century, urbanization has increasing and represented a major demographic and environmental change in developed countries. Urban living may offer a greater possibility to better health care, education and social services, but is also associated with increased exposure to air pollution, outdoors and indoors, loss of natural environments and biodiversity and lifestyle changes. Furthermore, this ever-changing urban environment has an impact on diseases patterns and prevalence, namely on noncommunicable diseases, such as asthma and allergy, and poses many challenges to understand the relationship between the changing on the urban environment and the children health.

The overall aim of this thesis was to study the role of the environmental determinants of paediatric asthma.

Study I aimed to review, summarize and compare recent quantitative data concerning different human environments - schools, housing, offices and other indoor settings - and seasonal variation of volatile organic compounds (VOC) concentrations that were detected, measured and reported both indoors and outdoors. Papers published from 2000 onwards were reviewed and 40 studies were included in this review. On average, higher mean concentrations of VOC were found indoors in cold season. Volatile organic compounds were commonly present in the indoor air and specific compounds presence and their concentrations may vary with the indoor environments and the seasons, indicating corresponding differences in indoor sources.

Associations between endocrine-disrupting chemicals exposure and asthma, respiratory symptoms and obesity were assessed in 845 schoolchildren in **study II**. Exposure to endocrine-disrupting compounds in classrooms was associated with an increased risk of asthma, prevalence of nasal obstruction symptoms in previous 3 months, as well as with obesity and percentage of body fat. Moreover, even at low levels of exposure, a significant relationship between compounds grouped by principal component analysis and current symptoms and body composition were observed.

Study III included 701 children from a cross-sectional study assembled in Porto, Portugal. This is the first community-based study evaluating the effect of schools' surrounding neighbourhoods on lung function, airway reversibility and inflammation, and autonomic nervous system activity. Our findings suggest that the presence of urban green areas has a positive effect on lung function. Further, we provide proof of concept for the role of the autonomic nervous system in mediating the interaction between the environment and the individual.

Study IV aimed to assess the effect of school neighbourhoods on obesity and body composition in schoolchildren. Data on 701 children (7-12 years old) from 20 primary schools were analysed. Green urban areas around schools showed a tendency to be associated with lower values of body mass index (BMI) and better body composition parameters compared with built areas. This study showed that the presence of urban green areas around schools may promote a framework of lifestyle or behaviours conducive to achieving a healthy weight.

Study V aimed to evaluate the effect of the indoor swimming pool environment on skin and gut microbiome. Skin and stool samples were collected from 29 elite swimmers and 34 football players. The microbiome was profiled by 16S rRNA gene amplicon sequencing approach. Skin microbial diversity was significantly lower in swimmers compared to non-water athletes, a significant difference in microbiome composition between the two groups was also observed. Alpha diversity was similar in stool samples from both groups; however, there was a significant difference in beta diversity between swimmers and non-water athletes. Our results suggest that the swimming pool training environment may have an important role in shaping skin and gut microbiome. These results emphasize the importance of training environment in the composition and diversity of microbiome, suggesting that adverse health effects may be mediated by changes on microbiome.

Resumo

Ao longo do último século observou-se uma tendência crescente e rápida na urbanização, representando uma grande mudança demográfica e ambiental, principalmente nos países desenvolvidos. Apesar das cidades poderem oferecer oportunidades de acesso aos serviços de saúde, educação e sociais, estão também associadas a um aumento da exposição à poluição do ar, no exterior e no interior, diminuição de ambientes naturais e da biodiversidade e alterações nos estilos de vida. A alteração crescente do meio ambiente está igualmente associada ao aumento da prevalência de algumas doenças, nomeadamente, de doenças crónicas como a asma e as alergias e, representando por isso diversos desafios na compreensão da relação entre as mudanças no ambiente na saúde das crianças.

O objetivo da presente tese é investigar o papel dos determinantes ambientais da asma e da alergia pediátrica.

O **estudo I** teve como objetivo apresentar uma revisão sistemática, sumariar e comparar estudos quantitativos que avaliaram a concentração de compostos orgânicos voláteis (COV) em diferentes espaços interiores - escolas, habitações, escritórios e outros ambientes interiores (), considerando as variações sazonais e a influência do ambiente exterior. Foram considerados os estudos publicados após o ano de 2000, tendo sido incluídos 40 estudos para efeitos de revisão. Foram reportadas concentrações médias mais elevadas de COV em ambientes interiores, do que nos exteriores, principalmente durante o período de aquecimento. Os COV são ubíquos no ambiente interior, mas a presença de compostos específicos e a sua concentração pode variar entre ambientes interiores e as estações do ano, correspondendo às diferentes fontes de emissão localizadas no interior dos espaços e no exterior.

A associação entre a exposição a disruptores endócrinos (EDCs) e a asma, sintomas respiratórios e obesidade foi avaliada em 845 crianças em idade escolar (**estudo II**). A exposição a EDCs no interior das salas de aula está associada a um aumento no risco de asma, prevalência de sintomas de obstrução nasal nos últimos 3 meses, bem como a um risco aumentado de obesidade e aumento da percentagem de massa gorda. A exposição a baixas concentrações de uma mistura de EDCs, avaliada com base na análise de componentes principais, está associada a um aumento de sintomas e alterações na composição corporal das crianças.

O **estudo III** incluiu 701 crianças de um estudo transversal realizado no Porto, Portugal. Este é o primeiro estudo de base populacional a avaliar o efeito do ambiente em redor das escolas na função pulmonar e reversibilidade das vias aéreas, na inflamação e na atividade do sistema nervoso autónomo. Os resultados sugerem a existência de um efeito positivo da presença de

áreas verdes urbanas em redor das escolas na função pulmonar. O estudo sugere ainda o papel do sistema nervoso autónomo na mediação da interação entre o ambiente e o indivíduo.

O **estudo IV** teve como objetivo avaliar o efeito do ambiente em redor da escola na obesidade e na composição corporal de crianças. Foram analisados os dados de 701 crianças, com idades entre os 7 e os 12 anos, de 20 escolas públicas primárias. Os resultados mostram uma tendência entre a presença de áreas verdes urbanas em redor da escola e valores mais baixos de índice de massa corporal comparativamente com as áreas urbanas construídas. Neste estudo observou-se ainda que a presença de áreas verdes urbanas pode promover estilos de vida e comportamentos conducentes a um peso saudável.

No **estudo V** foi avaliado o efeito do ambiente interior de uma piscina no microbioma da pele e intestino. Para tal, foram colhidas e analisadas amostras de pele e fezes de 29 nadadores de alta competição e de 34 jogadores de futebol. O microbioma foi analisado através da sequenciação do gene 16S rRNA. A diversidade do microbioma da pele foi significativamente menor nos nadadores em comparação com o dos jogadores de futebol. Observou-se também uma diferença significativa na composição do microbioma entre os dois grupos. A diversidade alfa foi semelhante nas amostras de fezes dos dois grupos; no entanto, observou-se uma diferença significativa na diversidade beta entre nadadores e jogadores de futebol. Os nossos resultados sugerem que o ambiente da piscina tem um efeito na diversidade e composição do microbioma, sendo que estas alterações podem estar associadas a efeitos adversos na saúde.

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Abbreviations

ACV	Average constriction velocity
ADV	Average dilation velocity
aHR	Adjusted hazard risk
ANS	Autonomic nervous system
ARIA	How indoor air quality can affect children allergies and asthma – study
Ars	Androgen receptors
ATS	American Thoracic Society
BD	Bronchodilatation
BF	Body fat
BHT	Butylated hydroxytoluene
BMI	Body mass index
BMR	Basal metabolic rate
CCL17	C-C motif chemokine ligand 17
CDC	Centres for Disease Control and Prevention
CEHAPE	Children's Environment and Health Action Plan for Europe
CI	Confidence interval
CTLA4	Cytotoxic T lymphocyte-associated protein 4
DBPs	Disinfection by-products
DCs	Dendritic cells
DES	Diethylstilboestrol
DDT	Dichloro-diphenyl-trichloroethane
EBC	Exhaled breath condensate
EDCs	Endocrine disrupting compounds
e-NANC	Excitatory non-adrenergic non-cholinergic
EPA	Environmental Protection Agency
ERS	European Respiratory Society
ERs	Estrogen receptors
ESRI	Environmental Systems Research Institute
FVC	Forced vital capacity
FEV1	Forced expiratory volume in the first second of FVC
FEF25-75	Forced expiratory flow in the middle portion of FVC
FFM	Free fat mass

FOXP3	Forkhead box P3
GC-ECD	Gas chromatography coupled with an electron capture detector
GC-FID	Gas chromatography coupled with flame ionization detector
GC-MS	Gas chromatography coupled with mass spectrometry
GC-MSD	Gas chromatography coupled with mass selective detector
GINA	Global Initiative of Asthma
GIS	Geographical information system
GWAS	Genome-wide association studies
HDM	House dust mite
HPA	Hypothalamic-pituitary-adrenal
HPLC	High performance liquid chromatography
IAQ	Indoor air quality
ICC	Intraclass correlation coefficient
ICS	Inhaled corticosteroids
IL	Interleukin
ILC2	Induce type 2 innate lymphoid cells
i-NANC	Inhibitory non-adrenergic non-cholinergic
I/O	Indoor/outdoor ratio
IOM	Institute of Medicine
IOTF	International Obesity Task Force
IPCS	International Programme on Chemical Safety
ISAAC	International Study of Asthma and Allergies in Childhood
MCV	Maximum constriction velocity
NANC	Non-adrenergic non-cholinergic
NDVI	Neighbourhood normalized difference vegetation index
NMDRs	Non-monotonic dose responses
PAHs	Polycyclic aromatic hydrocarbons
PAR-2	Protease-activated receptor-2
PCA	Principal component analysis
PC	Principal component
PM	Particulate matter
PNS	Parasympathetic nervous system
PPAR	Peroxisome proliferator activated receptors
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses

POPs	Persistent organic pollutants
OR	Odds ratio
OTUs	Operational taxonomic units
RXR	Retinoic X receptor
SCHER	Scientific Committee on Health and Environmental Risks
SD	Standard deviation
SNS	Sympathetic nervous system
SPSS	Statistical Package for the Social Sciences
SPT	Skin prick test
SWAN	Swimming pool environment impact on the human respiratory health research protocol
TBW	Total body water
TEDX	Endocrine Disruption Exchange Research Institute
TH	T helper
TRs	Thyroid receptors
TRP	Transient receptor potential
TRPA1	Transient receptor potential ankyrin
TRPV1	Transient receptor potential vanilloid type 1
TSLP	Thymic stromal lymphopoietin
T4CE	Tetrachloroethylene
T75	Total time taken by the pupil to recover 75% of its initial resting diameter after it reached the peak of constriction
UNEP	United Nations Environment Programme
VOC	Volatile organic compounds
WHO	World Health Organization

1. Introduction

The world is rapidly urbanizing with significant changes in lifestyles, behaviours and health [1]. As reported by the United Nations (2014), in 1950 one-third of people lived in urban areas but, by 2050, two-thirds of the world's population will be living in urban areas with reduced green spaces and limited contact with plants, animals and biodiversity [2]. Urban living offers many socio-economic opportunities, such as access to education, social services and healthcare, while the unhealthy diets, physical inactivity and exposure to urban air pollution outdoors and indoors points to many emerging environment and health hazards [1]. Rapid and often unplanned urban growth is also associated with environmental degradation, reduced green spaces and limited contact with biodiversity [3] and with increasing commercialization and widespread use of chemicals [4].

Over the past decades, urbanization and the Western lifestyle have been linked to the rising prevalence of inflammatory disorders, including asthma and allergic diseases. Epidemiological studies have demonstrated that several urban factors, such as traffic-related air pollution, and household characteristics, are associated with increased risk of asthma-related symptoms [5, 6]. Additionally, the rapid development of new building materials and consumer products have resulted in a changing pattern of production and use of chemical compounds, and consequently to an increase of their impact indoors [4]. Some of these chemicals have been associated with an increasing prevalence of asthma among children [7, 8]. However, the possible pathways whereby they influence the development of asthma are complex and interactive.

Indoor and outdoor environment has received particular attention in recent years, since people spend more than 90% of their lives inside buildings and this situation may be particularly distressing among children. Parental fears, loss of natural environments in urban cities, exposure to indoor chemicals, and the ever-increasing time in schools underlines the importance of a better understanding of the effect of environment exposure on children's health. In fact, and according to WHO Healthy Cities Project Office, connecting public health and urban planning is an opportunity to understand the impact of urbanization and the implications of urban exposures on health, providing information to build up successful community-based prevention efforts [9].

Despite the substantial body of evidence and the attention given to the importance of air pollutants and physical environments around homes [10-13], there is a need to better understand the effect of school environmental determinants, such as exposure to emerging pollutants and neighbourhood characteristics, on children health. Therefore, adapting the "Holobiont" concept [14], which conceptualize the dynamic interaction between host and its microbial community in an environmental context, this thesis considered three interactive levels: individual "*Who are you?*",

indoor environment “*Where are you?*” and neighbourhood “*Where do you live?*” levels (**Figure 1**). Similarly to “holobionts” (host and all of its symbiotic microbes) and their “hologenome”, the dynamic interaction between the human being and health is determined by many factors, including genetic, lifestyle, and environmental interactions. At the end, it is not only who you are, but also and mostly, where and how you live that determine your health [15]. Therefore, this thesis aimed to study the role of environmental determinants of paediatric asthma.

Figure 1 Levels of paediatric asthma determinants

2. Background

2.1. Level I: Who are you?

Individual

According to the Organization for Economic Co-operation and Development (OECD) the impact of environmental risk factors on health are diverse and complex in both severity and clinical significance [16]. The WHO reported that 24% of the global burden of disease and 23% of all deaths are attributable to environmental factors, being their effects not equally distributed across all age groups [17]. Children may be particularly susceptible to the adverse environmental effects, since their respiratory, immune and central nervous systems are not fully developed, and due to their size, physiology and activity level, children inhalation rates are higher than adults exhibiting also higher oxygen consumption and resting metabolic rate per unit of body weight [18, 19]. Additionally, environmental exposures at childhood may also increase disease risk later in life [20]. Environmental factors such as air pollutants have also been linked to onset, progression and manifestation of asthma by inducing epigenetic effects [21]. Peng *et al.* [21] showed that epigenetic age acceleration at mild childhood was associated with a higher odds of asthma, which may reflect perturbations in epigenetic maintenance.

Increasing urbanization and consequent environmental degradation through air pollution, loss of natural areas and lifestyle changes are contributing to the increased prevalence of non-communicable diseases [22]. The European Union Environment and Health Action Plan (EHAP) also highlights the concern about health effects associated with environmental determinants, including respiratory diseases, asthma and allergies, particularly those affecting vulnerable groups such as children [23].

2.1.1. Asthma definition

Asthma has been recognized since antiquity in many cultures, including the Chinese's, Hebrews', Greeks' and Romans' [24]. Hippocrates (4th century BC) described asthma as a complex of symptoms rather than a specific disease entity, which derived from the Greek word *αση*θ*μα*, meaning a short-drawn breath, hard breathing, or death rattle [25]. Hippocrates was also the first to report the association between asthma and environmental factors [25]. Moreover, in the 16th century, Georg Bauer described an association between environment and airway symptoms [24]. In 1860, Henry Hyde Salter proposed a formal definition of asthma based on airway remodelling, stating that "if it is at all severe and its attacks frequent, cannot long exist without inflicting

permanent injury to the lungs". Some years later, William Osler (1894) described the pathological changes within the asthmatic airways, recognized in bronchial hyperresponsiveness [24, 25].

Furthermore, over the years, the neuro-psychogenic origin of asthma has also been reported [24, 26]. For instance, Krimer, in 1819, was one of the first investigators to demonstrate the role of vagus nerves on the contractile action of the airway smooth muscle. In the beginning of the 20 century, the relationship between asthma and several allergies and its neural, inflammatory and vascular mechanisms gained more attention. In 1982, Sant' Ambrogio and Widdicombe reported that C-fiber receptors are stimulated by mediators and chemical irritants involved in allergic tissue reactions [26]. Additionally, Ingram [27] and Kaliner *et al.* [28] have showed changes in the autonomic regulation of the airways, which may lead to bronchospasm, airway edema and excessive mucous secretion. Recently, asthma has been associated with abnormal autonomic nervous system activity, characterized by a bronchial hypersensitivity to cholinergic and non-adrenergic non-cholinergic (NANC) constrictors, and a decreased sensitivity to adrenergic and NANC dilators [29].

Despite substantial advances in understanding the pathogenesis, genetics, and clinical features of asthma, a comprehensive definition remains difficult to construct. Recently, The Lancet Commissions described asthma as an "umbrella term" that varies in severity, onset, comorbidities, risk factors, triggers, in response to treatment, genetics and natural history [25, 30] This heterogeneity is reflected in the currently used definition of the Global Initiative of Asthma (GINA). In 2019, GINA defined asthma as an "heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation" [31].

2.1.2. Epidemiology of paediatric asthma

The prevalence of asthma has increased worldwide over the past decades and continues to increase particularly in developed western countries. The most recent estimate, from the Global Burden of Disease Study 2015, stated that there are more than 300 million people with asthma, and there may be an additional 100 million people with asthma by 2025, making it one of the most common noncommunicable disease [32]. Although the prevalence of asthma is higher in developed western countries, the disease is recognized worldwide. In developing countries, the prevalence of asthma tends to be ≤1%, much lower than the 10% usually found in developed western countries [33]. Within populations, and considering their level of development, the

prevalence of asthma follows an urban-rural gradient [34, 35]. In Portugal, data from the 4th Portuguese National Health Survey including 32644 adults (≥20 years-old) were analysed, and the reported prevalence of ever asthma (self-reported ever medical doctor diagnosis of asthma) was 5.3%, current asthma (any self-reported asthma symptoms within previous 12 months) 3.5%, current medicated asthma (self-reported use of any asthma drugs within previous 12 months) 3.0%, current severe asthma 1.4% (self-reported attending an emergency department because of asthma within 12 months), and incident asthma 0.2% (self-reported medical diagnosis of asthma within previous 12 months) [36].

In children, according to the International Study of Asthma and Allergies in Childhood (ISAAC) performed in several countries, this prevalence has also increased in many countries that are experiencing rapid increases in urbanization and westernization of lifestyle [37]. ISAAC consisted of 3 phases: phase I was conducted during 1991 to 1995 in 56 countries with 721601 children involved; phase II began in 1998 and was performed in 30 centres in 22 countries and included children aged 9–11 years; and phase III was performed 5 years after phase I in 98 countries, including 237 centres and 1187496 children, in order to examine changes in prevalence of symptoms after phase I. Between ISAAC phase I and phase III the prevalence of asthma symptoms increased in children (6–7 years) and adolescents (13–14 years) ranging from 11.1 to 11.6% and from 13.2 to 13.7%, respectively [38]. In ISAAC phase III, the prevalence of asthma among the 6- to 7-years-old children was 9.4%, being the lowest prevalence found in Northern and Eastern Europe (4.0%) and the highest in United Kingdom (20.0%) and in North America (20.0%). In the 13-14-year age group, the global prevalence of asthma was 12.6%, and the same trend was observed between the Northern and Eastern European countries (5.1%) and English languages centres (19.9%). In Portugal, the prevalence of asthma among children from 6 to 7 years was 9.4% and among adolescences from 13 to 14 years was 14.7% [39]. The differences in prevalence in most regions suggest that the factors that affect asthma vary between locations [37]. These factors may act differently in developed countries and in developing countries, and their interaction with lifestyles may also be important. There are likely to be several of environmental factors associated with the development process related to the global changes, including loss of protective factors, which may be associated with differences between regions [40].

In addition, the cause of this increase in asthma prevalence that began in the late 1970s [33] is still unclear, but it is consistent with a rise in other allergic diseases, such as rhinitis and atopic dermatitis, and autoimmune diseases: type 1 diabetes, multiple sclerosis and Crohn's disease [41]. Concomitantly, there has been a decrease in the incidence of many infectious diseases in

developed countries as a result of improved health and socio-economic conditions [41]. These trends cannot be explained only by genetic reasons, but by an interaction between multiple genetic and environmental factors [42]. The rapidity of these changing trends may suggest a profound role of environment determinants in the aetiology of asthma.

2.1.3. Mechanisms of asthma

Airway Inflammation

The immune system evolved to protect human being from a huge diversity of pathogenic organisms and environmental stressors. Progress has been made to understand the mechanisms associated with the heterogeneity of asthma. Papi *et al.* [30] reported the mechanisms and characteristic pathological features of asthma, considering three main features: eosinophilic (allergic and non-allergic), non-eosinophilic (neutrophilic type 1 and type 17 and paucigranulocytic), and mixed granulocytic inflammation.

Th2-type inflammation occurs in more than 80% of children and in around 50% of adults with asthma in association with sensitization to environmental allergens. The inflammatory infiltrate that accompanies Th2 lymphocyte responses is mainly composed of eosinophils, but also includes mast cells, basophils, neutrophils, monocytes and macrophages. Although the activity of Th2 CD4+ lymphocytes predominate in allergic asthma, roles for a range of other T cells in different asthma subtypes have been described, including the association of Th1 and Th17 cells with neutrophilic asthma [33, 43].

In eosinophilic allergic asthma, allergen sensitization also requires an interaction between specialized antigen-presenting airway dendritic cells (DCs) and T cells, which results into Th2-type T cells. Following allergic sensitization and consequent co-stimulatory interactions between DCs and T cells, adaptative Th2-type T cells produce several cytokines. These Th2-type T cells secrete the pro-allergic cytokines, IL-3, IL-4, IL-5, IL-9, IL-13 and granulocyte–macrophage colony-stimulating factor, which in turn leads to the IgE, mast cell and eosinophilic responses that are characteristic of allergic asthma. IL-5 and IL-4 are associated with the survival and maturation of eosinophils, and with B-cell isotype switching and IgE synthesis leading to mast cell activation, respectively. Many of the asthma-related allergens, including house dust mite (HDM), cockroach, animal and fungal allergens, exhibit enzymatic properties that enable allergens to penetrate the epithelial barrier and directly interact with mucosal DCs. During this process, quiescent DCs transform to express an array of cell adhesion and co-stimulatory molecules. These molecules are recognized by naive T cells, which interact with DCs to create an immunological synapse that

facilitates allergen presentation. Whereas a minority of allergen-specific Th2 cells migrate to the B cell follicle to initiate immunoglobulin class switching from IgM to IgE, others relocate to the airway mucosa, under the influence of chemoattractants, to elicit the Th2-type inflammatory response and the associated coordinated secretion of pro-allergic cytokines (**Figure 2**) [30, 33, 44, 45]. Furthermore, Th2 inflammation promotes long-term airway remodelling. This remodelling involves an increase in airway smooth muscle, thickening of the subepithelial reticular lamina, matrix deposition throughout the airway wall, angiogenesis, neuronal proliferation and increased production of mucus [33], underlying hyperresponsiveness of the airways. This structural changes in airways also contribute to the development and progression of asthma [30, 33].

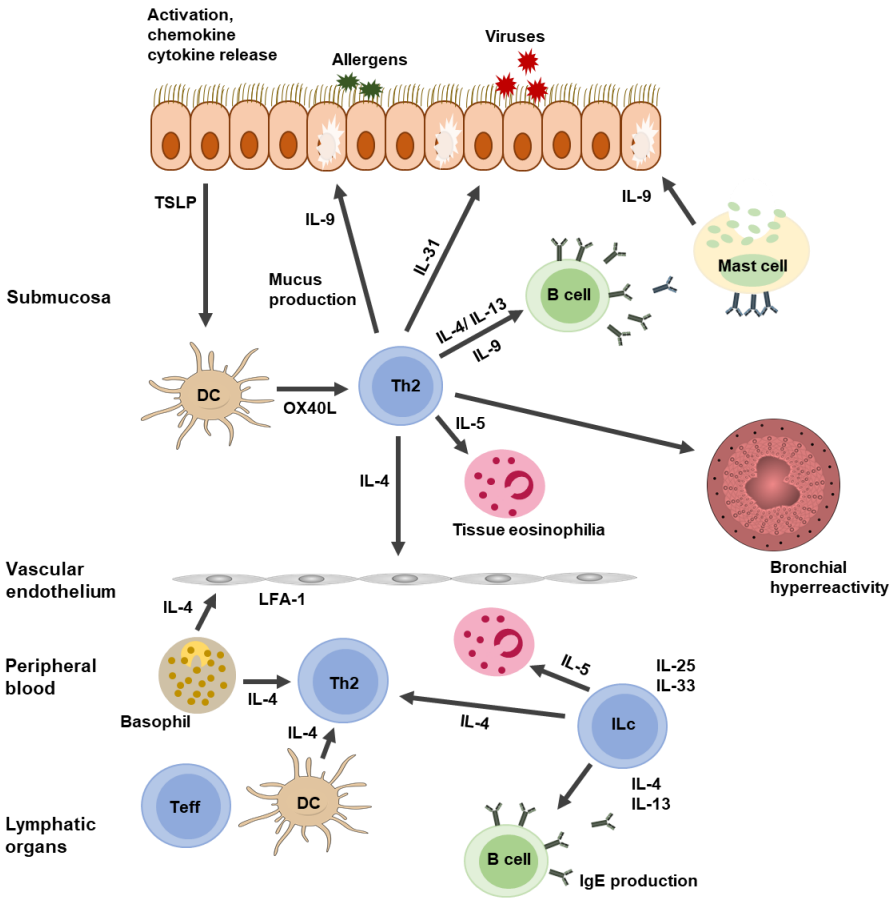

Figure 2 Mechanisms of allergic inflammation in asthma

Adapted from Papadopoulos *et al.* [46], 2012

In eosinophilic asthma, and potentially non-allergic asthma, the initiation of Th2-type immune responses occurs through secretion of the epithelial cell-derived cytokines IL-25, IL-33 and thymic

stromal lymphopoietin (TSLP). These cytokines also induce type 2 innate lymphoid cells (ILC2) to produce the Th2-type cytokines IL-5, IL-9 and IL-13. Continued stimulation of epithelial and smooth muscle cells, and fibroblasts by Th2-cell-derived and ILC2-derived IL13 would lead to airway hyper-responsiveness and remodelling.

Neurogenic mechanism

There is a complex interaction between inflammation and the neural control of airways tone, with effects of inflammatory mediators on neurotransmission and neurotransmitters, which in turn modulate the inflammatory response in the airways. The human airways are innervated by efferent (motor) and afferent (sensory) autonomic nerves, originating from vagal or spinal nerves, which regulate many features of airway physiology, including airway smooth muscle tone, mucus secretion, microvascular permeability, and the recruitment and activation of inflammatory cells [47, 48]. The parasympathetic nervous system (PNS) is the dominant neuronal pathway in the control of smooth muscle tone, inducing bronchoconstriction and mucus secretion in the airways when activated. The sympathetic nervous system (SNS) is less prominent than PNS within the human airways, but it is possible that adrenergic nerves may influence bronchomotor tone indirectly via prejunctional and β-adrenergic receptors [47].

In asthmatics patients, increased basal parasympathetic tone is observed [29, 49]. This results in constricted airways and an enhanced bronchoconstriction response to different inhaled agents that are known to stimulate sensory nerves [50, 51]. The majority of sensory nerves fibres are non-myelinated C-fibres, which act as afferent pathways and, also contains neurotransmitters of non-adrenergic, non-cholinergic (NANC) system, such as nitric oxide, vasoactive intestinal peptide, acetylcholine and norepinephrine, that can be released after activation and exhibit efferent functions. In turn, their activation, namely due to environmental exposure, may lead to increased afferent and efferent function, which in asthma, could contribute to bronchial hyperresponsiveness, inflammation and remodelling of the airway wall [52].

In addition to parasympathetic and sympathetic innervation, airways are also innervated by non-adrenergic, non-cholinergic system. In human airways, inhibitory NANC (i-NANC) system localized in the parasympathetic nerves is the only bronchodilator pathway in the human airways. i-NANC has been associated with the release of several neuropeptides, including vasoactive intestinal peptide, nitric oxide, peptide histidine methionine and pituitary adenylate cyclase-activating peptide, and a dysfunction of these systems may be involved in the inflammation or airway hyperresponsiveness in asthmatics patients [49, 53]. Additionally, these neurotransmitters

have been associated with the production of different cytokines (IL-2, IL-4 and IL-10), inhibition of T lymphocyte proliferation, regulation of isotype switching in B lymphocytes, mucus secretion, inhibition of Th1 cells [49, 53]. The excitatory NANC (e-NANC) system is mediated by the release of neuropeptides from a subpopulation of nonmyelinated sensory C-fibers in the airways, being associated with bronchoconstriction. C-fibers may be stimulated both by exogenous substances, including capsaicin, cigarette smoke and air pollutants, and by endogenous substances such as histamine, bradykinin and prostaglandins. After stimulation, neuropeptides (calcitonin gene-related peptide, tachykinins, substance P, secretoneurin and neurokinin A) of sensory nerves are released from the afferent nerves into the airway contributing to the asthma development by causing bronchoconstriction, mucus secretion, vascular hyperpermeability, cough and vasodilation, as well as by stimulating the recruitment and activation of eosinophils, dendritic cells, mast cells, T cell proliferation and cytokine production [49, 53].

Recent studies have suggested that transient receptor potential cation channels, including transient receptor potential ankyrin 1 (TRPA1) and transient receptor potential vanilloid 1 (TRPV1), which are expressed in sensory C-fibers, may be associated with the development of asthma and to the release of pro-inflammatory neuropeptides, including tachykinins, substance P and calcitonin gene-related peptide [54, 55]. The activation of TRP channels induces and increases vascular permeability, extravasation of plasma and leukocytes, mucus hypersecretion and airway constriction [49, 53]. Moreover, both channels can be activated by exogenous environmental irritants, such as particulate matter (PM), pollutants from diesel exhaust [nitric oxides (NO_x), and polycyclic aromatic hydrocarbons (PAHs)], ozone (O_3) and cigarette smoke, inducing airway neurogenic inflammation with the release of inflammatory neuropeptides, as well as inflammatory mediators such as TNF-α, leukotrienes, and IL-1β [56]. The neurogenic inflammation response after environmental irritants activation of airway TRPV1 and TRPA1 may contribute to asthma development, mucus hypersecretion and airway constriction [57, 58].

2.1.4. Determinants of disease expression

Asthma is a complex and multifactorial condition that comprises a range of heterogeneous phenotypes defined by their interaction between host and environmental factors [59]. Although genes play their role in initiation of different pathways of asthma disease, during the last decades several studies have identified the role of environmental changes in the current asthma epidemic [43]. Furthermore, environmental factors may affect human health at different moments of life, being a relevant risk factor for asthma over time (**Figure 3**). However, the risk of developing

asthma is likely to be greatest when both genetic and environmental risk factors are present simultaneously [60].

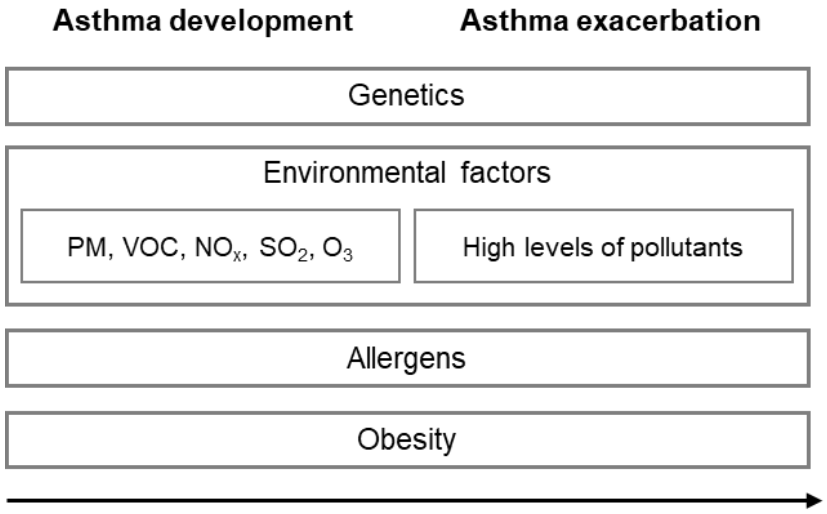

Figure 3 Determinants of asthma development and/or exacerbation

PM: particulate matter; VOC: volatile organic compounds; NO_x: nitric oxides; SO_2: sulphur dioxide; O_3: ozone

Adapted from Holgate *et al.* [33], 2015

Family and twin studies suggested that genetics plays an important role in the development of asthma [61]. In different studies it was reported that family history of asthma and atopy are important risk factors for asthma; with most odds ratio ranging between 2 and 4 when a first-degree relative has asthma [62]. Thomsen [61] also reported an increased risk of asthma in children with one or both parents diagnosed with asthma (25% and 50%, respectively). Studies with twins support that asthma is more likely to occur in an individual who has a genetically close relative(s) with the disease. For instance, the risk of asthma in monozygotic twins (75%) is greater than in dizygotic twins (35%). Additionally, genome-wide association studies (GWAS) have identified 18 genomic regions and more than 100 genes associated with asthma in eleven different populations [63]. ORMDL3-GSDM on chromosome 17 has been associated with asthma among children (OR=1.84, 95% CI 1.43; 2.42) [64]. However, the effects of all asthma-related loci are weak, accounting for a small proportion of the heritability of the disease [65].

In addition to genetic factors, a sex disparity is also well-established in asthma and it changes throughout life. Among younger children (0 to 14 years of age), the incidence and prevalence of asthma are higher among boys compared to girls although after puberty it is more prevalent in

girls. The mechanisms underlying differences between sexes are still unclear, but most authors have suggest that sex hormones modulate pathways associated with asthma pathogenesis [33, 66].

Subbarao *et al.* [63] and Beasley *et al.* [67] reviewed other risk factors and reported the role of several prenatal and childhood risk factors in the development of asthma, including tobacco smoke, prenatal diet and nutrition, maternal stress, prenatal antibiotic treatment, and mode of delivery. Atopy in early life is also a key factor of an individual's risk of asthma [68]. Several studies have demonstrated that atopic asthma represents a common form of asthma in paediatric age, being characterized by eosinophilic airway inflammation associated with specific IgE antibodies sensitization to several allergens [69]. In 2016, a cohort study, which followed 2607 new-borns over 16 years, indicated that the prevalence of asthma at any time is significantly higher among ever-allergic sensitized children compared to never-sensitized (26.3% and 13.3%, respectively). Tan *et al.* [70] also reported a strong association between atopy and the prevalence of asthma before the age of 13 years. Nevertheless, only a proportion of atopic individuals become asthmatic [71], suggesting the existence of environmental factors associated to the development of asthma in atopic individuals [68].

Environmental factors

Host determinants alone cannot explain the rapid rise in the prevalence of childhood asthma and allergies observed in most westernized countries in a single generation. Since asthma development is a dynamic process throughout childhood, it is also relevant to study the effect of environmental exposures.

Environmental factors, including outdoor and indoor air exposures, have been associated not only with asthma development but also with its exacerbation [72, 73]. It is widely acknowledged that exposure to outdoor air pollutants, including O_3, nitrogen dioxide (NO_2), sulphur dioxide (SO_2) and PM, can increases the risk of developing asthma [74, 75]. In fact, guidelines recommend that individuals with asthma should avoid outdoors when concentrations of these pollutants (O_3, NO_2, SO_2, and PM) are elevated, as they are related with asthma exacerbations, increased symptoms, and with a greater risk of hospitalization among children with asthma [74]. A growing body of evidence also shows that chronic exposure to traffic-related pollution, even at low levels within current standards, impairs lung function in childhood and increases the risk of clinically significant decreases in lung function. Children living in neighbourhoods whose air quality improved during the observation period had a more pronounced trajectories of lung function growth than those

living in neighbourhoods whose air quality did not improve [76]. While there is a large evidence linking ambient air pollution and children health [75, 77, 78], less is known about the impact of indoor air pollution on children health. Indoor environments represent an important contribution of the individual's environmental exposure due to i) the time that humans spend indoors, especially children; ii) the higher concentration of several pollutants indoors; and iii) the risk of encountering specific environmental factors associated with asthma and asthma-related symptoms, including chemical compounds as endocrine disruptors [74, 79].

Asthma and obesity

Asthma and obesity are two of the most highly prevalent and challenging public health conditions in children worldwide [80]. Together, asthma and obesity lead to substantial morbidity, impaired quality of life, and health care burden, mainly in developed countries [81]. During the past 20 years, the increase in asthma prevalence was similar to obesity, suggesting a possible link between the both conditions [82, 83]. In fact, obesity has been suggested as a major risk factor and disease modifier for asthma in children and adults [84], resulting in the obese-asthma phenotype [43]. In 2018, Lang *et al.* [85] reported an overall incidence rate for new cases of asthma in the population of 2.7 per 1000 patient years, ranging from 2.4 among children with normal weight to 3.2 per 1000 patient years among obese children (BMI ≥ 95^{th} percentile adjusted for sex and age). The proportion of physician-diagnosed asthma incidence in children with obesity that is attributed to obesity was 23% to 25%, being higher when compared to all children (10%). Whereas the incidence of asthma confirmed by spirometry among all children and in obese children attributable to obesity was 13% and 28%, respectively. Roughly one-quarter of the incidences of new asthma were directly attributable to obesity. A pooled analysis of 16 European cohorts, including 21130 children born from 1990 to 2008, reported an increased risk for incident obesity in children with physician-diagnosed asthma than those without asthma (aHR=1.66, 95% CI 1.18; 2.33). In addition, children with asthma had a higher risk for obesity (aHR=1.98, 95% CI 1.31; 3.00) than those without wheeze and asthma [80]. Similar results were observed in a meta-analysis showing that weight gain above the obesity threshold significantly increase the risk for incident asthma by 1.98 (95% CI 0.71; 5.52) in children [86], suggesting that obesity may play an important role on childhood asthma.

In addition to the risk of developing asthma, there is evidence suggesting that obesity increases the severity of respiratory symptoms by decreasing lung function and increasing airway hyperreactivity, contributing towards a more difficult-to-control asthma phenotype [86, 87], and is

also a determinant of shorter exacerbation-free time in children with asthma [88]. Ahmadizar *et al.* [89] provides a summary estimates of the relation between BMI and poor asthma control/exacerbations. This meta-analysis showed that both obese and overweight children have a slightly higher risk for severe asthma exacerbations (assessed by the risk of oral corticosteroid use), yet not for poor asthma control based on symptoms. Moreover, obesity is associated with a decreased response to bronchodilator medications in children and adolescents with asthma [89]. Obese-asthmatic children experienced a higher risk of exacerbation compared to non-obese children after initiation of therapy (inhaled corticosteroids (ICS) or combination therapy), being ICS monotherapy less effective [89]. The increased number of exacerbations and asthma symptoms associated with overweight/obesity is consistent with other study, including preschool children, demonstrating that overweight/obesity status was associated with greater likelihood of recent hospitalization for asthma [90]. Within the group not treated with a daily controller, overweight/obese children had more asthma symptom days (90.7 *vs.* 53.2, p=0.020) and exacerbations (1.4 *vs.* 0.8, p=0.009) than normal weight children. However, when obese and non-obese children were treated with ICS (daily, intermittent step up, or as-needed), their symptoms and exacerbations were similar [90]. Similar, obese children tend to have increased asthma severity, poorer disease control and lower quality of life [91-93]. Moreover, among children hospitalized for asthma, obesity is associated with longer length of stay and with the use of mechanical ventilation [91]. Although there are many common pathophysiologic and clinical similarities, obese children with asthma might also be more susceptible to having increased symptoms as consequence of exposure to indoor pollutants [94]. This reflects an obese-asthma phenotype that is complex and multifactorial.

2.2. Level II: Where are you?

Indoor environment

2.2.1. Indoor air

Most people are aware that outdoor air pollution can impact their health, but the less recognized, or even unsuspected, indoor air pollution can also have significant and harmful health effects. The Scientific Committee on Health and Environmental Risks (SCHER) reported that indoor air pollution is the 8th most important risk factor for disease, responsible for an estimated 2.7% of the global burden of disease. Estimates also show that 1.5–2 million deaths every year could be attributed to indoor air pollution [95].

According to the US Environmental Protection Agency (EPA) indoor levels of pollutants may be two to five times, and occasionally more than 100 times, higher than outdoor levels [96]. The levels of indoor air pollutants are of particular concern because most people spend about 90% of their time indoors, at home, school, workplaces, transportation vehicles or at indoor recreational places [97, 98], having a significant impact on individuals' health and quality of life [99, 100]. Indoor environments represent a mixture of outdoor pollutants, associated to traffic and industrial activities, which can penetrate the building envelope through infiltrations or ventilation specific openings (windows or other) or mechanical, as well as indoor pollutants, resulting from emissions from building materials, furnishings, cleaning products, heating and cooling systems, humidification devices, moisture processes, electronic equipment, pets, combustion sources (such as burning fuels, coal, wood, incense and candles) and also from the behaviours of occupants (smoking, painting,…) [99, 101].

Increasing urbanization, changing behaviours and use of materials and consumer products are also associated to qualitative and quantitative variations of indoor air quality (IAQ) over the years, underlining an increase in pollutants and their concentrations [101]. IAQ can be impaired by several chemicals, including carbon monoxide (CO), O_3, nitrogen oxides (NO, NO_2), radon, and VOC, PM and fibers, and by biological agents, such as bacteria, fungi and allergens [95] (**Figure 4**). Some of these indoor pollutants may be mainly influenced by their outdoor concentrations, have primarily indoor sources, or are influenced by both indoor and outdoor sources [101].

	Sources		Pollutants
Anthropogenic pollutants	Combustion	Unvented gas/kerosene heaters	CO, CO_2, NO_3, PAHs
		Biomass (wood/coal) for heating/cooking	CO, CO_2, NO_3, PM
		Tobacco smoke	CO, CO_2, PM, VOC, PAHs
		Wood (fireplaces), gas ranges	VOC
	New furniture, solvents, painting, adhesives, insulation, cleaning products, materials for offices		VOC, formaldehyde
	Building materials, water		Radon
Biological pollutants	Allergens	Dust, beds, carpets	HDM
		Dampness	Moulds
		Plants	Pollens
	Viruses, bacteria		Biological contaminants

Figure 4 The main indoor pollutants and their sources

CO: carbon monoxide; CO_2: carbon dioxide; NO_2: nitrogen dioxide; PAHs: polycyclic aromatic hydrocarbons; PM: particulate matter; VOC: volatile organic compounds; HDM: house dust mite

Adapted from Nicolino Ambrosino et al. [95], 2019

Air pollutants found indoors are broadly similar to those found outdoors, with similar biological mechanisms for impacting on human health [102]. However, the effects of exposure to indoor air pollution may be greater than those related to outdoor air, namely to vulnerable groups such as children, young adults, the elderly, or those suffering chronic respiratory and/or cardiovascular diseases [103, 104]. As aforementioned, children are one of the most susceptible groups, due to their physical characteristics [18, 19], as well as the time spent indoors. In the past decades, many studies have focused on the effect of indoor air pollutants on children health, including asthma and allergies. In 2000, the Committee on the Assessment of Asthma and Indoor Air of the Institute of Medicine (IOM) reviewed and summarized the evidence for associations between indoor air exposures, namely to biological and chemical stressors, and the exacerbation and development of asthma [105]. More recently, Kanchongkittiphon *et al.* [106] reviewed published articles on indoor exposures and exacerbation of asthma. Exposure to HDM allergens were associated with dust mite sensitization, which was in turn associated with asthma; the protease activity of HDM may act on airway epithelial cells and on the activation of protease-activated receptor-2 (PAR-2) triggering an innate immune response and the release of proinflammatory cytokines, such as interleukin-6 (IL-6) and IL-8 from airway epithelial cells [107]. Kanchongkittiphon *et al.* [106] also reported that exposure to chemical pollutants, such as formaldehyde, 2-ethyl-1-hexanol and di(2-

ethylhexyl) phthalate, was found to be associated with asthma development and airway inflammation in children. In line with previous studies, Patelarou *et al.* [108] stated that most of the individual VOC appeared to be significant risk factors for asthma with the highest OR for benzene (aOR=2.92; 95% CI 2.25; 3.80) followed by ethylbenzene (aOR=2.54; 95% CI 1.16; 5.57) and toluene (aOR=1.84; 95% CI 1.41; 2.41).

Among different indoor environments, schools, besides their homes, are one of the most important settings for children, since they spend at least one-third of their time in schools. According to Oliveira *et al.* [109], Portuguese children spend per day up to 10 hours of their time at school, mainly in classrooms, demonstrating the relevance of understanding the health effects of indoor air pollutants in this environment. Schools are also indoor settings with a high population density, in which different pollutants may remain for a long time due to insufficient ventilation and existence of indoor sources, and are often characterized by infrequent interventions and building maintenance [110, 111]. Therefore, characterization of indoor air pollution in school environments is of critical public health concern given the potential long-term adverse consequences from such exposures. Associations have been found between wheezing and high exposure to indoor formaldehyde, pinene, PM ($PM_{2.5}$ and PM_{10}) and CO in classrooms [112]. Additionally, children exposed to high benzene [112] and PM_{10} levels [113] were more likely to have nocturnal cough. Similar associations were observed between exposure to formaldehyde, VOC and $PM_{2.5}$ and asthma [111]. Fsadni *et al.* [112] reported that school IAQ is dependent on the school building and classroom characteristics and cleaning/maintenance schedules, and that exposure to several indoor air pollutants was associated with upper and lower airway inflammation. Recently, studies related to IAQ in schools in different European countries have showed that children who study and live in industrial areas have an increased risk of respiratory symptoms when compared to those living in other areas [114, 115]. The presence of moulds was also associated to adverse health effects such as asthma symptoms, coughing, wheezing and upper respiratory symptoms [116]. Although increased exacerbation of current asthma symptoms in children were associated with increased levels of *Penicillium*, *Aspergillus*, *Cladosporium*, and *Alternaria* species [117], Cavaleiro Rufo *et al.* [118] reported that classrooms with increased diversity scores showed a significantly lower prevalence of children with atopic sensitization. Among school indoor air pollutants, VOC could be a significant group of compounds since they are largely present indoors and thus can be released continuously and slowly over a long time, thus posing a higher risk to human health [119]. On the other hand, the specific VOC to which occupants are exposed nowadays are substantially different from those that occupants experienced 50 years ago [101], suggesting changes in type and concentration from day-to-day, month-to-month, year-to-year and

decade-to decade. Some VOC measured in schools have been associated with a variety of health effects and symptoms such as asthma and allergies [120-122]. The Children's Environment and Health Action Plan for Europe (CEHAPE) of WHO found a negative association between maximal expiratory flow at 75% of vital capacity (MEF_{75}) and formaldehyde, benzyl-butyl-phthalate and the sum of polybrominated diphenyl ethers. FVC and FEV_1 were also negatively associated with ethylbenzene, xylenes and tris(1,3-dichlor-2-propyl)-phosphate [123]. According to SCHER, in infants and children, exposure to VOC increases the risk of respiratory and allergic conditions, such as asthma, wheezing, chronic bronchitis, reduced lung function, atopy and severity of sensitisation, rhinitis and respiratory infections [95]. Additionally, in a national representative cross-sectional study in France, high concentrations of VOC in homes were associated with an increasing prevalence of asthma and rhinitis in adults [95]. However, a systematic review has demonstrated that the role of VOC in the development and exacerbation of asthma and allergic disease in children and also in adults is weak [124], suggesting that VOC exposure can influence the immune responses, increasing Th2 polarisation.

Although exposure to ubiquitous environmental pollutants has increased in recent years and the impact of these pollutants on human health have been widely explored, less attention has been paid to the potential health risks of exposure to VOC functioning as endocrine-disrupting compounds (EDCs) [125]. Indoor exposures to suspected endocrine disruptors have markedly increased [101] and many of these compounds may be absorbed by other indoor surfaces after being released to the air and continue to be desorbed long after the host material is removed [101]. Therefore, research on EDCs should focus on understanding factors influencing its indoor air concentrations and the effect of their exposure on human health, especially in children. Beyond chemical screening programs to identify chemicals with endocrine-disrupting activity, many questions remain about the way to assess implications of this exposure on health outcomes.

2.2.2. Volatile organic compounds identified as endocrine-disrupting compounds

Endocrine-disrupting compounds have been described following the discovery of the effects of the insecticide dichloro-diphenyl-trichloroethane (DDT) on workers in cotton fields [126]. According to Bouchard [126], in 1962, Rachel Carson in “Silent Spring”, describes a world where there is no more bird singing, because they have been eradicated by the environmental toxics. However, it was in the medical field that their mechanism of action was discovered: children of women who received diethylstilboestrol (DES), while they were pregnant, presented genital malformations, infertility and clear cell adenocarcinomas of the vagina. In addition, the second

generation presented a high rate of hypospadias in boys [126]. In 2002, the International Programme on Chemical Safety (IPCS) defined EDCs as "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations*" [127].

Growing urbanization and the rapid development of new building materials and consumer products have result in a changing pattern in both the production and use of chemical compounds and consequently in an increase of these compounds indoors [4]. Some chemicals used in building materials, furnishings, and consumer products have been showed to be EDCs [4], making them potentially important indoor contaminants/pollutants and suggesting that indoor exposure may be a greater contributor to overall EDCs exposure [4, 128]. Most of EDCs are synthetic organic chemicals used in a wide range of materials and goods and can be found indoors in wide variety of products, including building and furnishing materials, pesticides, along with cleaning products [129-131]. In addition to the time spent indoors, where EDCs concentrations can be more relevant than the outdoor concentrations [111, 132], humans are continuously exposed to a diverse number of EDCs not only through inhalation of air and particles, but also by contact with contaminated media (soil or surfaces), consumption of food and drinking water and through direct dermal contact (e.g. cosmetic products) [131].

Although ingestion of contaminated food and beverages have been pointed as the major pathways of exposure to EDCs [133], current data suggest that there is a chronic and sustained exposure to indoor EDCs through inhalation [134-136] (**Figure 5**).

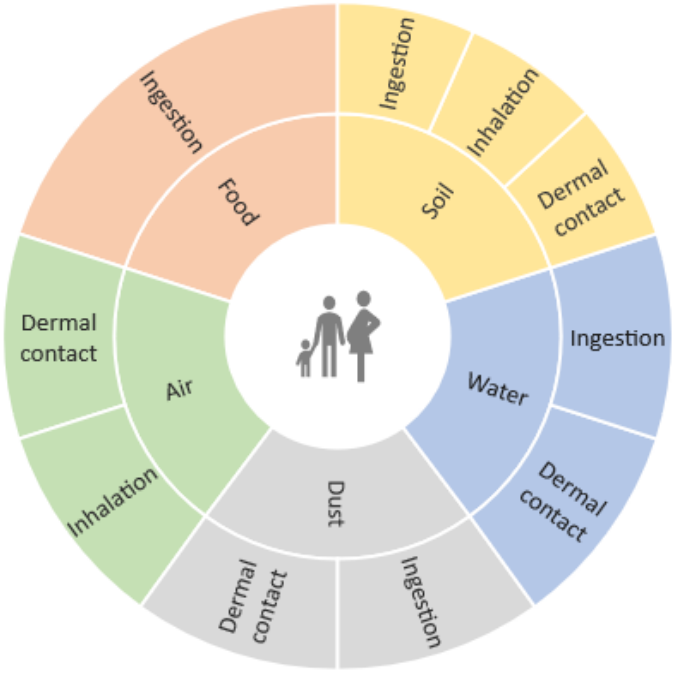

Figure 5 Routes of exposure to endocrine-disrupting compounds

Adapted from World Health Organization [131], 2012

While the development of synthetic chemical compounds has improved our daily life, the role of environmental chemicals in human health has increased in past years, suggesting that the effect of environmental exposure could constitute the paradox of progress [137], with EDCs exposure implicated in the development of several human diseases [138, 139]. Over recent decades, several studies recognized that EDCs can disturb many hormonal pathways, such as altered reproductive function in males and females, increased incidence of breast cancer, abnormal growth patterns, and neurodevelopmental delays in children, as well as changes in immune function [140]. The age at which an individual is exposed to an EDCs also has implications on health effects; during early foetal and childhood development, a wide variety of genes are activated and inactivated in a sequential manner, providing numerous targets for these environmental exposures. Exposure to EDCs during childhood can induce long-lasting effects persisting throughout adulthood and affecting future generations [131]. Indeed, it is now clear that exposure to EDCs during early development results in different health effects than exposures during adulthood. In general, higher concentrations of EDCs are require to cause toxicity in adults, and their effects only last as long as the EDCs is present [141]. Although there is growing evidence on the health effects of EDCs in adults, there is limited knowledge regarding the association

between exposure to EDCs during childhood and the development of asthma and/or allergies. This underlines the need of a better understanding of the EDCs exposure processes and health outcomes in children, who may be especially susceptible to effects at low concentration, considering the increasing amount of time spent indoors. Studies of potential health effects associated with EDCs have been hampered by lack of information about the major sources of exposure, exposure pathways, and exposure management/mitigation options.

This is further complicated by an infinite number of chemical combinations to which humans can be exposed. In an industrial, commercial or toxicological context, mixtures are sometimes restricted to intentional mixtures, such as adjuvants, stabilizers and excipients composing products like biocides, drugs or cosmetics. However, from a public health perspective, it is also relevant to consider the effect of exposure to simultaneously multiple chemicals from one or several sources and/or uses [142] that may or may not have the same mode of action and type of effect [143]. Given the widespread exposure to several EDCs, many of which with potential health effects in the general population, whether mixtures of EDCs affecting health is no longer a question limited to patients, but is of importance for all population groups, especially for children [143]. Furthermore, each chemical is present in a dose that may or not have an effect in the case to the exposure to the EDCs alone, although the mixture may have an effect [143]. In 2019, Yu *et al.* [144] hypothesized the possibility of potential interactive effects (synergistic or antagonistic) by the multiple EDCs in mixture that may be not reflected by the individual compounds.

For many years, the concept proposed by the Swiss physician and alchemist, Paracelsus in the 1500's that “the dose makes the poison” has been used by regulators to establish risk assessment profiles of chemicals. This concept predicts that higher doses of a chemical will cause greater harm than low doses. However, similar to hormones, even low concentrations of EDCs (from parts-per-trillion to parts-per-billion range) may have effects that are not predicted by effects at higher doses [145]. EDCs may also exert non-traditional dose-responses due to the dynamics of several receptor occupancy and saturation. Thus, low doses may have more impact on a target tissue than higher doses, and the effects may be entirely different [145-147]. This non-monotonic dose responses (NMDRs) may be due to different mechanisms, such as opposing effects induced by multiple receptors differing in their affinity, receptor desensitization, negative feedback with increasing doses, or dose-dependent metabolism modulation [148]. The NMDRs has been reported in several human studies, suggesting that the risk of diseases does not increase with an increasing level, but often tends to plateau or even decrease with increasing levels [149]. Additionally, the need to consider mixture effects when assessing the risks associated with EDCs

exposure has been widely recognized [150], since these compounds can be released as mixtures and interact within or between classes [146, 147].

According to the WHO, approximately 800 chemicals are known or suspected to be capable of interfering with various mechanisms of the endocrine system, such as receptor binding and/or hormone synthesis, potentially inducing adverse health effects in exposed individuals or populations and possibly playing a substantial role in many endocrine disorders [131, 139]. Initially, it was suggested that EDCs act through nuclear hormone receptors, including oestrogen and androgen receptors (ERs, ARs), progesterone receptors and thyroid receptors (TRs), among others [147]. Indeed, some recent studies have showed that EDCs are capable of acting through non-steroid receptors, transcriptional coactivators, enzymatic pathways involved in steroid biosynthesis and/or metabolism [147, 151], have a direct effect on genes [152] and an epigenetic impact [153], and may also target different organs and systems [154].

Additionally, exposure to EDCs may change cytokine production, Th1 and Th2 balance and activate the immune system [125, 155]. Kuo *et al.* [156] showed that human bronchial epithelial cells treated with different EDCs increased bronchial smooth-muscle cell proliferation and migration by increasing the secretion of chemokines (IL-8 and RANTES), suggesting a possible role for EDCs in asthma airway remodelling. These pollutants may also induce oxidative stress and epithelial damage to initiate or augment airway inflammation and reduce inhibitory T_{reg} function [157]. Furthermore, EDCs may mediate epigenetic changes through alterations in DNA methylation, which are proposed to play a role in the development of asthma [158]. Exposure to higher EDCs levels is related to lower methylation of TNFα 5′ CGI, which is associated with airway inflammation, hyperresponsiveness, the regulation of immune cells and a higher risk of asthma in children [158]. Similar to other air pollutants that have an irritant effect, EDCs may also stimulate airway C-fibre sensory nerves, which express transient receptor potential (TRP) cation channels; when exposed to irritants, TRP channels release neuropeptides locally, resulting in cough, airway irritation, mucous secretion, and bronchoconstriction mediated by the efferent pathways of the autonomic nervous system [56, 159]. Nassi *et al.* [160] and Cantero-Recasens *et al.* [161] reported the role of activation and/or increased expression of transient receptor potential vanilloid 1 (TRPV1) and ankyrin 1 (TRPA1) channels in the pathogenesis of asthma, providing evidence for the role of ANS in the regulation of airway function.

In addition, different mechanisms, such as mechanical, immunological, genetic, epigenetic, hormonal, and environmental pathways, may mediate the association between asthma and obesity [162]. Recently, EDCs have also been showed to possess obesogenic properties, being associated to weight gain by changing lipid homeostasis as well as promoting adipogenesis and

lipid accumulation. EDCs inappropriately regulate lipid metabolism and adipogenesis by targeting peroxisome proliferator activated receptors (PPARα, δ and γ) and the retinoic X receptor (RXR), which are essential for the control of intracellular lipid homeostasis as well as the proliferation and differentiation of adipocytes, and the aryl hydrocarbon receptor in adipocytes, thus increasing adipogenesis [133, 163]. Obesogenic effects of EDCs may also be mediated by the methylation of PPARγ and RXRα, changing the regulation of adipogenesis and increasing triglycerides accumulation, glucose metabolism, and insulin sensitivity in mature adipocytes [164, 165]. Another mechanism may be through changing the energy balance between energy intake and expenditure, namely by altering appetite, satiety and food preferences, by mimic, block or interfere with the production of leptin and ghrelin [166].

Evidence of a potential link between endocrine-disrupting chemicals and development of asthma

The focus of the effects of exposure to EDCs has earlier been on reproductive parameters and potential carcinogenic effects, but it has been recognized that different human systems can also be affected. Some researchers have suggested that exposure to EDCs, such as phthalates and bisphenol A, may contribute to the development of asthma in children. Two independent studies conducted in Sweden [167] and in Bulgaria [168] reported a statistically significant association between the concentration in house dust of di-2-ethylhexyl phthalate and allergic and respiratory symptoms in children. In Swedish homes, association between benzyl butyl phthalate in house dust and allergic symptoms was also found [167]. In addition, a systematic review found a positive association between PVC surface materials at home and the risk of asthma (OR=1.55, 95% CI 1.18; 2.05) and allergies (OR=1.32, 95% CI 1.09; 1.60) in children aged up to 12 years [169]. Donohue *et al.* [170] found an inverse association between maternal bisphenol A exposure during pregnancy and wheeze at 5 years (OR=0.7, 95% CI 0.5; 0.9); however, a positive association was found between bisphenol A concentrations at ages 3, 5 and 7 years and asthma (OR=1.5, 95% CI 1.1; 2.0; OR=1.4, 95% CI 1.0; 1.9; and OR=1.5, 95% CI 1.0; 2.1, respectively). In 2015, Gascon *et al.* [171] measured bisphenol A and metabolites of low- and high-molecular-weight phthalates in urine samples collected during the first and third trimesters in pregnant women and found a higher risk of asthma at age 7 years with increased prenatal exposure to bisphenol A and high-molecular-weight phthalates.

More recently, two studies observed that prenatal maternal urinary concentrations of biomarkers of exposure to EDCs are associated with a higher risk of asthma in children [172, 173]. In 2018,

Buckley *et al.* [173] quantified urinary phenol and phthalate biomarkers in third trimester maternal samples and found a positive association between bisphenol A (OR=3.00, 95% CI 1.36; 6.59) and 2,5-dichlorophenol (OR=3.04, 95% CI 1.38; 6.68) and asthma diagnosis among boys. An inverse association was found between wheeze in the past 12 months and low-molecular-weight phthalate metabolites (OR=0.27, 95% CI 0.13, 0.59) among girls and with benzophenone-3 among all children (OR=0.65, 95% CI 0.44, 0.96). Furthermore, Berger *et al.* [172] observed that prenatal maternal urinary concentrations of biomarkers of exposure to high-molecular-weight phthalates, particularly monocarboxyisooctyl phthalate, were associated with increased risk of asthma (OR=1.54, 95% CI 1.12; 2.12) and with lower FEV1 (β= −0.09, 95% CI −0.15; −0.03) and $FEF_{25-75\%}$ (β= −7.06, 95% CI −11.04; −2.90) ate age 7, after adjusting for additional chemical exposure and demographic characteristics.

To date, insufficient data exist about the public health risks posed by other commonly normally found in the indoor environment at low concentrations. There is indirect evidence from secular changes in childhood growth and reproductive development which suggests exposure to these agents may be significantly influencing human health trends.

2.2.3. Influence of chemical compounds on human microbiome

More than 100 trillion symbiotic microorganisms live on and within human beings, which together form the microbiota, playing an important role in human health and disease [174]. The microbial colonization process of human body begins at birth [175]. After birth, human microbiome composition is characterized by a succession of microbial diversity, which is affected by life events including birth gestational date, type of delivery, and by diet including feeding habits and methods (e.g. breast or bottle feeding) [176]. Infancy is a period of rapid colonization by microbial consortia that can shift in response to several events, including illness, changes in diet and environmental exposures [175]. From birth, the microbial diversity increases and converges toward an adult-like microbiome by the end of the first 3–5 years of life [176]. Although the composition of an adult microbiome remains relatively stable, the combination of multiple individual-specific factors, both endogenous (genetics, life stage, health status) and exogenous (diet, lifestyle, use of antibiotics and environmental exposures) shapes the microbiome, making every individual microbially unique [177, 178].

Beyond the individual-specific factors, one important determinant of community composition is the anatomical location [179]. The composition of human microbiome varies by anatomical site. Each body site is characterized by a distinct microbial community structure and function [180],

being shaped mostly by the environmental conditions, such as pH, humidity, temperature, substrate availability and oxygen, resulting in one or a few signature taxa within and between individuals that can change over time [181, 182].

Recently, several studies reported that exposure to some environmental chemicals can also disturb the human microbiome or, conversely, that the microbiome can play a role in the development of chemical toxicity, leading to adverse health outcomes [183-185]. Authors identified two different types of interactions between chemicals and microbiome: a) microbiome can directly metabolize some environmental chemical after ingestion or after their conjugation by the liver; and b) environmental chemicals can interfere with composition and/or metabolic activity of the human microbiome, which may affect the activity of endogenous metabolites or the toxicity of other chemicals that depend on microbiome for their metabolism [177, 183, 185]. Claus *et al.* [177] described the effect of some chemicals, including pesticides, metals, persistent organic pollutants (POPs) and artificial sweeteners, on microbiome composition and metabolic activity of gut microbes, suggesting that chemicals may change the community composition and function of a microbiome, but also might alter chemical exposure by damaging the metabolic capacity of the microbiome or changing the environment that supports microbiome-induced chemical metabolism. For example, chronic exposure to low doses of chlorpyrifos, an organophosphate insecticide commonly used to treat fruit and vegetable crops and vineyards, induced gut dysbiosis - a change in the microbial community structure, being associated with proliferation of *Bacteroides sp*. and decreased levels of *Lactobacillus sp*. and *Bifidobacterium sp*.. Furthermore, exposure to POPs has a major impact on the host-microbiome metabolic axis, through the activation of aryl hydrocarbon receptor signalling, and also in the decrease of the *Firmicutes/Bacteroidetes* ratio [177]. In addition, Rosenfeld [186] suggested that changes in hormone production induced by exposure to environmental chemicals, such as bisphenol A, can influence the gut microbiome composition. Exposure to disinfection by-products (DBPs) has also been linked to changes in composition and activity of gut microbiome [187, 188]. Evidence from mouse studies suggest that ingested DBPs were associated with elevated relative abundance of *Bacteroidetes* and dose-dependent changes in the ratio of *Firmicutes/Bacteroidetes* [187] and decreased levels of *Clostridium perfringens*, *Clostridium difficile*, *Enterobacteriaceae*, and *Staphylococcus* [188]. However, there are no studies about the dose–response relationship, namely on how the microbiome is changed by chemical dose changes, and also on how the dose–response relationship of microbiome perturbations is related to the dose relationship of the ultimate manifestation of toxicity [184], with no clear patterns on whether certain bacteria are especially vulnerable to a range of chemical exposures [186]. However, a specific or a mixture of chemical

compounds, its dose, the moment and duration of exposure are likely factors inducing different microbiome effects and the ability to recover after elimination of the exposure [186].

Several of the above studies reported the effect of exposure to environmental chemicals by ingestion on the gut microbiome [187, 188]. Nevertheless, exposure to such chemicals are also likely to occurs through other routes, including inhalation and dermal contact, being expected that these routes of exposure may also result in relevant human microbiome changes. Disruptions in the microbiome can in turn induce effects on host physiological responses and health [174, 189]. Thus, going forward is essential to understand the interaction between environmental exposures and microbiome, and consequent the impact on human health. Given the relevance of the microbiome to human health, persistent exposure to chemical compounds may be an unrecognized risk factor for dysbiosis, which has now been linked to several chronic non-communicable diseases. Although effects may be subtle, children are expected to be more susceptible to ecological disturbance, given that the microbiome is highly plastic and influenced by environmental factors during this age period [186] where microbiome structures appear to be more dynamic and developmental. Additionally, Cho *et al.* [179] reported other studies showing that human microbiome is completely resilient, and that returns to the *status quo ante* after perturbation. Nevertheless continued perturbations may result in the loss of recovery with implications to human health [190].

2.3. Level III: Where do you live?

Neighbourhood

Why does where you live matters? When it comes to your health, where you live matters. When our children go to school, what kinds of features do they see? Buildings, streets, or greenspaces? How do they get there? By car, bike, train, or walk? The places where we live are conceptualized by the idea of neighbourhood and the characteristics of built environment, from urban cities to neighbourhood, has been recognized as playing an important role in human health.

Urban environments are diverse, dynamic and complex playing an essential role in human health and wellbeing [1]. Although the features of urban living encourage rural migration, recent studies have showed that the advantages of urban life can be eroded by the adverse impacts of the urban environment, such as changes in diet patterns, sedentary lifestyle, exposure to air pollution and loss of greenspaces [191]. With hasty global urbanization, there is an increasing interest in understanding how urban settings and environment affect children's health.

2.3.1. Urbanization, land use and biodiversity

Urbanization is one of the most important global change processes, with approximately 54% of the world population living in cities [192]. If current trends continue, by 2050 the global urban population is estimated to be 6.3 billion, nearly doubling the 3.5 billion urban dwellers in 2010 [192]. While the process of urbanization has important implications for changes in demographic characteristics, unplanned and rapid urbanization can also cause profound impacts on environment and on physical landscape [193]. These changes of landscape are induced by residential, commercial or industrial land development processes and by new communication infrastructures, mainly controlled by social and economic factors that exceed the local conditions [194]. Even under scenarios of slower urbanization rates, urban areas face several challenges, such as climate change, environmental degradation, land changes, habitat fragmentation and loss of green areas [195].

Within a context of increasing urbanization, climate change and health effects, urban greenspaces are gaining a growing interest for their role as an important element for sustainable and healthy societies in an urban context [196]. Green spaces can greatly contribute to the urban ecosystem through air purification by absorbing certain airborne pollutants from the atmosphere, water and climate regulation and biodiversity while, providing benefits to urban residents (recreation, social interaction and inclusion, collective empowerment and health benefits and wellbeing), and

producing economic value by increasing the quality of landscapes [197] and the attractiveness of the city within the context of increasing city competition [197].

Additionally, green areas, including forests, parks and other natural areas, have been associated with better self-reported health and lower stress scores [198], increased physical activity, and improved health [199]. James *et al.* [200] reviewed and summarized the evidence on exposure to greenness and various health outcomes. A strong consistency was reported between greenness and physical activity, even after adjustment for a range of individual and area-level potential confounders, as well as with the lower risk of overweight or obesity. In addition, street tree density and other urban greenery have been associated with increased playtime outdoors, physical activity [200, 201] and lower prevalence of overweight/obesity among children [202]. A meta-analysis including 10 UK studies involving 1252 participants showed that physical activity in green places, even of short duration, significantly improved both self-esteem and mood [203]. Similar results were observed among 276 children residing in the city of Edinburgh. Findings suggested that higher use of greenspace in urban areas was positively associated with better quality of life, including friends and self-esteem sub-scales [204]. Additionally, several on-going studies have examined the health effects to forests and elements of forest settings [205, 206]. A review of field experiments conducted in 24 forests across Japan on the effects of *shinrin-yoku* (taking in the forest atmosphere, or “forest bathing”) showed that forest environments could lower concentrations of cortisol, decrease heart rate and blood pressure, increase parasympathetic nerve activity, and lower sympathetic activity compared with city settings [207]. Park *et al.* [206] reported a benefit associated with the practice of *shinrin-yoku* and nature therapy, suggesting that nature has healing and restorative properties that contribute to health and well-being. Authors reported a positive effect of *shinrin-yoku* on the immune system function, cardiovascular and respiratory system, depression and anxiety, mental relaxation and on human feelings of gratitude and selflessness. Even research involving the use of photos of green spaces have the same physiological effects. The nature views appeared to have a restorative effect with greater decreases towards baseline values after the stressor, induced by changes in the autonomic nervous system activity, compared to viewing built environments [208, 209].

Several studies from the last years have showed that exposure to greenness may also be protective of asthma and allergic diseases. Ruokolainen *et al.* [210] found an inverse association between higher forest and agricultural land within 2 to 5 km around children and adolescents’ homes and the risk of atopic sensitization, especially in rural areas characterized by less densely built-up areas and more native vegetation. Among children older than 6 years, a dose-dependent relationship was observed, suggesting a causal link. In Spain, a study involving 2472 children

investigated the effect of availability and accessibility of greenspace around homes in two biogeographical regions, Euro-Siberian and Mediterranean region, on respiratory health. Higher residential surrounding greenness and proximity to green spaces were associated with reduced risk for wheezing in Euro-Siberian, but not in Mediterranean region. In contrast, the risk of bronchitis increased with lower residential proximity to greenspaces in the Mediterranean region but no significant associations were observed in Euro-Siberian region [5]. Similar results were observed in two birth cohorts in Germany [211]. Fuertes *et al.* [211] reported a significant difference between Normalised Difference Vegetation Index (NDVI), which provides a measure of vegetation, and allergic rhinitis among two distinct areas. In urban areas, NDVI in living environment was positively associated with allergic rhinitis, while in rural areas, greenspaces have a protective impact. A recent study developed among 49 956 New Zealand children born in 1998 and follow-up until 2016 suggested that exposure to greenness and vegetation diversity were associated with a lower risk of asthma [212]. An increase of home neighbourhood greenspaces, measured as NDVI, across children's life course was associated with a 6% lower risk of asthma. Vegetation diversity (total number of natural land cover types) was also associated with a decrease risk of asthma. Both early- and late-life exposures to greenness were reported to be protective factor of asthma [212]. Within urban areas, exposure to higher neighbourhood greenness around home in early life was negatively associated with incidence of asthma during preschool years, but no association was observed in childhood (6-10 years) [213]. However, in New York, tree canopy around the prenatal residential address has been associated with an increased risk of asthma among 7-years-old children [214]. Beyond cultural and lifestyle characteristics, in their study Tischer *et al.* [5] reported that the type (natural or artificial) and quantity of urban greenspace, the introduction of non-native vegetation and air pollution levels may be important factors in its relation to respiratory health effects.

Furthermore, the current trend of urban growth has several environmental impacts on the surrounding ecosystems, land resources and consumption, structure and pattern of the urban area, namely on the conversion of green spaces into the built-up areas [215, 216]. Urban cities tend to agglomerate, forming urban clusters or corridors, along which transportation and other forms of development occur [217]. As a consequence, urban green space has come under increasing pressure during the urbanization process and this negatively affects the structure and function of ecosystems and consequently the biodiversity and the relationships between the natural environment and human microbiome [197, 216, 218, 219]. According to the biodiversity hypothesis, the reduced exposure of natural environments, and therefore microbial diversity, adversely affects the human microbiome and may lead to the inadequate stimulation of immune

regulatory circuits and clinical disease [220, 221] (**Figure 6**). This hypothesis builds upon the "old friends" concept, which highlights the long-term evolution of humans with old infections, commensal and environmental microbiotas that have a critical immune regulatory role to play [221].

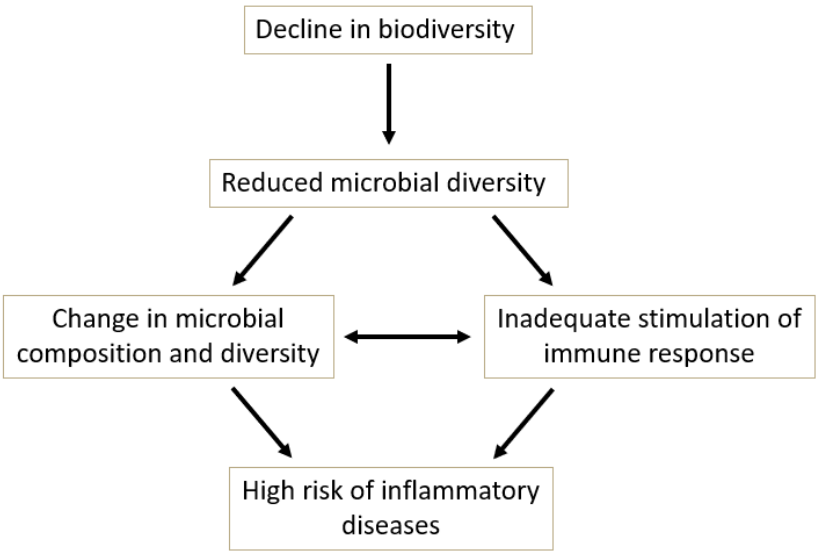

Figure 6 The biodiversity hypothesis

Adapted from Haahtela [221], 2019

Several studies reported a significant effect of living environment on the composition of human microbiome and consequently on asthma and allergic diseases [219, 222-224]. The border between Finland and Russia marks one of the sharpest boundaries between living standards, environment exposure and health. In Russian Karelia, people have small houses in the countryside with some cattle and domestic animals and produce much of their own food in small gardens. In contrast, Finnish Karelia has experienced major economic growth and rapid urbanization. Although the two current populations share partly the same ancestry, with about 15% of the current population of the Karelian Republic are Finns or Karelians, and the similar geoclimatic and vegetative conditions, the prevalence of asthma, hay fever and positive allergen-specific IgE levels to birch pollen, were significantly higher among children and young adolescents in Finnish than in Russian Karelia (8.8 *vs.* 1.6; 15.6 *vs.* 1.1; and 42.8 *vs.* 15.7, respectively) [222, 225]. Adults cohort showed that among those born after 1940s, sensitization to birch pollen increased in Finnish Karelia [225]. Furthermore, randomly selected children from the same areas were examined in 2003 and followed-up in 2010-2012 [222]. In 2003, a population-based study

of 2 generations (schoolchildren aged 7 to 16 years, and their mothers) was carried out in both regions to assess the effect of environment on generational differences. In Finnish Karelia the prevalence of atopy was significantly higher among children than their mother, whereas the opposite trend was found in Russia [226]. At the follow-up, adolescents from both areas differed in their skin and nasal bacterial community compositions, being diversity higher in skin and nasal Russian samples. The abundance of *Acinetobacter* was on average 3 and 4 times higher on skin and nasal epithelium in Russian Karelia, as compared to Finnish adolescents [222]. The contrasts between US Amish and Hutterite population [223] as well as urban and rural Mongolia [224] have provided similar results. Despite the similar genetic ancestries, Amish and Hutterite children revealed marked differences in the prevalence of asthma. Compared with the Hutterites, the Amish, who practice traditional farming and are exposed to an environment rich in microbes, showed a four times lower rate of asthma and distinct immune profiles. Additionally, the analysis of samples of mattress dust from Amish or Hutterite home showed different profiles in the abundance of bacteria [223]. In Mongolia, the prevalence of asthma, allergic rhinoconjunctivitis and allergic sensitization was also low in rural areas and increased with increasing urbanization, suggesting that rural living environment confer protection against allergic diseases [224].

The individual microbial composition and health tend to be affected by different environments. Supporting evidence is provided by studies showing that asthma and allergy are associated with lower environmental biodiversity and diversity in human microbiome composition. Hanski *et al.* [219] reported that environmental diversity around adolescents' homes influenced the composition of bacteria in their skin and atopy. Forest, agricultural land, and species richness of native flowering plants were positively correlated with diversity of gammaproteobacteria and *Actinobacteria* on the skin of adolescents and negatively associated with atopy. Additionally, a positive correlation was found between the relative abundance of gammaproteobacteria in skin healthy individuals and IL-10, one of the key anti-inflammatory cytokines in immunologic tolerance; however, a reverse correlation was observed in atopic individuals. Authors suggested that environmental biodiversity, human microbiome and human immune system are complex systems that interact with each other and that observed associations reflect immunologic responses developed by individuals with log-term exposure to environmental microbiome. Other studies highlighted the environment in which children grow up as an important factor affecting the composition of skin microbiome, which was related to their atopic diagnosis [227], and suggest that the size of this effect varies with age [228].

In 2018, a canine model was considered to assess the association between exposure to environment, skin microbiome and allergic symptoms. This model was adopted since dogs share

and are influenced by the living environment and lifestyle of their owners, and suffer increasingly from allergic diseases, providing an empirical evidence of such interaction. Compared to dogs living in rural environments with a large family and frequent animal contacts, dogs living in urban environments and exposed to urban-lifestyles, characterized by living in apartments, in a single-person family without other pets, have a higher prevalence of allergies. The composition of skin microbiome also differed in healthy and allergic dogs, being more heterogeneous in healthy dog families and associated with increasing area of arable land and forest in surroundings of birth and current home. Furthermore, the skin microbiome of dogs differed between rural and urban environments, suggesting that microbiome can have an important role in the development of allergic diseases. This assumed interplay between microbial exposure, host microbiome and allergic diseases highlight the importance of natural environments for health [229].

Several studies have also revealed that the diversity of bacterial communities has an important role in the immune system, being fundamental for immunological tolerance and tissue integrity [220]. A significant and positive correlation was observed between Proteobacteria (*Diaphorobacter*) and TLR2, which play a decisive role in recognizing microbes, such as Gram-positive bacteria, and bridging the innate and immune responses, inducing IL-10 [230].

This highlight the protective role of natural environment on human microbiome and health, namely on asthma development. The individual microbiome has an important role in our health, whereas human being hold the power to engineer the environment, both inner as well as the one surrounding us [231].

2.3.2. Walkability in urban environment

In the past few decades, increasing evidence suggests that features of the built environment are also associated with health-related behaviours such as physical activity, social connection and environment protection [232]. A key concept is *walkability*, which includes a combination of built environment factors such as street connectivity, residential density, net area retail and land use mix, that are conducive to walking (i.e. walking to destinations, including work, school, shopping) [233]. The street connectivity measures how well a street network provides multiple, direct and short routes to reach different destinations (≥3 intersecting streets). The residential density refers to the number of dwellings of residential land use, which is thought to be related to walking. Net area retail and land use mix expresses the diversity of land uses (commercial, residential, recreation/leisure, business/industrial, educational and others) within the individual's neighbourhood [233-235]. A high walkable neighbourhood is characterized by high residential

density, high street connectivity and high land use mix diversity [236], being an indicator of how user-friendly a neighbourhood area is for walking and biking [233].

Living in neighbourhoods characterized by higher walkability was found to be associated with more walking and cycling for transport and leisure, and with moderate to vigorous physical activity [237] and reduced obesity and overweight [238]. In children, high walkability is also positively associated with active park use and overall higher levels of physical activity. In a research on 13-15-year-old Belgian adolescents the average physical activity per day was associated with neighbourhood walkability; adolescents living in high-walkable neighbourhoods performed more moderate to vigorous physical activity than those who live in low-walkable neighbourhoods, but only in low-socioeconomic status neighbourhoods [239]. In 2011, Giles-Corti *et al.* [240] developed a school walkability index considering the traffic exposure and reported that children attending schools located in highly walkable areas were 3.63 times (95% CI 2.01; 6.56) more likely to walk to school than those attending schools in low walkability areas. However, children living in areas with high street connectivity and traffic were significantly less likely to walk to school (OR=0.32, 95% CI 0.22; 0.47). Previous studies also showed a positive association between active school travel and healthier body composition and level of cardiorespiratory fitness in children [241]. Recently, Simons *et al.* [242] reported an inverse association between home neighbourhood walkability and incidence of asthma and ongoing asthma in Toronto children after adjustment for neighbourhood and individual characteristics, highlighting the level of physical activity as a possible mechanism of association between the lower neighbourhood walkability and asthma. Furthermore, higher walkability around schools has been associated with positive attitudes towards children neighbourhood community, which were related to social interactions, social network and sense of community [243]. Although features of neighbourhoods, such as density, accessibility, and connectivity, may be predictors of air pollution levels [244], walkable neighbourhoods may also allow people to reduce their daily travel distance, by encouraging active and public transport modes, thereby decreasing vehicle emissions of air pollutants [245].

2.3.3. Air pollution in urban environment

Ambient air pollution and its health effects are also closely related with the scale of urbanization and the type and intensity of human activities. Cites at different stages of urbanization may have different sources of air pollution, including industry, transport, power generation, construction and household emissions [197]. For example, Denmark presents a satisfying urbanization growth with low air pollution (urbanization: 83.1%; air pollution intensity: 1.01 kt of carbon dioxide (CO_2)

equivalent per hundred billion dollars). However other countries, like Russia, presents high-level of urbanization and a serious problem regarding to air pollution (urbanization: 68.9%; air pollution intensity: 5.91 kt of CO_2 equivalent per hundred billion dollars), while Vietnam, maintain a low-level urbanization, air pollution has been a severe problem (urbanization: 21.7%; air pollution intensity: 5.66 kt of CO_2 equivalent per hundred billion dollars) [246]. Concern about environmental health and effect of air pollution has been increasing since the great smog of London, which killed 4000 people over the course of a few weeks in 1952 and caused 12000 excess deaths in the year after the event. The great smog of London half a century ago highlighted that air pollution can not only cause acute health effects but can also result in long-term effects [247].

According to WHO, nine out of ten people now breath polluted air, which kills 7 million people every year. An estimated 4.2 million premature deaths are linked to air pollution, mainly from heart disease, stroke, chronic obstructive pulmonary disease, lung cancer, and acute respiratory infections in children [248]. Children are at high risk of air pollution related disease and even extremely low-dose of pollutants during infancy and/or in early infancy can result in disease, disability, or death later in life [248].

Growing evidence supports the link between ambient air pollution exposure and the incidence of asthma in children. In a study involving 10 European cities, exposure to roads with high vehicle traffic were associated with 14% of all asthma cases in children and 15% of all exacerbations were attributed to exposure to road traffic pollutants [249]. In 2014, Guarnieri *et al.* [250] review the effect of different ambient air pollutants, including O_3, NO_2, SO_2 and PM on development and exacerbation of asthma in children. Authors suggested that these pollutants might have irritant and inflammatory effects on airway neuroreceptors and epithelium. Additionally, exposure to O_3 and nitrogen oxide has been reported as having an airway hyperresponsiveness effect [250]. In 2017, a meta-analysis, showed a positive association between asthma exacerbations and several air pollutants among children aged 0 to 18 years. In this subgroup, the association was significant for NO_2 (OR: 1.040; 95% CI 1.001; 1.081), SO_2 (OR: 1.047; 95% CI 1.009; 1.086), and $PM_{2.5}$ (OR: 1.022; 95% CI 1.000; 1.045) [251]. A recent population-based study also found that early-life exposures to NO_2 (OR: 1.25; 95% CI 1.10; 1.41 and $PM_{2.5}$ (OR: 1.25; 95% CI 1.06; 1.46) were positively associated with the risk of asthma development in childhood, per interquartile range increase in each pollutant (NO_2 IQR=8.51 ppb and $PM_{2.5}$ IQR=4.43 μ/m^3) [252]. Alotaibi *et al.* [253] estimated the number of new cases of asthma among children attributable to traffic related air pollution (TRAP) in USA in 2000 and 2010. In 2010, the incidence of asthma due to TRAP accounted for 18% (due to NO_2) to 36% (due to PM_{10}) of all cases. Most attributable cases

clustered in urban areas, being particularly prominent for NO_2. Moreover, authors found that children living in urban areas had twice the percentage of asthma cases attributable to NO_2 exposure as compared to children living in rural areas (30% versus 15% in 2000, and 20% versus 10% in 2010). The type and quantity of emitted pollutants are dependent on the type, age and condition of the vehicle and the fuel and road type [253]. Although the mechanisms by which pollutants induce the development or exacerbation of asthma are not completely clear, the UK's Committee on the Medical Effects of Air Pollutants identified the following four: oxidative stress and damage airway remodelling, inflammatory pathways and immunological responses, together with enhancement of respiratory sensitization to aeroallergens [254].

Urban green spaces not only provide balance for ecosystems but can also act as a buffer against exposure to air pollution, by removing pollutants from the atmosphere [12, 255]. Pollutants may be removed from the atmosphere through wet and dry deposition on the tree surface and/or by stomatal adsorption and absorption processes [256]. In Strasbourg from July 2012 to June 2013, public trees removed about 88 tons of air pollution, which varies with pollutants, amount of tree cover, condition and size, and seasons. In addition, sustaining healthy trees could improve air quality in cities by improving leaf surface area to remove pollutants by dispersing local pollutants or limiting dispersion towards sidewalks where people are often exposed to emissions of pollutants [257]. Similar results were observed in Gothenburg, Sweden [258], and in ten Italian metropolitan cities, authors found that concentrations of NO_2 and PAHs [258] and O_3 and PM_{10} [259], respectively, were affected by green areas. In contrast, Yli-Pelkonen *et al.* [260] suggested that forest vegetation in near-road urban environments in Helsinki does not improve local air quality. Authors reported that concentrations of gaseous air pollutants, including NO_2, O_3 and anthropogenic VOC, did not differ between tree-covered and adjacent open areas, while PM levels were significantly lower in tree-covered areas than in adjacent open. However, air pollution can also affect plant health and functions and limit pollutant dispersion, increasing local pollutants concentrations [257, 260]. Thus, greenspaces should be used as a complementary solution to reduce air pollution.

Schools neighbourhood

The complex interaction between human beings with urbanization is dependent not only on individual determinants such as gender, age, social or economic resources and lifestyles and behaviours, but also on urbanization, including air pollution, handiness of green areas and recreational facilities, neighbourhood safety and opportunities for mobility and physical activity

[261, 262]. Therefore, the assessment and identification of school environment impact (urban density, land-use mix and biodiversity) on health have become a priority and many recent studies have been conducted with the goal of better understand the impacts related to urbanization, identifying types of neighbourhoods, or characteristics of neighbourhoods that promote health benefits [263, 264].

Furthermore, urbanization represents a great opportunity to develop and implement policies to promote more sustainable, healthy and liveable cities. Thus, it is imperative to understand the association between the three levels – host, indoor environment and school neighbourhood and children health, namely on asthma and allergic diseases. This was highlighted already in the Parma Declaration where the European member countries committed themselves “to provide each child by 2020 with access to healthy and safe environments and settings of daily life in which they can walk and cycle to kindergartens and schools, and to green spaces in which to play and undertake physical activity” [265].

3. Aims

The overall aim of this thesis was to study the role of the environmental determinants of paediatric asthma.

Study-specific aims:

I. To review, summarize and compare existing quantitative data concerning spatial (schools, housing, offices and other indoor) and seasonal variation (cold and warm) of volatile organic compounds (VOC) concentrations that were detected, measured and reported both indoors and outdoors.

II. To assess the association between the indoor exposure to VOC identified as endocrine-disrupting compounds and asthma, respiratory symptoms and obesity in schoolchildren.

III. To evaluate the effect of school neighbourhood and their walkability on lung function, related airway inflammation and autonomic nervous system activity in children.

IV. To evaluate the effect of the school neighbourhood on obesity and body composition in schoolchildren.

V. To assess the effect of an indoor swimming pool exposure, an environment that has been associate with an increased asthma risk, on skin and gut microbiome.

4. Material and methods

4.1. Study design and participants

In **study I** original published studies from 2000 to 2015 were searched through PubMed, Scopus and Web of Science, following the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [266]. The search included the combined terms “volatile organic compounds” AND “indoor air” AND “outdoor air”. Additionally, a cross-reference check was performed to search for additional potential studies. All studies that fulfilled the following inclusion criteria were considered: published in English; VOC measured in both indoor and outdoor environments; and VOC measured in at least one season. The exclusion criteria were studies reporting modelled or estimated exposure.

Studies that reported mean concentration values for different VOC were considered and stratified by space (outdoor and indoor environments) and season (cold – winter and fall; warm – summer and spring). Indoor environments comprised 4 categories: schools, housing such as homes and hotels, offices, and other indoor settings. Other indoor settings included shopping malls, hospitals and dental clinics, restaurants, photocopy centres and museums. No restrictions were considered in the selection of studies according to analytical method that were used for identification and quantification of VOC. In the included studies, the most reported method was gas chromatography coupled with mass spectrometry (GC-MS), which was used in 18 studies, followed by gas chromatography coupled with flame ionization detector (GC-FID) (11 studies), whereas only 6 studies used gas chromatography coupled with mass selective detector (GC-MSD) and 1 used gas chromatography coupled with an electron capture detector (GC-ECD). Three studies reported the use of high-performance liquid chromatography (HPLC).

Initially, 1393 papers were retrieved from the search including overlapping publications across the 3 databases. After removal of duplicates (177), 1123 papers were excluded by title and abstract screening. Another 53 papers were removed after assessment of the full text as these did not meet inclusion criteria. Ultimately, 40 papers were included in this review. Twelve studies were conducted at schools, 21 in housing environments, 4 in offices and 7 in other indoor environments (**Figure 7**).

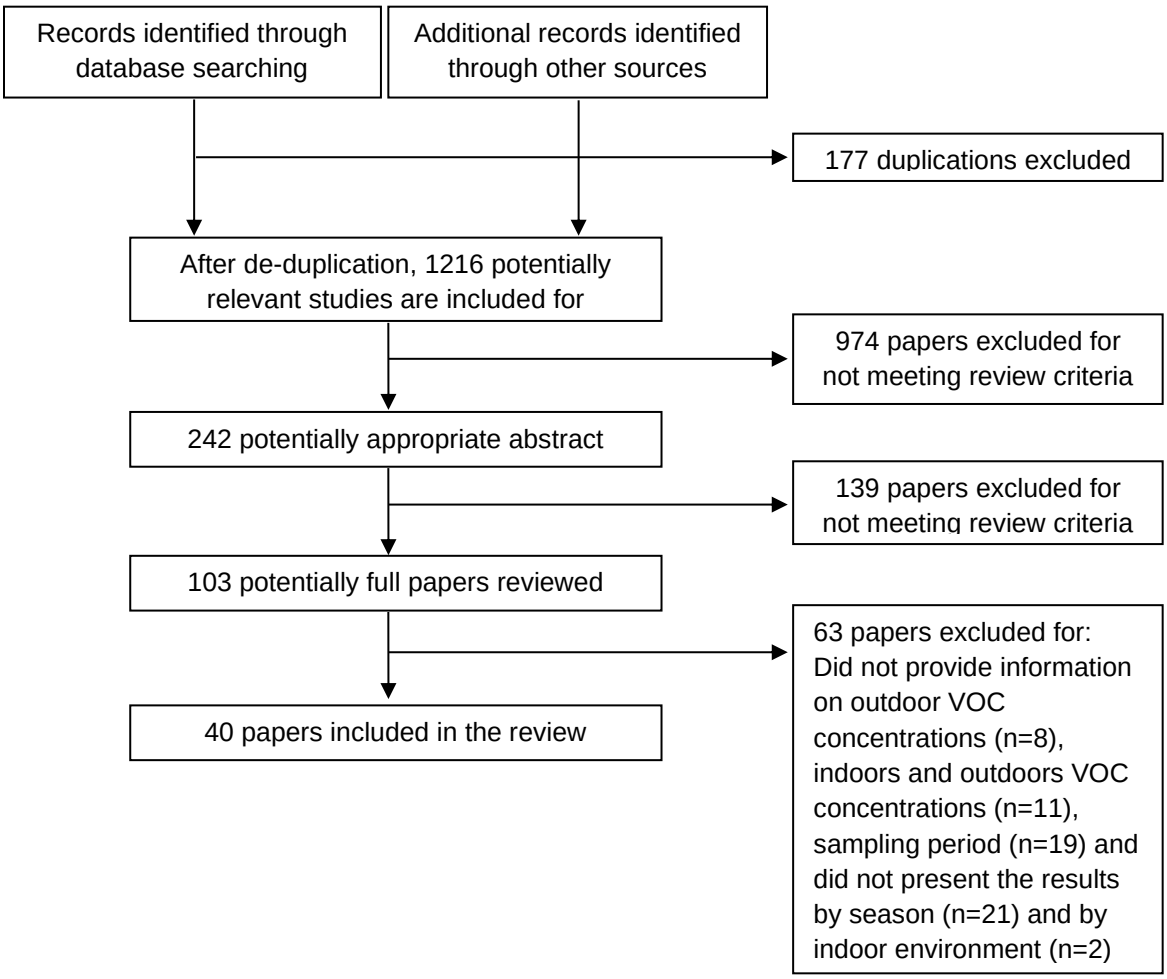

Figure 7 Systematic review flowchart

Studies II, III and **IV** included data from the ARIA (How indoor air quality can affect children allergies and asthma) project. ARIA is a cross-sectional study assembled in Porto, Portugal. The aim of ARIA was to contribute to a better understanding of the effects of the exposure to schools' indoor air on children's health, taking also into account the contribution of the home environment. Enrolment took place during the heating season in two different periods: from January to April 2014 and from October 2014 to January 2015.

From the 53 public primary schools of Porto Municipality, 20 schools with the highest number of students were selected (**Figure 8**). In each school, four classrooms of 3rd and/or 4th grades were selected among those with similar conditions and representative of the school building, corresponding to a total of 71 assessed classrooms. Classrooms were selected based on the International Study of Asthma and Allergies in Childhood (ISAAC) Phase II criteria, where children

of this age group were more likely to understand the procedures than 6-7-year-old children and to be more compliant that 13-14-year-old adolescents [267], highest density of occupation, full-week occupation time by the same class, and, if possible, on the location of classrooms on different floors [268].

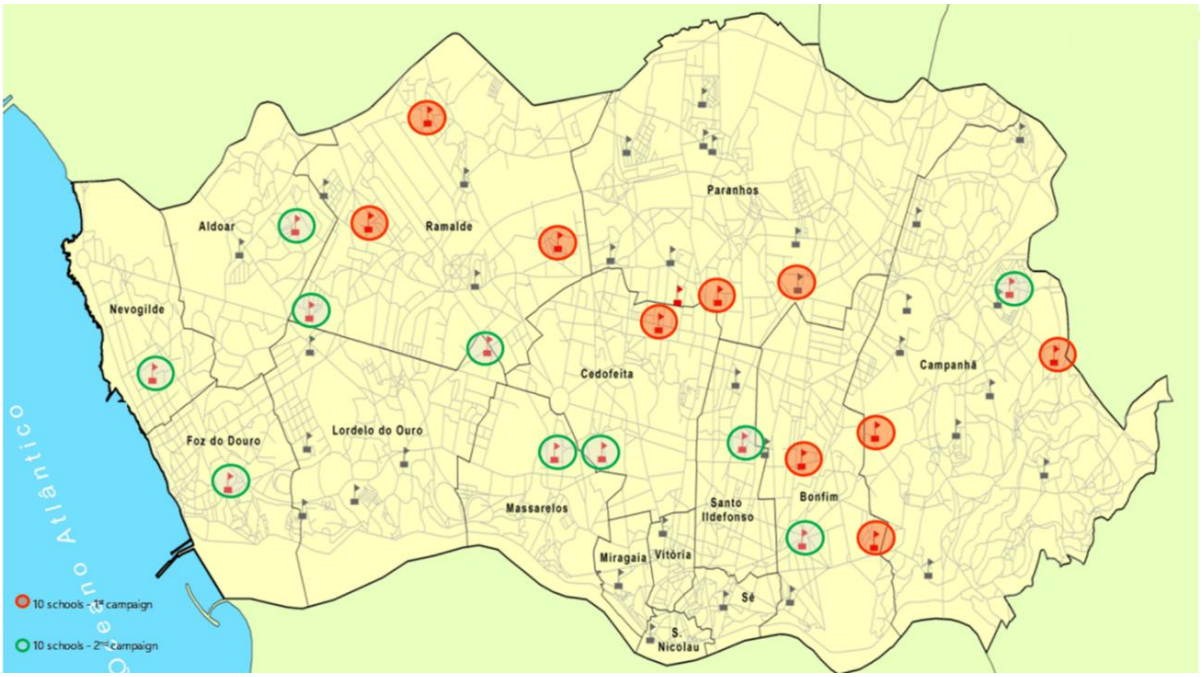

Figure 8 Location of the 53 primary schools at Porto according to the participation on this study

Red circles correspond to the first 10 schools (first campaign) and red circles correspond to the last 10 schools (second campaign)

In total, 1602 children (7-12 years old) were invited to participate (**Figure 9**). After receiving the written consent from their legal guardians, 916 children were enrolled in the study (participation rate of 57.2%). Among them, 58 children refused to perform clinical tests. Considering a confidence level of 95%, the estimated confidence interval for the sample size was 2.42, showing a high statistical power.

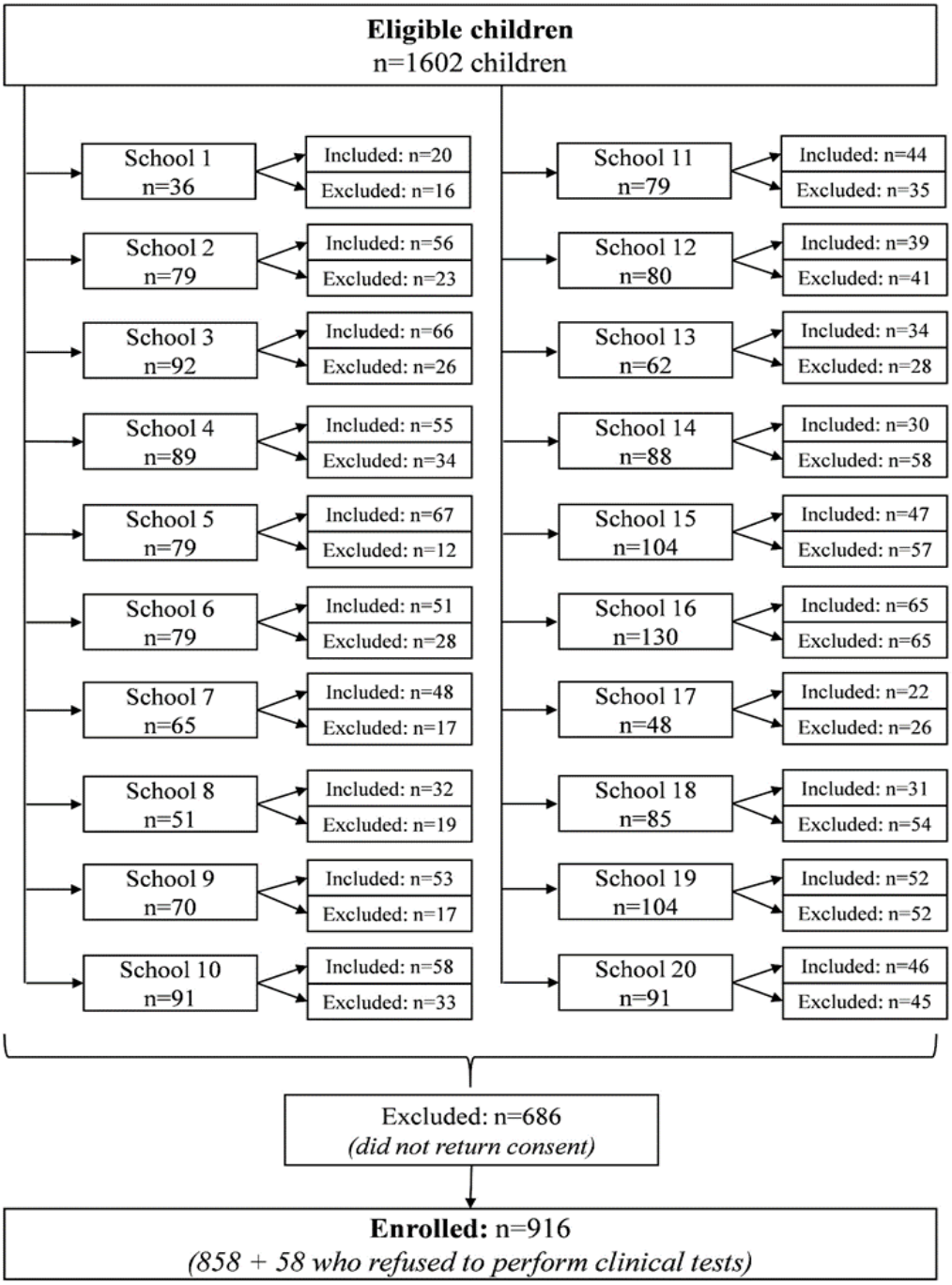

Figure 9 Flow of participants through each phase of the study

Study V included data from the SWAN (Swimming pool environment impact on the human respiratory health research protocol) study. SWAN was a cross-sectional study assembled in Porto, Portugal, between February and April 2017 (ClinicalTrials.gov Identifier: NCT03017976). This study aimed to assess the influence of exposure to swimming pool environment on

respiratory symptoms and diseases, and on cancer outcomes among swimmers and swimming pool attendants. In total, 33 regular and competitive swimmers (12-21 years old), from one indoor swimming pool, were invited to participate. Thirty-four football players from B team, sub-14/15 and sub-13 levels (11-18 years old), considered as non-water competitive athletes, were also invited to participate. Among swimmers, 3 did not continue in the study. Among non-water competitive athletes, all athletes return the signed informed consent form and participated in the study. Thus, this study was based on data from 30 swimmers (66.7% girls) and 34 non-water competitive athletes (20.6% girls).

4.2. Participants assessment

In the **studies II**, **III**, **IV** and **V**, a self-administered ISAAC-based questionnaire [269] was filled out by the children's caregivers and reviewed by a research nurse. A physical and clinical assessment was also performed by trained health professionals. A summary of the study outcomes and instruments is showed in **Table 1**.

Table 1 Summary of study outcomes and instruments

Outcome	Instruments	Study	Reference
Symptoms	Standardized questionnaire based on ISAAC's	II-V	Asher *et al.* [269]
Anthropometry			
BMI	Tanita™ BC-418 Segmental Body Analyzer	II-V	CDC, WHO, IOTF [270-272]
Body fat			McCarthy *et al.* [273]
Allergic sensitization	QuickTestTM applicator with allergen batch (Hall Allergy, Netherlands)	II-V	GINA [274]
Lung function			
Spirometry	Spirobank (MIR, Italy)	II-V	Miller *et al.* [275]
Airway inflammation			
Exhaled NO	NObreath analyzer (BedfontScienctific Ltd)	II-V	Dweik *et al.* [276]
EBC pH	pH meter (pHenomenal® pH 1100 H)	III	Horvath *et al.* [277] + mi
Autonomic nervous system	Pupillometry, PLR-200™ Pupillometer (NeurOptics Inc, CA, USA)	II, III, V	Muppidi *et al.* [278]
Microbiome	16S ribosomal RNA	V	Costello *et al.* [279]

mi: manufacturer instructions; BMI: body mass index; NO: nitric oxide; EBC: exhaled breath condensate.

4.2.1. Questionnaires

A self-administered ISAAC-based questionnaire [269] was used to collect information on social, demographic and behavioural characteristics and including questions regarding the respiratory/allergic health and current symptoms (previous 3 months) of children. Wheezing and cough symptoms were defined as a positive answer to the questions “In the past 12 months, has your child had wheezing or whistling in the chest?” and to any of the two following questions “In the last 12 months, has your child suffered coughing at night?” or “In last year, has your child suffered coughing more than three months?”, respectively. Asthma symptoms were defined considering the presence of wheezing and cough symptoms, shortness of breath and chest tightness. Children were considered to have current symptoms if there was a positive answer to the question “During the past 3 months, has your child had any of the following symptoms?”: “Itching on the face or neck”, “Eye irritation (redness, dryness, itch)”, “Swollen eyes”, “Runny nose”, “Nasal obstruction/blocked nose”, “Dry throat”, “Sore throat”, “Irritative cough”, “Breathing difficulties”, “Feeling like getting a cold” and “Nausea”. Children were considered to have allergic rhinitis if there was a positive answer to the question “Has your child ever had a problem with sneezing, or a runny nose or blocked nose when he/she did not have a cold or the flu?” and a positive skin prick test (SPT). Current allergic rhinitis was defined as a positive answer to the question “In the past 12 months, has your child had a problem with sneezing, or a runny nose or blocked nose when he/she did not have a cold or the flu?” and a positive SPT. Family history of asthma or allergy was recorded as a positive answer to the question “Are there any allergic disorders in the family, including asthma and allergies?”.

Parental education level was used as socioeconomic status indicator and was recorded as the number of successfully completed years of formal schooling. Children were classified according to the parent with the higher education level. Parental education level was categorized into three classes: ≤ 9 years; ≥ 10 years and ≤ 12 years; and ≥ 13 years.

Energy intake was recorded using the interviewer-administered 24-hour dietary recall method [280]. Parents or caregiver of the children were questioned accurately about their children food, and drinks consumption, even reporting brands, and consuming time, and place. The software Food Processor® (ESHA Research, USA) was used to convert food into energy intake.

4.2.2. Clinical and physical evaluation

Anthropometry

Weight (Kg), body fat percentage, body fat mass (Kg), free fat mass (FFM, Kg), total body water (TBW, %) and basal metabolic rate (BMR, Kcal) were measured using a digital scale (Tanita™ BC-418 Segmental Body Analyzer). Height in centimetres (cm) was measured with a portable stadiometer. Body fat percentage was classified in accordance with sex-specific centile curves for body fat in children [273]. Body mass index was calculated using the ratio of weight/height2 (Kg/m^2) and classified according to the age- and sex-specific percentiles defined by the US Centers for Disease Control and Prevention (CDC) [270], World Health Organization (WHO) [271], and International Obesity Task Force (IOTF) [272].

Skin-prick-tests

Skin-prick-tests were performed on children's forearm using a QuickTestTM applicator containing house dust mite, mix of weeds, mix of grasses, cat dander, dog dander and *Alternaria alternata*, negative control, and a positive control consisting of histamine at 10mg/mL (Hall Allergy, Netherlands). Results were read 15 minutes afterwards and atopy was defined by a positive SPT to at least one of the allergens [274].

Lung function

Lung function and airway reversibility were assessed by spirometry according to ATS/ERS guidelines [275] using a portable spirometer (MIR Spiro bank, A23-04003237) before and 15 minutes after inhalation of 400µg of salbutamol. Different asthma and asthma-like conditions were considered: i) *Medical diagnosis of asthma*: self-reported medical diagnosis; ii) *Positive bronchodilation (BD+)*: at least a 12% and over 200mL increase in forced expiratory volume in 1 second (FEV1) after bronchodilation; iii) *Medical diagnosis of asthma and symptoms or BD+*: at least a 12% and over 200mL increase in FEV1 after bronchodilation or a self-reported medical diagnosis with reported symptoms (wheezing, dyspnoea or dry cough) in the past 12 months; iv) *Medical diagnosis of asthma or BD+:* at least a 12% and over 200mL increase in FEV1 after bronchodilation or a self-reported medical diagnosis (**Table 2**).

Table 2 Definitions of asthma

Variable	Definition
Medical diagnosis of asthma	self-reported medical diagnosis
Positive bronchodilatation (BD+)	at least a 12% and over 200mL increase in FEV1 after bronchodilation
Medical diagnosis of asthma and symptoms or BD+	at least a 12% and over 200mL increase in forced expiratory volume in 1 second (FEV1) after bronchodilation or self-reported medical diagnosis with reported symptoms (wheezing, dyspnoea or dry cough) occurring in the past 12 months
Medical diagnosis of asthma or BD+	at least a 12% and over 200mL increase in FEV1 after bronchodilation or self-reported medical diagnosis

Airway inflammation

Airway inflammation was assessed by measuring the fractional exhaled nitric oxide level using a NObreath analyzer (BedfontScienctific Ltd) in accordance with the ATS guidelines [276]. Furthermore, exhaled breath condensate (EBC) was collected from the children by breathing (regular tidal volumes and respiratory rate) 10 to 15 minutes to an exhaled air condensing system (portable Turbo DECCS) [277]. EBC samples were transferred to sterile tubes and stored at -80ºC until laboratorial analysis. Deaeration was performed by bubbling oxygen through the sample for 10 minutes at 0.3 L/min [281, 282]. The pH of the EBC was measured before and 10 minutes after deaeration with oxygen using a calibrated pH meter (pHenomenal® pH 1100 H) with an accuracy of ±0.005 pH units. The pH meter was calibrated before each measurement using solutions with pH values of 4, 7, and 10.

Pupillometry

Pupillometry is a simple noninvasive technique that can provide valuable data concerning the functioning of both branches of the autonomic nervous system [209, 278, 283, 284]. According to Purves D *et al.* [285], the pupil light reflex is the reflex by which a change in pupil size occurs in response to light intensity. Under the direct control of the autonomic nervous system (ANS), the pupil light reflex reflects the balance between the parasympathetic (PNS) and sympathetic nervous system (SNS) [285]. The pupil light reflex pathway has been previously reported by Wang *et al.* [286], showing that as response to light reflex, neurons of the PNS innervate circular fibers of the iris, causing pupillary constriction, whereas excitation by SNS neurons causes the radial

fibers to produce dilation of the pupil. Therefore, the pupillary response to an external light stimulus might provide an indirect means to assess the ANS activity. The pupil diameter, average and maximum construction velocities (ACV and MCV, respectively), latency and constriction amplitude are related to parasympathetic activity, while average dilation velocity (ADV) and the total time taken by the pupil to recover to 75% of the initial resting pupil size after reaching peak of constriction (T75) are measures of sympathetic activity [278].

Pupillometry was performed with a portable infrared PLR-200 pupillometer (NeurOptics PLR-200™ Pupillometer, NeurOptics Inc., CA). The children spent at least 15 minutes in a semi-dark and quiet room to allow pupil adjustment to the low level of light, after which they were instructed to focus on a small object 3 metres away with the eye that was not being tested, keeping their head straight and eyes wide open during targeting and measurement (**Figure 10**). The eye was briefly illuminated by light-emitting diodes with a single light stimulus having a peak wavelength of 180 nm. A pupillary light response curve for each eye was recorded for each child. During pupillometry, the eye is stimulated with a flash of light, and a rapid sequence of digital images captures the pupil diameter when it constricts and then redilates to its original size. Data were recorded for each child for the diameter of the pupil before (initial) and at constriction peak (minimal), in millimetres; for the relative constriction amplitude, as a percentage; for the MCV, ACV and ADV, all in mm/s; and T75, in seconds. All pupillary data reported were from one eye, since no side-to-side difference was observed in the pupil responses ($p<0.05$).

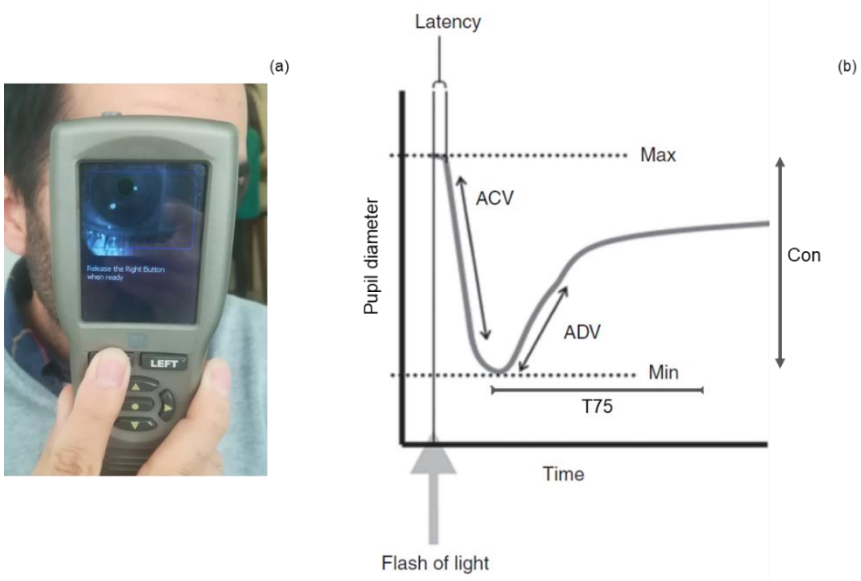

Figure 10 Pupillometry measurement (a) and results (b)

Max: diameter of the pupil before the constriction; min: diameter of the pupil at constriction peak; ACV: average construction velocity; MCV: maximum construction velocity; Con: constriction amplitude; are related to parasympathetic activity, while ADV: average dilation velocity; T75: total time taken by the pupil to recover to 75% of the initial resting pupil size after reaching peak of constriction are measures of sympathetic activity

Adapted from Patwari *et al.* [287], 2012

4.2.3. Microbiome sample collection and analysis

Sample collection

Microbiome sample collection was performed in **study V**. Sterile nylon swabs (FLOQSwabs™, COPAN) were used to collect all samples. Skin samples were collected by lightly pressing a swab dipped in sterile 0.15M NaCl and 0.1% Tween 20 solution against the skin on a 5×5cm area of the volar surface of the forearm of the participant's dominant hand. Skin samples were collected in two different moments: before (T0) and after 2 hours of swimming training (T1) to assess the effect of swimming pool environment. Regarding to non-water competitive athletes, samples were collected in one moment: before training (T0). Stool samples were collected using dry swabs by swabbing used bathroom tissue. Costello et al. [279] found that swabbing off of used bathroom tissue was adequate for obtaining material for microbial analysis, considering a similar laboratory approach. Stool sample were collected by each participant on the same day that the skin samples were collected (within 24 hours). Stoll samples have been showed to provide an adequately representative sample of the gut microbiome [288]. After collection, all samples were transferred to -80°C for storage. These samples were subsequently transported on dry ice to Institute of

Biotechnology, DNA Sequencing and Genomics Laboratory, University of Helsinki, Finland and maintained at −80°C until DNA extraction.

DNA extraction, PCR and sequencing

Sample DNA was extracted using FastDNA SPIN Kit for Soil (MP Biomedicals), according to manufacturer's instructions. Each extraction batch included a blank with no template DNA. PCR amplification was carried out in a PTC-225 thermal cycler (MJ Research). PCR amplification of the V3-V4 region of the 16S ribosomal RNA (rRNA) gene was performed in two steps. The first step was run with 2x25 µL technical replicates of each sample. This step was done with the primers 341F1-4 and 785R1-4. The cycling conditions were: initial denaturation at 98°C for 30 seconds, followed by 15 cycles at 98°C for 10 s, 55°C for 30 s, and 72°C for 10 s, and a final extension for 5 minutes at 72°C. The PCR products were purified with Exonuclease I and FastAP (Thermosensitive Alkaline Phosphatase, Thermo Scientific). A second PCR was performed with TruSeq and Index 8bp primers. The cycling conditions were: initial denaturation at 98°C for 30 seconds, followed by 18 cycles at 98°C for 10 s, 65°C for 30 s, and 72°C for 10 s, and a final extension for 5 minutes at 72°C. DNA extraction kit blank and PCR blanks (also with no DNA template) were amplified and sequenced for identification of potential contaminating DNA The final PCR products were purified with Agencourt® AMPure® XP magnetic beads (Beckman Coulter, CA, USA) and pooled. All samples were sequenced in a single run on MiSeq (Illumina, San Diego, CA).

4.3. Exposure assessment

4.3.1. Indoor air

The indoor levels of specific VOC were measured in each classroom over one week, from early Monday until late Friday, during regular daily activities and under representative conditions of occupancy and use of the classrooms in heating period. Indoor samples were collected at a height of about 1-1.5 m above the floor, which is the breathing zone, and at least at 1 meter of distance to a wall, a door or an active heating system [289].

Volatile organic compounds were collected passively onto thermally desorbed adsorbents tubes containing Tenax® TA (60/80 mesh). After sampling, the Tenax tubes were thermally desorbed (Dani STD 33.50) and compounds were quantified by gas chromatography (Agilent Technologies 6890N) coupled to a mass spectrometry detector (GC-MS) (Agilent Technologies 5973), according to the standard ISO 16000-6 [290]. Compounds concentrations were calculated based

on their own response factor or, in case of inexistence of the specific standards, toluene's response factor was used. Cyclodecane was injected simultaneously as an internal standard, to assess the sample loss during desorption. The uncertainty of the analytical method calculated for toluene is ±5.1%. All samples were collected in duplicate.

Formaldehyde and acetaldehyde were sampled by Radiello® passive devices (RAD 165, Sigma Aldrich) and determined using isocratic reverse phase high performance liquid chromatography (HPLC) (Agilent Technologies, 1220 Infinity LC) with a UV detector operated at 360 nm, according to the standard ISO 16000-4 [291]. Aldehydes were identified and quantified by comparing their retention times and peak areas with those of standard solutions. As an internal quality control, duplicate samplings were collected in one school per each three.

The concentrations of 13 VOC and 2 aldehydes identified as EDCs by *The Endocrine Disruption Exchange Research Institute* (TEDX) [292] were considered, including toluene, o-xylene, m/p-xylene, hexane, ethylbenzene, styrene, cyclohexanone, butylated hydroxytoluene (BHT), benzene, benzaldehyde, tetrachloroethylene (T4CE), 2-butoxyethanol, 2-ethyl-1-hexanol, formaldehyde and acetaldehyde (**Table 3**).

Table 3 Indoor air concentrations of endocrine-disrupting chemicals in classrooms (n=71)

Parameter (µg/m^3)	Classrooms (n) with concentration >LQ µg/m^3	Median (25^{th}-75^{th} percentile)	Minimum-Maximum
Toluene	71	9.11 (5.61-14.1)	1.44-109.0
o-xylene	64	2.35 (0.98-3.49)	0-35.1
m/p-xylene	65	4.07 (2.16-6.88)	0-114.0
Hexane	5	22.9 (0-94.6)	0-568.5
Ethylbenzene	53	1.13 (0-2.63)	0-37.3
Styrene	58	0.36 (0.03-0.88)	0-6.97
Cyclohexanone	5	9.89 (0-14.0)	0-28.3
Butylated hydroxytoluene	10	0.89 (0-5.12)	0-5.12
Benzene	68	0.74 (0.37-1.37)	0-3.08
Benzaldehyde	60	10.1 (6.88-17.6)	0-48.7
Tetrachloroethylene	8	0 (0-<LQ)	0-5.58
2-butoxietanol	14	8.99 (5.47-61.5)	0-188.4
2-ethyl-1-hexanol	43	3.66 (2.15-5.57)	0-45.3
Formaldehyde	71	16.4 (12.4-21.1)	0.31-37.9
Acetaldehyde	71	7.57 (5.68-9.54)	0.39-12.8

LQ: Limit of quantification

4.3.2. Urban land use

The land use near each school was calculated based on the European Urban Atlas using a geographical information system (GIS). The Urban Atlas (https://www.eea.europa.eu/data-and-maps/data/copernicus-land-monitoring-service-urban-atlas) city information is currently the most up-to-date, harmonized database for the European Union countries, offering a high-resolution land-use map of cities (population ≥ 100,000), mapped using a total of 20 land use classes of which the 17 are urban classes (**Table 4**). The scale of Urban Atlas is 1:10.000 and the minimum mapping unit is 0.25 ha for the artificial surfaces and 1 ha for the other surfaces and data have been produced from sensors with spatial resolution of 2.5 meters [293].

Table 4 The Urban Atlas nomenclature

Class code	Nomenclature
11100	Continuous Urban Fabric (Sealing Degree > 80%)
11210	Discontinuous Dense Urban Fabric (Sealing Degree 50% - 80%)
11220	Discontinuous Medium Density Urban Fabric (Sealing Degree 30% - 50%)
11230	Discontinuous Low-Density Urban Fabric (Sealing Degree 10% - 30%)
11240	Discontinuous Very Low-Density Urban Fabric (Sealing Degree < 10%)
11300	Isolated Structures
12100	Industrial, commercial, public, military and private units
12210	Fast transit roads and associated land
12220	Other roads and associated land
12230	Railways and associated land
12300	Port areas
12400	Airports
13100	Mineral extraction and dump sites
13300	Construction sites
13400	Land without current use
14100	Green urban areas
14200	Sports and leisure facilities
20000	Agricultural, Semi-natural areas and Wetlands
30000	Forests
50000	Water bodies

A circular buffer of 500 metres around each primary school address was created (**Figure 11**). This buffer was based on reasonable walking distances described by Browson and colleagues [294], corresponding to approximately 6 minutes' walking distance for children [295].

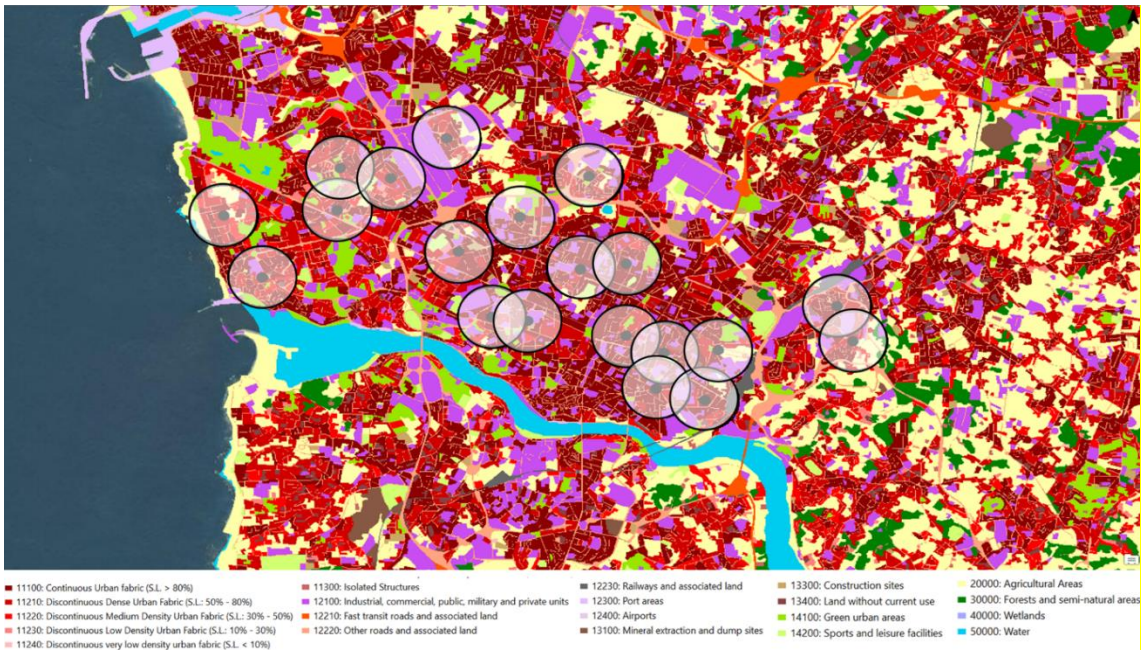

Figure 11 Neighbourhood land use around the 20 evaluated primary schools in Porto

Each school was represented by a point and a circular buffer of 500 metres

4.3.3. Walkability

The term walkability has been used to conceptualise a combination of built environment factors such as street connectivity, residential density, net area retail and land use mix, that are conducive to walking (i.e. walking to destinations, including work, school, shopping) [233]. Walkability is an objective indicator of how user-friendly a neighbourhood area is for walking and biking [233] and has been associated with physical activity and active transportation [296]. However, features of neighbourhoods that are associated with walkability may also be predictors of air pollution levels [244].

The walkability index was calculated based on street connectivity, residential density, and land use mix (expressed as an index of entropy), within the 500-metres buffer. This calculation has been previously described and determined across Porto neighbourhoods by Ribeiro and colleagues [297]. The street connectivity was calculated from the density of street junctions within

the primary school's neighbourhood considering the data from Porto's street map using the Environmental Systems Research Institute (ESRI). Only the streets that allowed pedestrian circulation were considered, freeways were removed. Residential density in each neighbourhood was obtained by calculating the density (number/area) of households based on the Portuguese population census of 2011. Whether the circular buffer cut census tracts, the number of the households that exist in the intersection area was estimated based on data from the *Instituto Nacional de Estatística* of 2011 [298]. Land use mix expresses the diversity of land use within school's neighbourhood and 6 types of land uses (commercial, residential, recreation/leisure, business/industrial, educational and others) were defined [299]. Residential and commercial data was obtained from *Instituto Nacional de Estatística* and ESRI and Urban Atlas were used to validate areas previously classified as residential and commercial areas and to identify leisure, business/industrial and educational areas. The entropy index was calculated for each neighbourhood and the values ranged between zero (low land use diversity) to one (high land use diversity) [300, 301].

After calculating these three components (connectivity, residential density, and land use mix), walkability index was calculated for each school's neighbourhood and the raw values were normalized using z-scores. The walkability index was calculated by adding weighted z-scores according to the following formula (equation 1):

$$\text{Walkability} = (2 * z - \text{connectivity}) + (z - \text{residential density}) + (z - \text{land use mix}) \quad \text{(equation 1)}$$

This formula is an adapted version of the formula of Frank and colleagues [233]. Residential density and land use mix were weighted by a factor of one, whereas street connectivity z score was weighted by a factor of two. This was based on prior evidence regarding reported utilitarian walking distances and the resulting strong influence of street connectivity on non-motorised travel choice [233]. Next, the values were normalized, and walkability index varies from zero (least walkable) to one (most walkable). Primary schools' neighbourhoods were characterized according to tertiles (from low to high) of neighbourhood walkability (**Figure 12**).

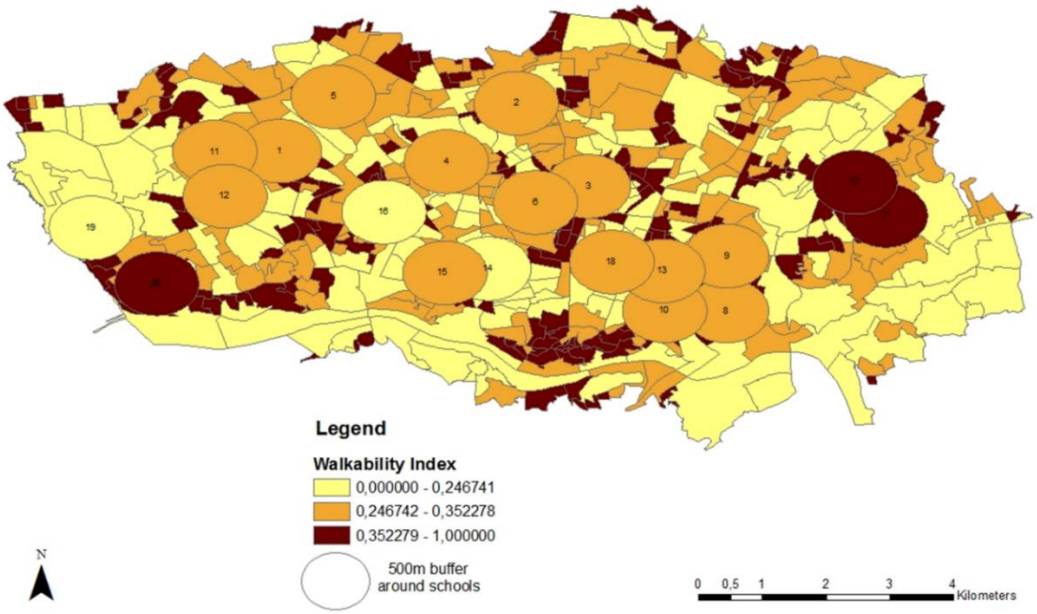

Figure 12 Neighbourhood walkability index around each primary school

Each school, represented by a number and a circular buffer of 500 metres, was characterized according to tertiles of neighbourhood walkability

4.4. Statistical analyses

For the **study I**, distribution of quantitative variables was determined using the Shapiro-Wilk test and Mann–Whitney or Kruskal-Wallis test were used to test comparisons between VOC concentrations, indoor and outdoor, and seasons for the 4 indoor environments considered. All the analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 17.0, and the level of statistical significance was set at 0.05.

In **study II**, the Kolmogorov-Smirnov test was used to check continuous variables for normality. The Mann-Whitney test was used to compare variables between girls and boys. Significant differences were reported with an α-value of less than 5% ($p<0.05$). Principal component analysis (PCA) was used to identify major EDCs patterns based on the 15 individual chemical compounds. Varimax rotation was performed to simplify the interpretation of the factor loading structure. The number of components was chosen based on eigenvalues greater than one. The PCA divided the EDCs into two principal components (PC1 and PC2), which were included in each PC if the correlation coefficient was equal to or greater than 0.30. PC1 had a higher absolute correlation with toluene, o-xylene, m/p-xylene, ethylbenzene, styrene and benzene, while PC2 had a higher

absolute correlation with cyclohexanone, BHT, benzene, benzaldehyde, formaldehyde and acetaldehyde (**Table 5**).

Table 5 Rotated component matrix of endocrine-disrupting chemicals

Compounds	Components and factor loadings	
	PC1	PC2
Toluene	0.85*	-0.01
o-xylene	0.97*	-0.03
m/p-xylene	0.97*	0.00
Hexane	-0.10	-0.04
Ethylbenzene	0.97*	0.00
Styrene	0.45*	0.14
Cyclohexanone	-0.05	-0.37*
Butylated hydroxytoluene	-0.09	0.40*
Benzene	0.54*	-0.34*
Benzaldehyde	-0.07	-0.33*
Tetrachloroethylene	0.16	0.00
2-butoxietanol	-0.02	0.26
2-ethyl-1-hexanol	-0.03	-0.10
Formaldehyde	0.01	0.86*
Acetaldehyde	-0.05	0.78*

*Compounds which were included in each principal component (PC)

Generalized linear models and multinomial logistic regression models were used to measure the effect of individual or combined EDCs on asthma, on the presence of respiratory symptoms in the previous 3 months, obesity and body composition in the schoolchildren. Crude model (model 0); model 1, adjusted for age and sex; and model 2, additionally adjusted for the following variables: a) BMI (according to the CDC criteria), atopy and parental education; b) asthma (according to the medical diagnosis of asthma and symptoms or BD+ criteria), parental education, physical activity and energy intake; c) asthma (medical diagnosis of asthma and symptoms or BD+) and parental education were considered. PCA and mixed effect models were analysed using RStudio software, version 1.0.

In **study III** and **IV**, the Kolmogorov-Smirnov test was used to check continuous variables for normality. The Mann-Whitney test was used to compare variables between girls and boys.

Significant differences were defined according to an α-value of 5% ($p<0.05$). Principal component analysis was used to identify major neighbourhood patterns based on 20 land use classes. Varimax rotation was performed to simplify the interpretation of the factor loading structure. A fixed number of factors were extracted, and two principal components were selected on that basis (PC1 and PC2). Between the two factors, PC1 had higher absolute correlation with discontinuous dense urban fabric, discontinuous medium-density urban land, green urban areas, and water bodies; while PC2 had higher absolute correlation with construction sites, land without current use, and railways. PC1 was characterized as green urban areas, and PC2 as built areas (**Table 6**).

Table 6 Rotated component matrix of land uses classes

Land uses classes	**Components**	
	PC1	**PC2**
Construction sites	-0·125	0·785*
Continuous Urban Fabric	-·0313	-0·732
Discontinuous Dense Urban Fabric	0·662*	0·335
Discontinuous Low-Density Urban Fabric	0·369	0·139
Discontinuous Medium Density Urban Fabric	0·911*	0·016
Forests	0·021	0·238
Green urban areas	0·468*	-0·373
Industrial	-0·687	0·125
Land without current use	0·267	0·610*
Other roads	-0·263	0·166
Railways	-0·177	0·717*
Sports	0·020	-0·199
Water bodies	0·833*	0·080

* Land uses classes which were included in each principal component

Afterwards, the PC1 and PC2 scores were ranked from 1 to 20, and the rank numbers were divided by 20. Mixed-effect models with a random effect of school were used to measure the effect of schools on lung function, airway inflammation and autonomic nervous system in children (**study III**) and on BMI and body composition (**study IV**). The intraclass correlation coefficient (ICC) and the proportion of explained variation were used to quantify the effect of schools and to quantify the effect of individual, neighbouring environment, and walkability on the school effect.

Intraclass correlation coefficient can be interpreted as the proportion of total variance in each outcome that could be attributed to school neighbourhood. The effect of schools' neighbourhoods on children health were analysed using a multilevel with individual-level and neighbourhood-level factors. All individual- and neighbourhood-level (as median values) factors were used in the multilevel analysis as independent variables. In **study III**, two models were considered for the analysis: crude model (model 0 and 1), and an individual-neighbourhood level model (mixed effects model, model 3). Model 0, only included the PC1 and PC2 score or walkability; Model 1, the null model, baseline model without any exposure variable; Model 2a is additionally adjusted for PC1 and PC2 score or walkability, age, sex, asthma, WHO z-score for BMI and family history of asthma or allergy; Model 2b is additionally adjusted for PC1 and PC2 score or walkability, age, sex, asthma, atopy, WHO z-score for BMI and family history of asthma or allergy; Model 2c is additionally adjusted for PC1 and PC2 score or walkability, age, sex, asthma, and WHO z-score for BMI.

In **study IV**, four models were considered for the analysis: crude model (model 0 and 1), neighbourhood-level model (model 2), and an individual-neighbourhood level model (mixed effects model, model 3). Model 0 only included the PC1 and PC2 score; Model 1, the null model, baseline model without any exposure variable; Model 2 is adjusted for PC1 and PC2 score; and Model 2 is additionally adjusted for age, sex, parental education, physical activity, energy intake and asthma. PCA, mixed-effect models and ICC were computed using the software RStudio, version 1.0.

In **study V**, the sequence analysis, including Operational Taxonomic Unit (OTU) clustering and taxonomy assignment, was performed in mothur software [302]. Mothur was used to process the sequence data and to perform taxonomic assignments following the OTU approach from the MiSeq Standard Operating Procedure [302-304]. The main sequence processing was performed in 7 steps: 1) reduction of sequencing errors; 2) alignment of the dataset to an external database; 3) pre-clustering the sequences, followed by a pre-specified similarity threshold for sequence merging; 4) identification and removal of chimeric sequences produced by PCR amplification; 5) removal of undesirable sequences derived from chloroplasts, mitochondria, *Archaea* and Eukaryota; 6) removal of singleton sequences; and 7) clustering and taxonomic classification of the remaining sequences using the OTU-based approach [304].

All singleton sequences were excluded to aid in processing the data as well as to reduce the number of unique sequences that are likely to be caused by sequencing errors. All OTUs of the genera *Ralstonia*, *Shewanella*, and *Halomonas* were removed as probable contaminants based on sequenced blank, as well as reported cases in the literature [305]. Alpha diversity, which

quantifies both species richness and evenness of the microbial communities, was estimated with the inverse Simpson's index and compared between groups using Wilcoxon test. Beta diversity, which quantifies community composition similarity between samples, based on Bray-Curtis dissimilarity, was compared with adonis, an implementation of permutational multivariate analysis of variance (PERMANOVA). Significant differences were defined according to an α-value of 5% ($p<0.05$). Statistical analyses were performed using the software RStudio, version 1.0.

4.5. Ethical approval

All studies were conducted in accordance to the Declaration of Helsinki for Medical Research Involving Human Subjects. The ARIA and SWAN studies were approved by the University Health Ethics Committee, Porto, Portugal. The children's legal guardians received written information about the purpose of the study and all study participants, or their legal caregivers have given their written informed consent.

5. Results

5.1. Participants

The characteristics of the children participating in **studies II, III** and **IV** are presented in **Table 7**. Among the 858 included children, 13 were excluded due to poor-quality data obtained for lung function parameters. The prevalence of atopy, rhinitis and current rhinitis was 35.4%, 13.4%, and 31.9%, respectively. The prevalence of asthma ranged between 6.5% (medical diagnosis of asthma criteria) and 12.3% (medical diagnosis of asthma or BD+ criteria). The prevalence of overweight or obesity was nearly 30%.

Table 7 Characteristics of participants (studies II-IV)

	Studies II-IV		
	Total n=845	**Girls n=416**	**Boys n=429**
Age [years (mean, min.-max.)]	9.0 (8.0-9.0)	9.0 (8.0-9.0)	9.0 (8.0-9.0)
Parental education [n (%)]			
0-9 years	219 (32.1)	97 (29.6)	122 (34.5)
10-12 years	201 (29.5)	103 (31.4)	98 (27.7)
≥ 13 years	262 (38.4))	128 (39.0)	134 (37.9)
Asthma [n (%)] defined by ∓			
Medical diagnosis	53 (6.5)	27 (6.7)	26 (6.3)
Positive bronchodilatation (BD+)	57 (6.7)	35 (8.4)	22 (5.1)
Medical diagnosis and symptoms or BD+	80 (9.5)	**49 (11.8)**	**31 (7.2)**
Medical diagnosis or BD+	104 (12.3)	60 (14.4)	44 (10.3)
Symptoms in previous 3 months [n (%)]			
Itching on the face or neck	54 (7.1)	22 (5.9)	32 (8.4)
Eye irritation	97 (12.6)	41 (10.8)	56 (14.4)
Swollen eyes	58 (7.8)	29 (7.9)	29 (7.7)
Runny nose	345 (45.5)	181 (48.0)	164 (42.0)
Nasal obstruction	404 (53.1)	199 (53.1)	205 (53.1)
Dry throat	154 (20.5)	81 (21.7)	73 (19.4)
Sore throat	302 (39.7)	**168 (44.6)**	**134 (31.2)**
Irritative cough	232 (30.5)	119 (31.7)	113 (29.4)
Breathing difficulties	82 (10.8)	44 (11.7)	38 (9.9)
Wheezing symptoms [n (%)]	78 (9.2%)	38 (9.1)	40 (9.3)

Wheezing previous year [n (%)]	64 (31.4)	31 (25.6)	35 (24.8)
More than 4 wheezing episodes previous year [n (%)]	16 (7.8)	9 (8.8)	7 (6.9)
Cough symptoms [n (%)]	97 (11.5)	50 (12.0)	47 (11.0)
Asthma symptoms [n (%)] ∫	119 (14.1)	59 (14.2)	60 (14.0)
Atopy [n (%)] ǂ	296 (35.4)	145 (35.3)	151 (35.6)
Rhinitis [n (%)]	101 (13.4)	**40 (10.8)**	**61 (16.0)**
Current rhinitis [n (%)]	89 (31.9)	**36 (26.1)**	**53 (37.6)**
Body fat percentage (%)	21.4 (18.2-26.2)	**23.2 (20.2-27.6)**	**19.5 (16.7-23.8)**
Body fat mass (Kg)	6.6 (5.0-9.6)	**7.4(5.6-10.4)**	**6.0 (4.6-8.4)**
Total body water (%)	17.9 (16.0-20.4)	**17.5 (15.7-20.1)**	**18.2 (16.4-20.7)**
BMI [n (%)]			
CDC			
Underweight	39 (4.6)	21 (5.0)	18 (4.2)
Normal weight	575 (68.0)	281 (67.5)	194 (68.5)
Overweight	128 (15.1)	67 (16.1)	61 (14.2)
Obese	103 (12.2)	47 (11.3)	56 (13.1)
WHO			
Underweight	12 (1.4)	5 (1.2)	7 (1.6)
Normal weight	541 (64.0)	260 (62.5)	281 (65.5)
Overweight	172 (20.4)	98 (23.6)	74 (17.2)
Obese	120 (14.2)	53 (12.7)	67 (15.6)
IOTF			
Underweight	75 (8.9)	42 (10.1)	33 (7.7)
Normal weight	539 (63.8)	253 (60.8)	286 (66.7)
Overweight	168 (19.9)	88 (21.2)	80 (18.6)
Obese	63 (7.5)	33 (7.9)	30 (7.0)
Percentage of body fat			
Underfat	8 (1.0)	3 (0.7)	5 (1.2)
Normal	556 (66.7)	286 (70.1)	270 (63.5)
Overfat	134 (16.1)	63 (15.4)	71 (16.7)
Obese	135 (16.2)	56 (13.7)	79 (18.6)
EBC pH [n (%)]			
<7.4	527 (62.4)	268 (64.4)	259 (60.4)
≥7.4	274 (32.3)	122 (29.3)	151 (35.2)
Exhaled NO (ppb)	11.0 (6.0-20.0)	**10.0 (6.0-17.5)**	**12.0 (6.0-21.0)**
Pupillometry			

Baseline pupil diameter (mm)	5.3 (4.7-5.9)	5.3 (4.6-5.9)	5.4 (4.8-5.9)
Final pupil diameter (mm)	3.4 (3.0-3.8)	3.4 (2.9-3.8)	3.4 (3.0-3.8)
ACV (mm/s)	4.0 (3.6-4.4)	4.0 (3.5-4.4)	4.0 (3.6-4.4)
MCV (mm/s)	5.3 (4.7-5.9)	**5.2 (4.6-5.8)**	**5.4 (4.8-6.0)**
Constriction amplitude (%)	35.0 (32.0-38.0)	**35.0 (32.0-38.0)**	**36.0 (33.0-39.0)**
ADV (mm/s)	1.1 (1.0-1.3)	1.1 (1.0-1.3)	1.2 (1.0-1.3)
T75 (s)	1.7 (1.2-2.1)	1.7 (1.1-2.1)	1.7 (1.2-2.2)

Data reported as median (25^{th}, 75^{th} percentile) unless otherwise stated. Significant differences in bold. FEV_1: forced expiratory volume in the first second of forced vital capacity; BD: bronchodilation; BMI: body mass index; CDC: US Centres for Disease Control; WHO: World Health Organization; IOTF: International Obesity Task Force; EBC: Exhaled breath condensate; ACV: Average constriction velocity; MCV: Maximum constriction velocity; ADV: Average dilation velocity; T75: the total time taken by the pupil to recover 75% of the initial resting pupil size after it reached the peak of constriction; ʃreported symptoms (wheezing, dyspnoea or dry cough) occurring in the past 12 months; ‡defined by a positive skin prick test to at least one of the allergens; ₸The following asthma definitions were adopted: i) *Medical diagnosis of asthma*: self-reported medical diagnosis; ii) *Positive bronchodilatation (BD+)*: at least a 12% and over 200mL increase in FEV1 after bronchodilation; iii) *Medical diagnosis of asthma and symptoms or BD+*: at least a 12% and over 200mL increase in forced expiratory volume in 1 second (FEV1) after bronchodilation or self-reported medical diagnosis with reported symptoms (wheezing, dyspnoea or dry cough) occurring in the past 12 months; iv) *Medical diagnosis of asthma or BD+*: at least a 12% and over 200mL increase in FEV1 after bronchodilation or self-reported medical diagnosis

The characteristics of athletes from **study V** are presented in **Table 8**. The number of girls and boys were significantly different between both groups of athletes, being the number of girls higher among swimmers. The prevalence of asthma defined based on positive bronchodilatation and atopy were higher among swimmers compared to non-swimmers (12.5% *vs.* 5.9%; and 58.1% *vs.* 33.3%, respectively).

Table 8 Characteristics of the participants (study V)

	Swimmers			**Non-swimmers**		
	Total n=29	**Girls n=20**	**Boys n=9**	**Total n=34**	**Girls n=7**	**Boys n=27**
Age [years (mean ± SD)]	15.1 (2.2)	14.8 (2.4)	15.7 (1.8)	14.9 (1.8)	12.6 (1.3)	15.5 (1.3)
BMI (z-scores CDC)	3.32 (3.14; 3.42)	3.37 (3.14; 3.42)	3.28 (3.04; 3.37)	3.28 (3.14; 3.37)	3.42 (3.37; 3.48)	3.28 (3.09; 3.37)
Asthma [n (%)] defined by₸						
Medical diagnosis	0	--	--	0	--	--
Positive bronchodilatation	4 (12.5)	3 (13.6)	1 (10.0)	2 (5.9)	2 (7.4)	3 (13.6)
Atopy [n (%)]	18 (58.1)	10 (47.6)	8 (80.0)	11 (33.3)	1 (14.3)	10 (38.5)

Skin samples (n)						
T0	28	19	9	34	7	27
T1	29	20	9	--	--	--
Stool samples (n)	22	16	6	32	7	25

Data reported as mean (standard deviation) unless otherwise stated; BMI: body mass index; CDC: US Centres for Disease Control; T0: before training; T1: after training; [T]The following asthma definitions were adopted: i) *Medical diagnosis of asthma*: self-reported medical diagnosis; ii) *Positive bronchodilatation:* at least a 12% and over 200mL increase in FEV1 after bronchodilation

5.2. Volatile Organic Compounds in indoor environments (study I)

Individual VOC in different indoor environments

For specific VOC detected at quantifiable levels in both seasons, higher mean concentrations were found during the cold season for most VOC in all indoor environments.

In schools, most studies evaluated VOC levels in both seasons, reporting higher concentrations during the cold season. The concentrations ranged between non-detectable to 160 µg/m^3, and the highest level was observed for m/p-xylene. For housing environments, a greater number of specific VOC were identified, mostly measured in both seasons. Concentrations ranged from non-detectable to 293 µg/m^3, being higher for toluene in cold season. In offices, the levels of specific VOC ranged between 0.05 and 532 µg/m^3, being higher for benzene in cold season (404 µg/m^3) and lower for 1,2,3 trimethylbenzene in warm season. In other indoor environments, including commercial spaces, hospitals, restaurants, dental settings, photocopy centres and museums, concentrations were higher in the cold season and the levels ranged between 7.32x10^{-4} (benzene) and 185.1 µg/m^3 (toluene), values observed in cold season. Comparing all individual VOC for each different indoor environment, no significant differences were observed between VOC concentrations and seasons.

Seasonal variations in indoor/outdoor ratio

In the school environments, a total of 31 compounds were measured both indoors and outdoors at concentrations that ranged from 0.51 µg/m^3 (1,2,4-trimethylbenzene) to 23 µg/m^3 (methyl-isobutyl-ketone) in cold season. I/O ratios higher than the unity were observed for the majority of VOC in both seasons, but higher ratio values were noticed in cold season; in fact, only 3 VOC (benzene, trichloroethylene and hexane) presented an I/O ratio lower than 1.

In housing environments, the I/O ratio was higher than 1 for most specific VOC, being higher in the warm season. The concentration of formaldehyde, 1,2,3-trimethylbenzene and acetaldehyde

in both seasons was significantly higher indoors. Although higher indoor levels of ethylbenzene and 1,3,5-trimethylbenzene were observed in the cold season; the indoor concentration of toluene, styrene, heptane, nonane, decane and 1,2,4-trimethylbenzene was significantly higher in warm season. Although no significant differences were found between indoor and outdoor levels for specific VOC measured in offices and other indoor environments, a I/O ratio greater than 1 was observed for most VOC.

5.3. Exposure to indoor endocrine-disrupting chemicals, childhood asthma, obesity and autonomic nervous system activity (study II)

Exposure to indoor EDCs and asthma, respiratory symptoms and obesity

Classrooms with higher levels of o-xylene, m/p-xylene, ethylbenzene, benzene, 2-ethyl-1-hexanol and higher PC1 scores had a higher number of children with asthma (**Table 9**). Higher levels of several EDCs were also found in classrooms with a higher number of children reporting respiratory symptoms in the previous 3 months. Runny nose was more prevalent in classrooms with higher concentrations of cyclohexanone and 2-ethyl-1-hexanol (**Table 9**). Additionally, higher classroom concentrations of toluene, m/-xylene, styrene and cyclohexanone showed a tendency to be associated with an increased number of overweight and obese children (**Table 9**). The results showed that classrooms with higher concentrations of toluene, o-xylene, m/p-xylene, ethylbenzene, 2-ethyl-1-hexanol and higher PC1 scores but lower PC2 scores had significantly higher numbers of children reporting nasal obstruction symptoms (**Table 9**).

Table 9 Endocrine-disrupting compounds levels and asthma, respiratory symptoms in the previous 3 months and body mass index

	Median (25^{th}-75^{th} percentile)		**Spearman's rho**	***p* value**
	No	**Yes**		
Toluene (µg/m^3)				
Symptoms in previous 3 months				
Nasal obstruction	8.8 (5.3; 11.8)	10.1 (5.7; 14.9)	--	0.006
Dry throat	8.9 (5.6; 13.4)	10.3 (6.0; 14.9)	--	0.027
Sore throat	8.9 (5.3; 13.4)	10.1 (5.7; 14.9)	--	0.015
BMI (WHO criteria)				0.023
Underweight	--	6.0 (3.1; 12.4)	--	
Normal weight	--	8.9 (5.5; 14.1)	--	
Overweight	--	9.1 (5.6; 14.1)	--	
Obese	--	10.6 (6.9; 14.7)	--	
o-xylene (µg/m^3)				

Medical diagnosis asthma or BD+[Ŧ]	2.2 (1.0; 3.5)	2.7 (1.3; 4.0)	--	0.016
Symptoms in previous 3 months				
Nasal obstruction	2.0 (1.0; 3.1)	2.5 (1.1; 3.9)	--	0.008
m/p-xylene (µg/m^3)				
Medical diagnosis asthma or BD+[Ŧ]	3.8 (2.1; 6.2)	5.0 (2.3; 7.6)	--	0.015
Symptoms in previous 3 months				
Nasal obstruction	3.7 (2.1; 6.1)	4.3 (2.2; 7.5)	--	0.016
Dry throat	3.8 (2.0; 6.7)	4.9 (2.6; 7.5)	--	0.030
Sore throat	3.8 (2.0; 6.2)	4.2 (2.2; 7.5)	--	0.042
BMI (WHO criteria)				0.023
Underweight	--	2.9 (1.6; 8.2)	--	
Normal weight	--	3.8 (2.1; 6.7)	--	
Overweight	--	4.2 (2.1; 6.1)	--	
Obese	--	5.0 (3.2; 7.4)	--	
z-score CDC	--	--	0.072	0.036
z-score WHO	--	--	0.069	0.045
Ethylbenzene (µg/m^3)				
Medical diagnosis asthma[Ŧ]	1.0 (0.0; 2.6)	1.7 (0.6; 4.0)	--	0.031
Medical diagnosis asthma or BD+[Ŧ]	1.0 (0.0; 2.5)	1.7 (0.6; 3.6)	--	0.002
Medical diagnosis asthma and symptoms or BD+[Ŧ]	1.0 (0.0; 2.5)	1.6 (0.4; 3.6)	--	0.024
Symptoms in previous 3 months				
Nasal obstruction	1.0 (0.0; 2.5)	1.2 (0; 2.8)	--	0.021
Styrene (µg/m^3)				
BMI (percentage of body fat criteria)				0.046
Underfat	--	0.1 (0.0; 0.6)	--	
Normal	--	0.3 (0.03; 0.9)	--	
Overfat	--	0.6 (0.2; 1.2)	--	
Obese	--	0.4 (0.03; 0.9)	--	
z-score CDC	--	--	0.091	0.008
z-score WHO	--	--	0.090	0.009
Cyclohexanone (µg/m^3)				
Symptoms in previous 3 months*				
Runny nose	0.7 (3.2)	1.3 (4.9)		0.006
BMI (percentage of body fat criteria)*				0.017
Underfat	--	0.0 (0.0)	--	
Normal	--	0.8 (3.6)	--	
Overfat	--	0.9 (4.2)	--	
Obese	--	2.0 (5.6)	--	
z-score CDC	--	--	0.078	0.023
z-score WHO	--	--	0.080	0.020
Butylated hydroxytoluene (µg/m^3)				
Symptoms in previous 3 months				

Dry throat	2.1 (0.0; 5.1)	0.8 (0.0; 4.1)	--	0.005
Benzene (µg/m^3)				
Medical diagnosis asthma or BD+$^{\text{Ŧ}}$	0.7 (0.4; 1.3)	1.0 (0.4; 1.5)	..	0.033
Symptoms in previous 3 months				
Itching on the face or neck	0.7 (0.4; 1.4)	1.2 (0.4; 1.9)		0.027
4-chloroethylene (µg/m^3)				
BMI (CDC criteria)				0.020
Underweight	--	0.3 (0.8)	--	
Normal weight	--	0.4 (1.2)	--	
Overweight	--	0.5 (1.4)	--	
Obese	--	0.1 (0.6)	--	
2-butoxietanol (µg/m^3)				
Symptoms in previous 3 months				
Eye irritation	9.0 (5.5; 61.5)	15.2 (6.6; 118.7)	--	0.031
2-ethyl-1-hexanol (µg/m^3)				
Medical diagnosis asthma$^{\text{Ŧ}}$	3.4 (2.1; 5.6)	4.8 (3.4; 9.6)	--	0.003
Medical diagnosis asthma or BD+$^{\text{Ŧ}}$	3.4 (2.1; 5.6)	4.5 (2.8; 6.8)	--	0.017
Symptoms in previous 3 months				
Swollen eyes	3.4 (2.1; 5.3)	5.3 (2.4; 7.1)	--	0.012
Runny nose	3.3 (2.1; 5.3)	4.5 (2.4; 5.6)	--	0.006
Irritative cough	3.4 (2.0; 5.3)	4.6 (2.8; 5.6)	--	0.007
PCA1				
Medical diagnosis asthma or BD+$^{\text{Ŧ}}$	-0.3 (-0.5; -0.03)	-0.2 (-0.4; -0.004)	--	0.023
Symptoms in previous 3 months				
Nasal obstruction	-0.3 (-0.5; -0.04)	-0.2 (-0.4; -0.004)	--	0.017
PCA2				
Symptoms in previous 3 months				
Nasal obstruction	-0.01 (-0.5; 0.7)	-0.1 (-0.7; 0.6)	--	0.036

*Data reported as mean (standard deviation); $^{\text{Ŧ}}$The following asthma definitions were adopted: i) *Medical diagnosis of asthma*: self-reported medical diagnosis; ii) *Positive bronchodilatation (BD+)*: at least a 12% and over 200mL increase in FEV1 after bronchodilation; iii) *Medical diagnosis of asthma and symptoms or BD+*: at least a 12% and over 200mL increase in forced expiratory volume in 1 second (FEV1) after bronchodilation or self-reported medical diagnosis with reported symptoms (wheezing, dyspnoea or dry cough) occurring in the past 12 months; iv) *Medical diagnosis of asthma or BD+*: at least a 12% and over 200mL increase in FEV1 after bronchodilation or self-reported medical diagnosis; BMI: body mass index; CDC: US Centers for Disease Control; WHO: World Health Organization

After adjustment, the risk of positive bronchodilation by exposure to higher levels of benzene increased by 1.5-fold (OR=1.49, 95% CI 1.01; 2.53). Toluene, o-xylene, m/p-xylene and ethylbenzene were significantly associated with nasal obstruction (OR=1.01, 95% CI 1.00; 1.03; OR=1.04, 95% CI 1.01; 1.08; OR=1.01, 95% CI 1.00; 1.03; and OR=1.04, 95% CI 1.01; 1.09, respectively). The levels of hexane, styrene, cyclohexanone, BHT and 2-butoxyethanol were also

associated with obesity, and the level of cyclohexanone was associated with increased child BMI. Additionally, positive associations were found between cyclohexanone, BHT, acetaldehyde and body composition (**Table 10**). Furthermore, a positive association was found between PC1 and the risk of obese-asthma (OR=1.43, 95% CI 1.01; 1.98) and between PC2 and overweight (OR=1.51, 95% CI 1.28; 1.79). In addition, PC1 and PC2 were associated with nasal obstruction, and PC2 was associated with breathing difficulties and lean body mass (**Table 10**), although the concentrations of the EDCs were relatively low. Higher concentrations of individual EDCs and higher PC1 and PC2 scores were also positively associated with asthma, and child BMI and respiratory symptoms; however, after adjustment, the effect size estimates were similar but not statistically significant.

Table 10 Association between endocrine-disrupting compounds and asthma, respiratory symptoms in the previous 3 months, and body mass index

	OR (95% CI)		
	Model 0	**Model 1**	**Model 2**
Toluene			
Symptoms in previous 3 months [a]			
Nasal obstruction	1.01 (1.00; 1.02)	1.01 (1.00; 1.02)	1.01 (1.00-1.03)
o-xylene			
Symptoms in previous 3 months [a]			
Nasal obstruction	1.03 (1.00; 1.07)	1.03 (1.00; 1.07)	1.04 (1.01; 1.08)
m/p-xylene			
Symptoms in previous 3 months [a]			
Nasal obstruction	1.01 (1.00; 1.02)	1.01 (1.00; 1.02)	1.01 (1.00; 1.03)
Hexane			
BMI (WHO criteria) [b]			
Underweight	Reference	Reference	Reference
Normal weight	1.00 (0.99; 1.02)	1.55 (1.54; 1.56)	1.18 (1.17; 1.19)
Overweight	1.00 (0.99; 1.02)	1.55 (1.54; 1.56)	1.18 (1.17; 1.19)
Obese	1.00 (0.99; 1.02)	1.55 (1.54; 1.56)	1.18 (1.17; 1.19)
Ethylbenzene			
Symptoms in previous 3 months [a]			
Nasal obstruction	1.03 (1.00; 1.08)	1.03 (1.01; 1.08)	1.04 (1.01; 1.09
Styrene			
BMI (WHO criteria) [b]			
Underweight	Reference	Reference	Reference
Normal weight	1.20 (0.69; 2.06)	1.59 (0.39; 6.52)	1.65 (1.45; 1.88)
Overweight	1.20 (0.67; 2.13)	1.53 (0.37; 6.34)	1.54 (1.30; 1.83)
Obese	1.34 (0.76; 2.37)	1.81 (0.44; 7.51)	1.78 (1.52; 2.10)
Cyclohexanone			
BMI (WHO criteria) [b]			
Underweight	Reference	Reference	Reference
Normal weight	1.02 (0.88; 1.20)	1.59 (0.01; 1.59E2)	2.45 (2.36; 2.55)

Overweight	1.04 (0.88; 1.22)	1.61 (0.01; 1.61E2)	2.54 (2.43; 2.65)
Obese	1.06 (0.90; 1.24)	1.61 (0.01; 1.61E2)	2.53 (2.41; 2.65)
z-score CDC*	0.02 (0.005, 0.04)	0.02 (0.001; 0.04)	0.03 (0.01; 0.05)
z-score WHO*	0.03 (0.007, 0.05)	0.02 (0.002; 0.04)	0.03 (0.01; 0.06)
Body composition [b*]			
Body fat (%)	0.18 (0.08; 0.27)	0.17 (0.07; 0.26)	0.21 (0.10; 0.32)
Butylated hydroxytoluene			
BMI (WHO criteria) [b]			
Underweight	Reference	Reference	Reference
Normal weight	1.35 (0.43, 4.18)	1.36 (0.4; 4.26)	1.23 (1.18; 1.29)
Overweight	1.33 (0.43, 4.14)	1.35 (0.43; 4.22)	1.25 (1.18; 1.32)
Obese	1.33 (0.43, 4.13)	1.35 (0.43; 4.22)	1.23 (1.16; 1.31)
Body composition [b*]			
Lean body mass	0.10 (0.02; 0.19)	0.09 (0.01; 0.17)	0.10 (0.01; 0.19)
Total body water	0.08 (0.01; 0.14)	0.06 (0.006; 0.12)	0.07 (0.01; 0.14)
Benzene			
Positive bronchodilatation [Ŧa]	1.67 (1.02, 2.66)	1.64 (1.00; 2.65)	1.49 (1.01; 2.53)
2-butoxyethanol			
BMI (Percentage of body fat criteria)[b]			
Underfat	Reference	Reference	Reference
Normal	1.81 (0.08, 43.1)	4.36 (4.34; 4.38)	3.90 (3.85; 3.95)
Overfat	1.81 (0.07, 43.1)	4.35 (4.33; 4.38)	3.90 (3.85; 3.96)
Obese	1.80 (0.07, 42.9)	4.33 (4.30; 4.36)	3.81 (3.72; 3.91)
Acetaldehyde			
Body composition [b*]			
Lean body mass	0.07 (-0.04; 0.18)	0.10 (-7.66E-4; 0.21)	0.16 (0.02; 0.30)
PC1			
Obese-asthma [a]	1.45 (1.04; 1.94)	1.38 (0.98; 1.86)	1.43 (1.01; 1.98)
Symptoms in previous 3 months [a]			
Nasal obstruction	1.25 (1.09; 1.47)	1.24 (1.08; 1.45)	1.32 (1.13; 1.58)
BMI (WHO criteria) [b]			
Underweight	Reference	Reference	Reference
Normal weight	0.89 (0.57; 1.39)	0.90 (0.57; 1.41)	0.89 (0.80; 1.01)
Overweight	0.79 (0.49; 1.27)	0.79 (0.49; 1.27)	0.80 (0.69; 0.92)
Obese	0.92 (0.58; 1.47)	0.94 (0.59; 1.51)	0.85 (0.73; 0.98)
BMI (Percentage of body fat criteria)[b]			
Underfat	Reference	Reference	Reference
Normal	1.40 (0.46; 4.32)	1.39 (0.47; 4.14)	1.39 (1.23; 1.57)
Overfat	1.29 (0.42; 4.03)	1.27 (0.42; 3.84)	1.17 (0.97; 1.40)
Obese	1.34 (0.43; 4.15)	1.33 (0.44; 4.01)	1.29 (1.10; 1.51)
PC2			
Symptoms in previous 3 months [a]			
Nasal obstruction	0.86 (0.74; 0.99)	0.85 (0.73; 0.99)	0.83 (0.71; 0.98)
Breathing difficulties	0.80 (0.63; 1.01)	0.78 (0.61; 1.00)	0.74 (0.55; 0.97)
BMI (WHO criteria) [b]			
Underweight	Reference	Reference	Reference
Normal weight	1.19 (0.66; 2.15)	1.18 (0.65; 2.13)	1.18 (1.04; 1.33)
Overweight	1.12 (0.61; 2.05)	1.15 (0.63; 2.11)	1.24 (1.06; 1.45)
Obese	1.08 (0.58; 1.98)	1.12 (0.60; 2.07)	1.10 (0.93; 1.30)
BMI (Percentage of body fat criteria)[b]			

Underfat	Reference	Reference	Reference
Normal	1.35 (0.65; 2.82)	1.33 (0.63; 2.78)	1.31 (1.15; 1.48)
Overfat	1.41 (0.67; 2.97)	1.37 (0.65; 2.92)	1.51 (1.28; 1.79)
Obese	1.16 (0.55; 2.45)	1.17 (0.55; 2.48)	1.10 (0.93; 1.30)
Body composition [b*]			
Lean body mass	0.33 (0.03; 0.63)	0.33 (0.03; 0.63)	0.40 (0.04; 0.76)

*Data reported as β (95% CI); ₸The following asthma definitions were adopted: i) *Medical diagnosis of asthma*: self-reported medical diagnosis; ii) *Positive bronchodilatation (BD+)*: at least a 12% and over 200mL increase in FEV1 after bronchodilation; iii) *Medical diagnosis of asthma and symptoms or BD+*: at least a 12% and over 200mL increase in forced expiratory volume in 1 second (FEV1) after bronchodilation or self-reported medical diagnosis with reported symptoms (wheezing, dyspnoea or dry cough) occurring in the past 12 months; iv) *Medical diagnosis of asthma or BD+*: at least a 12% and over 200mL increase in FEV1 after bronchodilation or self-reported medical diagnosis; Obese-asthma: defined based on CDC criteria for obesity and Medical diagnosis of asthma and symptoms or BD+; CI: confidence interval; BMI: body mass index; CDC: US Centres for Disease Control; WHO: World Health Organization; IOTF: International Obesity Task Force;
Model 0 is null model; Model 1 is adjusted for age and sex; Model 2 is additionally adjusted for: [a]BMI (according to CDC criteria), atopy and parental education; [b]asthma (according to medical diagnosis asthma and symptoms or BD+ criteria), parental education, physical activity, energy intake

Association between EDCs and autonomic nervous system activity

Children exposed to higher levels of m/p-xylene and benzaldehyde exhibited decreased baseline pupil diameters; additionally, children exposed to higher levels of toluene, ethylbenzene and benzene exhibited decreased ADV (**Table 11**). Toluene, cyclohexanone and benzene were also negatively associated with constriction amplitude. Moreover, a negative association was found between both formaldehyde and acetaldehyde and ACV and between benzaldehyde and the baseline and final pupil diameters (**Table 11**). Nevertheless, toluene, o-xylene, m/p-xylene, ethylbenzene and benzene were also positively associated with constriction velocity (MCV and ACV), whereas 2-butoxyethanol, formaldehyde and acetaldehyde were positively associated with constriction amplitude. Positive associations were found between T4CE and MCV and between cyclohexanone and ACV. In addition, PC1 was positively associated with both MCV and ACV (**Table 11**), and PC2 was positively associated with baseline pupil diameter and constriction amplitude. However, higher PC2 scores were associated with lower values of MCV and ACV (**Table 11**).

Table 11 Association between endocrine-disrupting compounds and autonomic nervous system activity

	OR (95% CI) *		
	Model 0	**Model 1**	**Model 2** [c]
Toluene			
MCV	-0.004 (-0.01; -7.79E-4)	-0.004 (-0.01; -4.17E-4)	0.005 (8.00E-4; 0.009)
ACV	-0.003 (-0.006; -7.14E-4)	-0.003 (-0.006; -4.70E-4)	0.004 (0.001; 0.007)
Constriction amplitude	-0.02 (-0.004; 6.51E-4)	0.009 (-0.04; 9.76E-4)	-0.02 (-0.04; -0.004)
ADV	-0.001 (-0.003; -2.18E-4)	-0.001 (-0.003; -1.51E-4)	-0,002 (-0.003; -2.00E-4)
o-xylene			
MCV	-0.01 (-0.02; -0.002)	-0.01 (-0.02; -0.003)	0.01 (0.002; 0.02)
ACV	-0.01 (-0.02; -0.003)	-0.01 (-0.02; -0.003)	0.01 (0.003; 0.02)
m/p-xylene			
Baseline pupil diameter	-0.004 (-0.007; -0.001)	-0.004 (-0.007; -0.001)	-0.003 (-0.006; -4.14E-5)
MCV	-0.005 (-0.008; -0.001)	-0.005 (-0.008; -0.002)	0.005 (0.001; 0.008)
ACV	-0.004 (-0.006; -0.001)	-0.004 (-0.006; -0.001)	0.004 (0.001; 0.006)
Ethylbenzene			
MCV	-0.01 (-0.02; -0.002)	-0.01 (-0.02; -0.002)	0.01 (0.002; 0.03)
ACV	-0.01 (-0.02; -0.003)	-0.01 (-0.02; -0.003)	0.01 (0.003; 0.02)
ADV	-0.004 (-0.008; -1.55E-4)	-0.004 (-0.009; -1.93E-4)	-0.005 (-0.01; -1.00E-4)
Cyclohexanone			
ACV	-0.01 (-0.02; 7.69E-4)	-0.01 (-0.002; 0.001)	0.01 (0.002; 0.03)
Constriction amplitude	-0.09 (-0.17; -0.002)	-0.08 (-0.17; 0.004)	-0.10 (-0.20; -0.01)
Benzene			
MCV	-0.15 (-0.24; -0.05)	-0.12 (-0.22; -0.02)	0.15 (0.04; 0.26)
ACV	-0.13 (-0.20; -0.06)	-0.12 (-0.19; -0.04)	0.14 (0.06; 0.22)
Constriction amplitude	-0.59 (-1.10; -0.09)	-0.50 (-1.10; 0.005)	-0.74 (-1.30; -0.18)
ADV	-0.001 (-0.003; 0.001)	-0.004 (-0.009; -1.93E-4)	-0.05 (-0.09; -0.007)
Benzaldehyde			
Baseline pupil diameter	-0.01 (-0.02; -0.005)	-0.01 (-0.02; -0.003)	-0.01 (-0.02; -0.004)
Final pupil diameter	-0.007 (-0.01; -0.002)	-0.006 (-0.01; -0.001)	-0.008 (-0.01; -0.03)
4-chloroethylene			
MCV	-0.06 (-0.12; 0.001)	-0.07 (-0.14; -0.01)	0.07 (0.005; 0.14)
2-butoxyethanol			
Constriction amplitude	0.01 (0.002; 0.02)	0.01 (0.003; 0.02)	0.02 (0.005; 0.03)
Formaldehyde			
ACV	0.01 (0.003; 0.02)	0.01 (0.003; 0.02)	-0.010 (-0.02; -0.003)
Constriction amplitude	0.08 (0.03; 0.13)	0.08 (0.03; 0.13)	0.10 (0.05; 0.16)
Acetaldehyde			
ACV	0.03 (0.01; 0.05)	0.04 (0.02; 0.05)	-0.03 (-0.05; -0.010)
Constriction amplitude	0.14 (0.003; 0.28)	0.17 (0.03; 0.31)	0.16 (0.004; 0.31)
PC1			

MCV	-0.09 (-0.16; -0.02)	-0.09 (-0.16; -0.02)	0.10 (0.02; 0.18)
ACV	-0.07 (-0.12; -0.02)	-0.07 (-0.12; -0.02)	0.08 (0.03; 0.14)
PC2			
Baseline pupil diameter	0.11 (0.04; 0.17)	0.09 (0.02; 0.16)	0.09 (0.01; 0.16)
MCV	0.09 (0.02; 0.17)	0.09 (0.01; 0.17)	-0.10 (-0.19; -0.02)
ACV	0.11 (0.06; 0.17)	0.11 (0.06; 0.17)	-0.12 (-0.18; -0.06)
Constriction amplitude	0.70 (0.32; 1.09)	0.72 (0.33; 1.12)	0.86 (0.44; 1.29)

*Data reported as β (95% CI); CI: confidence interval; MCV: maximum constriction velocity; ACV: average constriction velocity; ADV: average dilation velocity;
Model 0 is null model; Model 1 is adjusted for age and sex; Model 2 is additionally adjusted for asthma (according to medical diagnosis asthma and symptoms or BD+ criteria) and parental education

5.4. School environment, lung function and autonomic nervous system activity (study III)

Among the 858 participating children, 146 were excluded owing to lung function poor-quality data. Thus, this study (**study III**) was based on data from 701 children (50.9% girls). Of those, almost 9.4% reported wheezing symptoms, and 12% reported cough symptoms. The prevalence of asthma, rhinitis, current rhinitis, and atopy were 10.7%, 13.0%, 30.4%, and 35.5%, respectively.

School neighbourhood, lung function and autonomic nervous system activity

An increased proportion of built areas in the school neighbourhood was associated with significantly lower values of FVC (model 0: β=-5.13, 95% CI -9.36; -0.91; model 2: β=-4.98, 95% CI -10.3; -0.35), while green areas showed a tendency to be associated with higher values of FVC, FEV1 and FEF25-75%. The highest ICC were observed for FEV1 and FVC (0.40% and 0.04%, respectively), indicating that approximately 1% of the total variation in these parameters was found between schools. After adjustment for age, sex, asthma, WHO z-score for BMI and family history of asthma or allergy, the neighbouring environment explained 98%, 96%, and >99.9% of the effect of school on FVC, FEV1, and FEF25-75%, respectively (model 5, **Table 12**). No significant associations were observed between green or built areas and pupillometry parameters. Still, a positive trend was found between built areas and pupillometry parasympathetic parameters (ACV, MCV and constriction amplitude). After adjustment, estimates of ICC for pupillometry suggested that between 0% and 22% of the total variance was at the school level. The neighbouring environment explained 6% of the effect of school on MCV, 8% of its effect on ADV, 11% of its effect on constriction amplitude and 13% of its effect on T75 (model 5, **Table 12**).

Table 12 Multilevel model analysis of the association between individual and neighbouring environment and lung function, pH, exhaled NO and pupillometry parameters explained by school

Outcome	β (95% CI)		School ICC	Variance	Explained variation*
	PC1	PC2			
FVC					
Model 0	2.17 (-1.98; 6.33)	-5.13 (-9.36; -0.91)	---	---	---
Model 1	---	---	1.78%	4.48	Reference
Model 2[a]	3.66 (-3.01; 10.3)	-1.33 (-7.87; 5.02)	0.04%	0.08	98.2%
FEV_1					
Model 0	2.78 (-1.07; 6.63)	-3.11 (-7.02; 0.81)	---	---	---
Model 1	---	---	2.13%	4.53	Reference
Model 2[a]	1.54 (-4.58; 7.65)	1.07 (-4.93; 7.07)	0.40%	0.16	96.5%
$FEF_{25\%-75\%}$					
Model 0	5.05 (-1.27; 11.4)	-0.50 (-6.94; 5.93)	---	---	---
Model 1	---	---	0.37%	2.14	Reference
Model 2[a]	-5.19 (-16.2; 5.77)	4.86 (-5.89; 15.6)	7.10E-7%	3.71E-6	>99.9%
EBC pH					
Model 0	0.02 (-0.20; 0.24)	-0.05 (-0.17; 0.17)	---	---	---
Model 1	---	---	2.04%	4.33	Reference
Model 2[a]	0.12 (-0.53; 0.77)	-0.002 (-0.65; 0.64)	9.32%	0.09	97.8%
Exhaled NO					
Model 0	0.20 (-0.02; 0.41)	-0.14 (-0.35; 0.07)	---	---	---
Model 1	---	---	3.98%	0.03	Reference
Model 2[b]	-0.12 (-0.68; 0.44)	-0.37 (-0.93; 0.18)	6.76%	0.05	-54.6%
Baseline pupil diameter					
Model 0	-0.09 (-0.32; 0.14)	-0.03 (-0.26; 0.19)	---	---	---
Model 1	---	---	20.3%	0.158	Reference
Model 2[c]	-0.12 (-0.84; 0.60)	0.02 (-0.70; 0.75)	22.1%	0.178	-12.5%
Final pupil diameter					
Model 0	-0.08 (-0.24; 0.08)	-0.10 (-0.26; 0.06)	---	---	---
Model 1	---	---	14.1%	0.053	Reference
Model 2[c]	-0.10 (-0.51; 0.31)	-0.07 (-0.48; 0.35)	15.1%	0.059	-10.3%
ACV					
Model 0	-0.11 (-0.29; 0.07)	0.17 (-0.01; 0.35)	---	---	---
Model 1	---	---	15.7%	0.075	Reference
Model 2[c]	-0.05 (-0.56; 0.45)	0.23 (-0.28; 0.74)	16.7%	0.081	-8.24%
MCV					
Model 0	-0.06 (-0.32; 0.19)	0.26 (0.01; 0.51)	---	---	---

Model 1	---	---	12.1%	0.122	Reference
Model 2[c]	0.03 (-0.61; 0.68)	0.26 (-0.39; 0.91)	12.7%	0.118	-6.12%
Constriction amplitude					
Model 0	-0.25 (-1.56; 1.04)	1.17 (-0.12; 2.45)	---	---	---
Model 1	---	---	8.38%	2.07	Reference
Model 2[c]	-0.07 (-2.80; 2.65)	1.50 (-1.26; 4.27)	7.52%	1.85	10.9%
ADV					
Model 0	-0.02 (-0.10; 0.07)	0.06 (-0.03; 0.14)	---	---	---
Model 1	---	---	8.57E-8%	8.10E-11	Reference
Model 2[c]	-0.02 (-0.13; 0.09)	0.06 (-0.05; 0.17)	7.82E-8%	7.48E-11	7.60%
T75					
Model 0	0.21 (0.01; 0.42)	-0.13 (-0.33; 0.06)	---	---	---
Model 1	---	---	4.53%	0.023	Reference
Model 2[c]	0.31 (-5.07E-4; 0.63)	-0.10 (-0.42; 0.22)	3.89%	0.020	13.0%

*corresponds to the proportion of between-schools variance that could be explained by exposure and individual characteristics; PC1: discontinuous dense urban fabric, discontinuous medium density urban land, green urban areas, and water bodies; PC2: construction sites, land without current use, and railways; 95% CI: 95% confidence interval; ICC: intra-class correlation coefficient; FVC: forced vital capacity; FEV_1: forced expiratory volume in the first second of FVC; FEF_{25-75}: forced expiratory flow in the middle portion of FVC; EBC: Exhaled breath condensate; ACV: Average constriction velocity; MCV: Maximum constriction velocity; ADV: Average dilation velocity; T75: the total time taken by the pupil to recover 75% of its initial resting diameter after it reached the peak of constriction.

Model 0 only included the PC1 and PC2 score; [a] Model 1 is null model, baseline model without any exposure variable; Model 2[a] is additionally adjusted for age, sex, asthma, WHO z-score for BMI and family history of asthma or allergy; Model 2[b] is additionally adjusted for age, sex, asthma, atopy, WHO z-score for BMI and family history of asthma or allergy; Model 2[c] is additionally adjusted for age, sex, asthma, and WHO z-score for BMI

School walkability, lung function and autonomic nervous system activity

Neighbourhood walkability explained >99.9% of the school effect on FVC, FEV1 and FEF25-75%. Regarding ANS response, neighbourhood walkability explained 11% and 18% of the parasympathetic outcomes (constriction amplitude and MCV, respectively) and 7% and 29% of the pupillometry sympathetic parameters (ADV and T75, respectively) (**Table 13**). Lung function and exhaled NO decreased non-significantly with neighbourhood walkability, while a positive association was observed for EBC pH level. After adjustment for age, sex, asthma and WHO z-score for BMI, a significant negative association between walkability around schools and constriction amplitude (β=-1.62, 95% CI –2.87; -0.37) and T75 (β=-0.19, 95% CI –0.36; -0.02) was observed. Additionally, walkability showed a tendency to be associated with lower values of ACV, MCV and baseline pupil diameter.

Table 13 Multilevel model analysis of the association between individual and walkability and lung function, pH, exhaled NO and pupillometry parameters explained by school

Outcome	Walkability	School		
	β (95% CI)	ICC	Variance	Explained variation*
FVC				
Model 0	-0.58 (-2.79; 1.63)	---	---	---
Model 1	---	1.78%	4.48	Reference
Model 2[a]	-2.62 (-6.00; 0.77)	1.32E-6%	2.51E-6	>99.9%
FEV_1				
Model 0	-1.02 (-3.07; 1.02)	---	---	---
Model 1	---	2.09%	4.53	Reference
Model 2[a]	-2.63 (-5.71; 0.46)	4.47E-7%	7.09E-7	>99.9%
$FEF_{25\%-75\%}$				
Model 0	-1.27 (-4.74; 1.98)	---	---	---
Model 1	---	0.37%	2.14	Reference
Model 2[a]	-0.72 (-6.33; 4.89)	5.27E-7%	2.77E-6	>99.9%
EBC pH				
Model 0	0.09 (-0.03; 0.21)	---	---	---
Model 1	---	3.48%	0.03	Reference
Model 2[a]	-0.004 (-0.34; 0.33)	8.52%	0.08	>-99.9%
Exhaled NO				
Model 0	-0.05 (-0.17; 0.07)	---	---	---
Model 1	---	3.98%	0.03	Reference
Model 2[b]	-0.07 (-0.38; 0.23)	8.07%	0.06	-90.6%
Baseline pupil diameter				
Model 0	-0.22 (-0.34; -0.09)	---	---	---
Model 1	---	20.4%	0.158	Reference
Model 2[c]	-0.18 (-0.54; 0.17)	22.1%	0.178	-12.5%
Final pupil diameter				
Model 0	-0.04 (-0.13; 0.05)	---	---	---
Model 1	---	14.1%	0.053	Reference
Model 2[c]	-0.05 (-0.26; 0.16)	15.1%	0.059	-10.3%
ACV				
Model 0	-0.22 (-0.32; -0.12)	---	---	---
Model 1	---	14.7%	0.070	Reference
Model 2[c]	-0.21 (-0.46; 0.03)	16.7%	0.081	-16.4%
MCV				
Model 0	-0.31 (-0.45; -0.17)	---	---	---
Model 1	---	12.3%	0.112	Reference
Model 2[c]	-0.29 (-0.60; 0.02)	10.1%	0.091	18.0%
Constriction amplitude				

Model 0	1.94 (-2.65; -1.23)	---	---	---
Model 1	---	8.38%	2.073	Reference
Model 2[c]	-1.62 (-2.87; -0.37)	7.52%	1.847	10.9%
ADV				
Model 0	0.01 (-0.04; 0.06)	---	---	---
Model 1	---	8.57E-8%	8.10E-11	Reference
Model 2[c]	0.005 (-0.06; 0.07)	7.82E-8%	7.48E-11	7.60%
T75				
Model 0	-0.17 (-0.29; -0.06)	---	---	---
Model 1	---	4.53%	0.023	Reference
Model 2[c]	-0.19 (-0.36; -0.02)	3.19%	0.016	29.3%

*corresponds to the proportion of between-schools variance that could be explained by exposure and individual characteristics; 95% CI: 95% confidence interval; ICC: intra-class correlation coefficient; FVC: forced vital capacity; FEV_1: forced expiratory volume in the first second of FVC; FEF_{25-75}: forced expiratory flow in the middle portion of FVC; EBC: Exhaled breath condensate; ACV: Average constriction velocity; MCV: Maximum constriction velocity; ADV: Average dilation velocity; T75: the total time taken by the pupil to recover 75% of its initial resting diameter after it reached the peak of constriction.
Model 0 only included the PC1 and PC2 score; [a] Model 1 is null model, baseline model without any exposure variable; Model 2[a] is additionally adjusted for age, sex, asthma, WHO z-score for BMI and family history of asthma or allergy; Model 2[b] is additionally adjusted for age, sex, asthma, atopy, WHO z-score for BMI and family history of asthma or allergy; Model 2[c] is additionally adjusted for age, sex, asthma, and WHO z-score for BMI

5.5. School neighbourhood, obesity and body composition (study IV)

This study was based on data from 845 children (49.2% girls). Considering the different classifications of BMI, the prevalence of overweight and obesity ranged between 15.1% (CDC) and 20.4% (WHO) and between 7.5% (IOTF) and 14.2% (WHO).

An increased proportion of built areas around school neighbourhood was associated with significantly higher values of BMI z-scores (model 3: β=0.48, 95% CI 0.05; 0.90 for CDC and β=0.54, 95% CI 0.07; 1.01 for WHO); while green areas showed a tendency to be associated with lower values of BMI. Similar results were observed between increased built areas and BMI classified according to age- and sex-specific percentiles (**Table 14**). The highest ICC were observed for BMI z-scores (1.02% and 1.41%), indicating that approximately 2% of the total variation in this parameter was found between schools. After adjustment, the neighbouring environment explained at least 64% of the effect of school on BMI defined by percentiles.

No significant associations were observed between green or built areas and body fat mass, free fat mass, and total body water. Still, a negative trend was found between green areas and these parameters. After adjustment, a significant positive association was observed between built areas around schools and body fat percentage (model 3: β=2.56, 95% CI 0.39; 4.73). Estimates of ICC

for body composition suggested that between 0% and 1.32% of the total variance was at the school level. The neighbouring environment explained 85.1% of the effect of school on body fat mass, 89.0% of its effect on free fat mass and 46.6% of its effect on total body water (**Table 14**).

Table 14 Multilevel model analysis of the association between individual and neighbouring environment and body mass index and body composition explained by school

				School	
	β (95% CI)		ICC (%)	Variance	Explained
Outcome	**PC1**	**PC2**			**variation***
z-scores CDC					
Model 0	0.08 (-0.18; 0.33)	0.28 (0.02; 0.54)	---	---	---
Model 1	---	---	1.30	0.02	Reference
Model 2	-0.15 (-0.58; 0.28)	0.48 (0.05; 0.90)	1.41	0.02	-12.0
z-scores WHO					
Model 0	0.06 (-0.24; 0.36)	0.33 (0.04; 0.63)	---	---	---
Model 1	---	---	1.20	0.02	Reference
Model 2	-0.20 (-0.68; 0.28)	0.54 (0.07; 1.01)	1.02	0.02	13.0
BMI CDC					
Model 0	-0.10 (-0.27; 0.08)	0.22 (0.05; 0.39)	---	---	---
Model 1	---	---	1.18	0.007	Reference
Model 2	-0.43 (-1.77; 0.08)	0.29 (0.05; 0.54)	0.29	0.002	74.7
BMI WHO					
Model 0	-0.02 (-0.20; 0.15)	0.20 (0.02; 0.37)	---	---	---
Model 1	---	---	0.75	0.004	Reference
Model 2	-0.35 (-1.05; 0.14)	0.28 (0.04; 0.51)	1.29E-6	7.37E-9	99.9
BMI IOTF					
Model 0	-0.06 (-0.23; 0.11)	0.15 (-0.01; 0.32)	---	---	---
Model 1	---	---	0.57	0.003	Reference
Model 2	-0.42 (-1.88; 0.05)	0.24 (0.01; 0.47)	1.27E-6	6.76E-9	99.9
Body fat cut-offs					
Model 0	-0.0001 (-0.18; 0.18)	0.20 (0.02; 0.38)	---	---	---
Model 1	---	---	1.30	0.008	Reference
Model 2	-0.10 (-0.36; 0.17)	0.27 (0.005; 0.53)	0.47	0.003	64.1
Body fat percentage					
Model 0	-0.44 (-1.84; 0.96)	1.60 (0.22; 2.98)	---	---	---
Model 1	---	---	0.96	0.34	Reference
Model 2	-1.39 (-3.61; 0.81)	2.56 (0.39; 4.73)	1.32	0.45	-30.1
Body fat mass					
Model 0	-0.56 (-1.55; 0.43)	1.00 (0.02; 1.97)	---	---	---
Model 1	---	---	2.00E-6	3.59E-7	Reference

Model 2	-1.10 (-2.48; 0.28)	1.10 (-0.24; 2.44)	2.96E-7	5.35E-8	85.1
Free fat mass					
Model 0	-0.07 (-1.17; 1.02)	0.75 (-0.33; 1.84)	---	---	---
Model 1	---	---	1.62	0.35	Reference
Model 2	-0.63 (-2.08; 0.81)	-0.06 (-1.46; 1.35)	0.21	0.04	89.0
Total body water					
Model 0	-0.06 (-0.87; 0.74)	0.64 (-0.15; 1.44)	---	---	---
Model 1	---	---	1.97	0.23	Reference
Model 2	-0.57 (-1.77; 0.64)	0.16 (-1.02; 1.34)	1.21	0.12	46.6

*corresponds to the proportion of between-schools variance that could be explained by exposure and individual characteristics; PC1: discontinuous dense urban fabric, discontinuous medium density urban land, green urban areas, and water bodies; PC2: construction sites, land without current use, and railways; 95% CI: 95% confidence interval; ICC: intra-class correlation coefficient; BMI: body mass index; CDC: US Centres for Disease Control; WHO: World Health Organization; IOTF: International Obesity Task Force. Model 0 - only included the PC1 and PC2 score;
Model 1 - is null model, baseline model without any exposure variable; Model 2 - is adjusted for age, sex, parental education, physical activity, energy intake and asthma

5.6. Swimming pool environment, skin and gut microbiome in swimmers (study V)

The most common genera found on the skin of elite swimmers were *Enhydrobacter*, *Streptococcus, Halomonas* and *Shewanella*, comparatively to *Streptococcus*, *Staphylococcus, Enhydrobacter, Halomonas* and *Corynebacterium* found in non-water athletes (**Figure 13**). However, *Halomonas* and *Shewanella* may be contaminants detected due to the low biomass of skin samples. A significant difference was observed in the composition of microbial communities between both groups of athletes (R^2= 0.11, p<0.01). Regarding to richness and evenness of bacteria, alpha diversity was significantly lower in swimmers than in non-water competitive athletes (W= 557, p= 0.027).

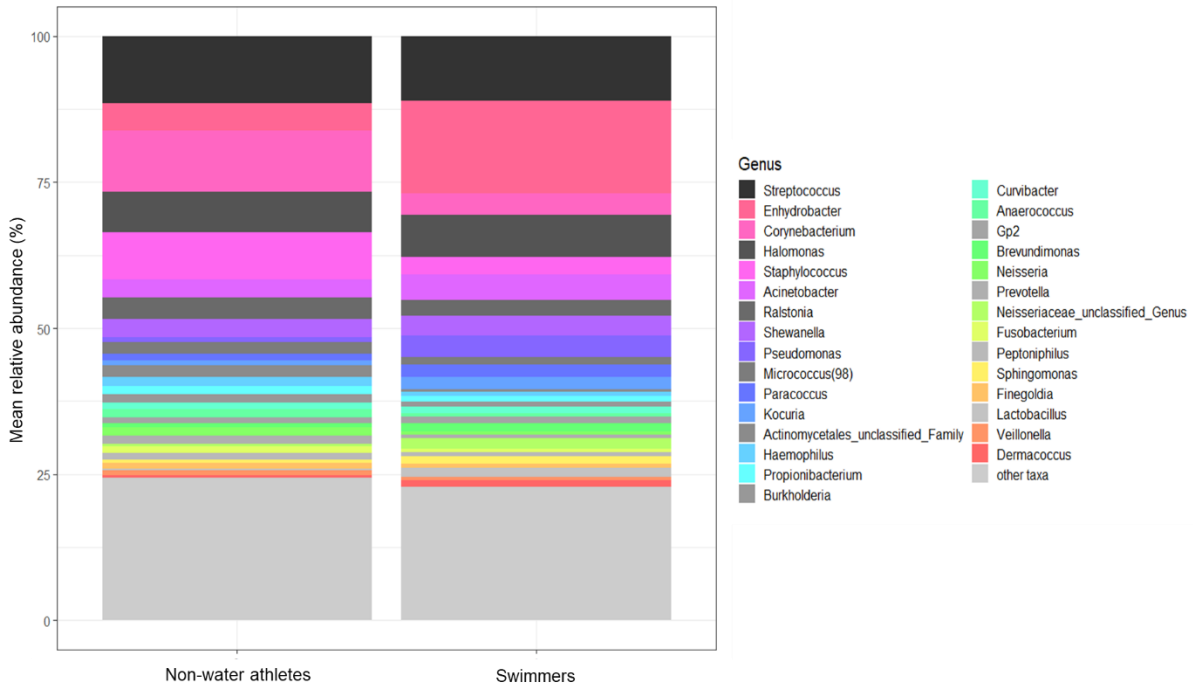

Figure 13 Mean relative abundance at the bacterial genus level for skin samples from non-water competitive athletes (n=34) and swimmers (n=29)

Bar plots display the skin microbiome profiles using the V3-V4 region of the 16S rRNA gene. Each bar represents a group of athletes' samples, with each colour representing different genera

In stool samples, the most common genera found were *Bacteroides*, *Faecalibacterium*, *Prevotella* and *Roseburia*, both in swimmers and non-water athletes. However, *Lachnospiracea* and *Blautia* were increased in swimmers and *Ruminococcaceae* and *Bifidobacterium* in non-water athletes (**Figure 14**). A significant difference was observed in the composition of microbial communities between both groups of athletes (R^2=0.04, *p*=0.03). For the stool samples, there were no significant differences between groups in richness and evenness of the microbial communities (W= 309, *p*= 0.606).

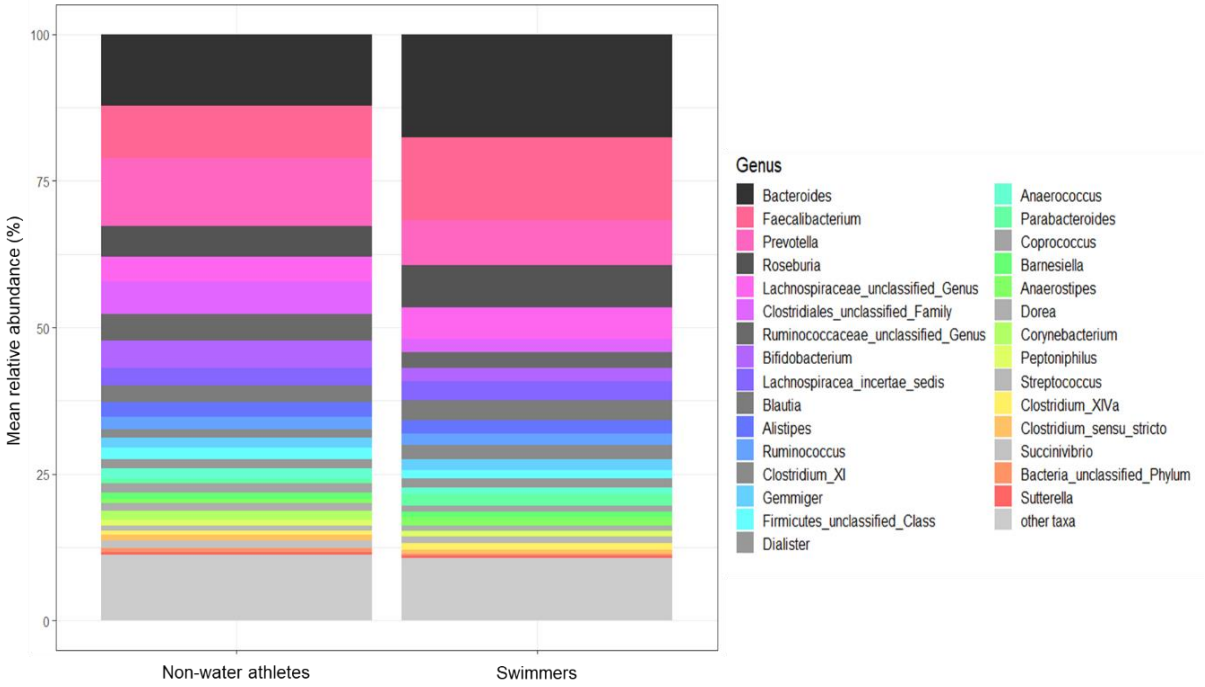

Figure 14 Mean relative abundance at the bacterial genus level for stool samples from non-water competitive athletes (n=34) and swimmers (n=29)

Bar plots display the gut microbiome profiles using the V3-V4 region of the 16S rRNA gene. Each bar represents a group of athletes' samples, with each colour representing different genera

6. Discussion

6.1. Summary of the results

This thesis was focused on the school environmental determinants of paediatric asthma and allergy, considering three dynamic and interactive levels – the individual, the indoor environment and school neighbourhood. Although the pathways whereby they influence the development of asthma are complex and interactive, this thesis also explored the role of the autonomic nervous system in mediating the interaction between the environment and asthma in children. The main findings and discussion are presented following the proposed levels, starting with participants' individual characteristics and progressing to the analysis of the school indoor environment and neighbourhood. Furthermore, we included the assessment of the effect of an extreme indoor environment – swimming pool training environment – on the complex composition and diversity of athletes' microbiome.

Growing urbanization and westernization of lifestyles have been associated to an increase in the prevalence of asthma in children, following a urban-rural gradient, being lower in less urbanized regions (Africa and Indian subcontinent, lower than 6%) and higher in more urbanized areas (North America, Oceania and United Kingdom, higher than 20%) [39]. The prevalence of asthma found in this study ranged between 6.5% and 12.3%, which is similar to the national Portuguese data obtained within ISAAC phase III (9.4% for children aged 6–7 years old and 14.7% for adolescents aged 13–14 years) and also from Porto center (5.7% for children aged 6–7 years old and 15.1% for adolescents aged 13–14 years) [306]. Additionally, the prevalence of asthma observed in this study is lower than those observed in other urbanized regions [39], however it is expected that increasing urbanization may affect the development of asthma. The higher prevalence of asthma led us to investigate the role of exposure to indoor VOC identified as endocrine-disrupting compounds (**study II**), and the physical environmental characteristics around primary schools (**studies III** and **IV**) as determinants of asthma in children as well as the impact of an extreme indoor environment, such as swimming pool, on skin and gut athletes' microbiome (**study V**), as a crucial factor in the development and maintenance of appropriate immune function.

The physical environmental characteristics included the assessment of land use, defined as built and urban green areas, and the walkability index, considering a circular buffer of 500 metres around each primary school address. A wide range of neighbourhood features were considered using GIS (geographical information system): street connectivity, residential density, industrial and commercial areas, construction sites, green urban areas, sports and leisure activities, forests.

Understanding the factors, such as indoor environment, that underlie changes in the composition of the skin and gut microbiome, could also provide insights into how to promote health by targeting indoor exposures and microbial community.

The main results showed that both environmental levels – indoor and neighbourhood - are associated with health effects in schoolchildren, namely with asthma, respiratory symptoms, body composition and obesity, suggesting a dynamic interaction between human-beings and the environment on their health status. Furthermore, exposure to swimming pool environment may also be associated with changes in composition and diversity of human microbiome, driving to dysbiosis. The systematic review (**study I**) highlighted the relevance of indoor environments in children's environmental exposure to VOC. Higher concentrations of specific compounds were found indoors when compared to outdoors, including in school's environments. This suggests that indoor sources, occupant activities and behaviours may have a great contribution to indoor concentrations of VOC. Therefore, the putative effect of indoor VOC, in particular those that are identified as endocrine-disrupting compounds, in asthma, respiratory symptoms and obesity in schoolchildren was extensively investigated (**study II**). In this study, EDCs were selected among the VOC found in each classroom and their effects as individual compounds as well as mixtures were analysed. As hypothesized, a positive association was observed between exposure to EDCs and child health, particularly with asthma, obesity and the presence of current respiratory symptoms. In addition, evidence from this study supports the assumption that even low concentration of EDCs in classrooms may affect schoolchildren health, also highlighting the negative effects of exposure to VOC concentrations, and to both individual and co-exposure to these endocrine disrupting-compounds. Similar to holobionts, the health status of human beings is also determined by the outdoor environment. This challenged us to additionally understand the effect of school neighbourhood and walkability on lung function, autonomic nervous system activity (**study III**), obesity and body composition in schoolchildren (**study IV**). Both studies demonstrated that school neighbourhood, namely built areas characterized by construction sites, land without current use and railways, has an effect on child health. In particular, we found that the presence of urban green areas has a positive effect on lung function (**study III**). It has been recognized that obesity and body composition is associated with a higher risk of developing asthma and to a more difficult-to-control asthma phenotype [86, 87], being key determinants of childhood asthma. Thus, we explored the role of school neighbourhood on obesity and body composition in children. We observed that built areas around schools were also positively associated with children's obesity, suggesting that land use around school may be essential to promote a framework of lifestyle or behaviours conducive to achieving a healthy weight (**study**

IV). Our results also provide proof of concept for the role of the autonomic nervous system in mediating the interaction between the environment and the individual. Specifically, indoor exposure to EDCs and built areas in school neighbourhood may be associated with a change in autonomic balance with an increase in vagal activity.

Overall, this thesis provides valuable evidences to a better understand of the role of school environmental determinants in children's health. In addition to schools, we also studied the effect of exposure to swimming pool training environment, as an important recreational environment, on skin and gut microbiome among competitive athletes. Our findings suggested that a daily and prolonged exposure to an extreme environment, such as swimming pool, may be associated with significant long-term effects in the composition and diversity of skin and gut microbiome among swimmers compared to non-water competitive athletes, and consequently to dysbiosis and adverse health effects. Exposure to swimming pool training environment may shape skin and gut microbiome to an alternative stable state and the magnitude of this disturbance may also differ across anatomical sites.

6.2. The role of indoor school environment

Implications of spatial and seasonal variations of volatile organic compounds (study I)

Our systematic review showed that VOC are commonly found in indoor air, varying their concentration between indoor environments and seasons. The study showed that higher concentrations of VOC can be found indoors, suggesting that indoor sources, occupant activities and behaviours contribute to higher concentrations of VOC. The VOC identified in each indoor environment and their concentrations varied considerably between studies, which may be due to differences in sampling methods and geographic settings, as well as fluctuations in air exchange rates, occupants' behaviours and outdoor air concentrations. Similar in all indoor environments (schools, homes, offices and other indoor settings), the mean concentration of most compounds was higher in the cold season, suggesting a time variation in the composition and levels of VOC between seasons, and providing important contribution to assess the effects of these compounds on indoor air quality and consequently on occupant health. Several compounds exhibited an I/O ratio higher than 1 corroborating the role of indoor sources in indoor concentrations. Nevertheless, other VOC presented an I/O ratio close to or below 1, which suggested that for those compounds indoor concentrations were also affected by out-to-indoor air penetration, namely from traffic and industrial-related sources. On average, higher mean concentrations of indoor VOC were found in housing and offices environments; however, exposure to VOC is particularly important in schools

where children spend time together in restricted area. Furthermore, schools can be one of the most significant indoor spaces among all buildings due to the high density of children for a long period of time during the day. On the other hand, and according to Babayigit *et al.* [307] schools environments are affected by one or more potential indoor sources of VOC, including the use of cleaning products, furniture, goods, food, paints, pens, markers, and electrical devices, which may adversely affect children health.

Indoor endocrine-disrupting VOC, childhood asthma and obesity (study II)

Exposure to VOC identified as endocrine-disrupting compounds in classrooms was associated with an increased risk of asthma and obesity and an increased body fat percentage, as well as with an increased prevalence of nasal obstruction symptoms in the previous 3 months. Significant associations between compounds grouped by principal component analysis and obese-asthma, current respiratory symptoms, obesity and body composition were also observed, even for low levels of EDCs. Furthermore, our results showed that exposure to individual or combined EDCs is associated with changes in the autonomic nervous system, specifically parasympathetic dysautonomia assessed through pupillometry [308, 309], thus suggesting that EDCs may increase parasympathetic activity , resulting in a subsequent increase in the risk of asthma and obesity.

Surprisingly, our findings showed that even low-level exposures of EDCs may increase the risk of asthma, the presence of respiratory symptoms in the previous 3 months or obesity in schoolchildren. These results suggest that no safe exposure level exists, especially when children are constantly exposed to these compounds. Indeed, other studies have showed that exposure to lower concentrations of EDCs may cause even stronger effects than higher doses, especially considering that exposure is lifelong [145-147]. Similar to natural hormones, even low concentrations of EDCs (in the parts-per-trillion to parts-per-billion range) may have influence on human metabolism. Several studies have showed that very low levels of individual or combined EDCs were associated with reduced intelligence, disrupting reproductive system and cause other health problems [145-147]. Thus, the statement by Paracelsus that “the dose makes the poison” may not apply to EDCs exposure because of potential low-dose effects, which cannot be predicted by the effects of exposure to higher concentrations [145].

We evaluated the effects of EDCs either alone or in combination, since these compounds can be released as mixtures and interact within or between classes, and combinations of low doses of EDCs that are individually inactive may cause a biological effect [146, 147]. According to

Kortenkamp [310] and Oziol *et al.* [311], it is important to understand the effects of co-exposure to EDCs, which may interact additively, synergistically or antagonistically, with potentially unknown intrinsic effects. However, most previous studies have focused primarily on the effects of exposure to individual semi-volatile organic compounds such as phthalates and bisphenol on respiratory health [170, 171, 312] and obesity in children [163, 313] and on compounds that interact with the oestrogen, androgen and thyroid hormone systems [130, 131]. Our results suggested that exposure to individual or combined EDCs may interfere with other systems, increasing the risk of asthma, the presence of respiratory symptoms in the previous 3 months and obesity in schoolchildren, and these results contribute to the understanding of the potential health risk of co-exposure. Additionally, exposure to combined EDCs represented by PC1 (positive loadings on toluene, o-xylene, m/p-xylene, ethylbenzene, styrene, and benzene) may also increase the risk of "obese-asthma", suggesting that obese children with asthma may be more sensitive than healthy children to EDCs.

Individual EDCs, such as toluene, hexane, styrene, o-,m/p-xylene, ethylbenzene, cyclohexanone, BHT, benzene and 2-butoxyethanol, and combined EDCs obtained in our study by principal component analysis were significantly associated with different health effects, showing that different EDCs may target different organs and systems, and that diverse mechanisms may be involved. EDCs may stimulate airway C-fibre sensory nerves, which express TRP cation channels; when exposed to irritants, these channels release neuropeptides locally, resulting in cough, airway irritation, mucous secretion, and bronchoconstriction mediated by the efferent pathways of the autonomic nervous system [56, 159]. Nassi *et al.* [160] and Cantero-Recasens *et al.* [161] reported the role of activation and/or increased expression of TRPV1 and TRPA1 channels in the pathogenesis of asthma, providing evidence for the role of ANS in the regulation of airway function. Additionally, Baillie-Hamilton [314] reported that exposure to different chemicals, such as EDCs, is also associated with weight gain, possibly by interfering with the normal activity of the ANS. The ANS plays an important role in the communication between the gastrointestinal system and the central nervous system, as a mediator of the sense of satiety after gastric distension, in the release of gut hormones, in the modulation of satiety, and in the release of leptin and insulin, which regulate body weight and adiposity [315].

Different mechanisms, such as mechanical, immunological, genetic, epigenetic, hormonal, and environmental pathways, may mediate the association between obesity and asthma [162]. Our results also suggested another possible noninflammatory mechanism underlying obese-asthma: disruption of the ANS by leptin, due to exposure to indoor EDCs. In fact EDCs can mimic, block or interfere with the production of leptin [316], a consequence that may also be involved in

increasing the risk of asthma and obesity [317]. The lack of leptin signalling has been showed to influence lung physiology and mechanics, including bronchoconstriction, and associates with asthma development by disrupting the activity of the parasympathetic nervous system [318, 319].

Our results suggest that both individual exposure and co-exposure to low concentrations of EDCs in classrooms, increasing the ANS parasympathetic activity may affect child health, specifically obesity, asthma, obesity and the presence of respiratory symptoms in the previous 3 months, highlighting the negative health effects of indoor exposure to EDCs and the recommendation to minimize VOC exposure in the classroom indoor environment.

6.3. The role of school neighbourhood

School environment, lung function and autonomic nervous system (study III)

Our findings showed that the built areas around schools may have an effect on children's health, specifically on lung function and on autonomic nervous system activity. Our results suggested that effects of environment on lung function may be partly neurogenically mediated, as schools' neighbourhood walkability explained up to 14% and 30% of the effect of school on parasympathetic and sympathetic activity, respectively.

Recent studies have showed evidence of beneficial associations between greenness and health outcomes. Urban green spaces not only provide balance for ecosystems but also promote physical activity, psychological well-being, and public health in urban populations [199]. Greenness may influence health by promoting physical activity and opportunities for social interactions, decreasing the risk of many chronic diseases and psychophysiological stress and reducing air pollution levels, noise, and heat exposure [200]. In children, exposure to green areas has been associated with reduced obesity and sedentary behaviours [15, 200]. Ruokolainen and colleagues have showed that the amount of forest and agricultural land around homes are inversely associated with the risk of atopy in children [210]. These findings provide support for a role of natural environmental on the regulation of the Th1, Th2 immune response mediated by the children commensal microbiome [210, 320]. Furthermore, in children living in greener areas of Vancouver, as measured by the normalized differential vegetation index, had a slightly reduced risk of incidence of asthma (aOR=0.96; 95% CI 0.93; 0.99) [213]. Similarly, lower asthma prevalence in areas with greater tree density in New York City has been reported [321]. Nevertheless, no individual-level studies are available to compare with our findings; however, these associations are similar to the reported results of previous studies on the association between greenness and asthma. Although several studies reported the role of greenness as a

buffer against exposure to air pollution and the positive effect of greenspaces in urban context [12, 200], air pollution can also affect plant health and functions and limit pollutant dispersion and thus increase local pollutant concentration [257, 260] and affect human health. Airway obstruction in children is often triggered by environmental factors. Previous studies have reported associations between exposure to urban areas and adverse respiratory health effects, especially in children For instance, the ESCAPE meta-analysis with data for 5921 children from five European birth cohorts reported that annual exposures to NO_2, NO_x, PM_{10}, and $PM_{2.5}$ were associated with reduced lung function [322]. The negative impact of exposure to urban environment has also been further reinforced by Mudway *et al.* [323], in which exposure to urban air pollutants, particularly to NO_x and NO_2, was inversely associated with lung function, and by Gauderman *et al.* [324], showing that reductions in pollution delivered significant improvements in FEV1 and FVC.

In addition to the positive effect of the presence of urban green areas on children lung function, our results proposed that autonomic nervous system may play a role in mediating the interaction between the environment and the individual. Air pollutants, such as PM, O_3, and NO_2, can activate the TRPV1 and TRPA1 cation channels on airway C-fibre sensory nerves, and cause several responses, such as bronchoconstriction, mucus secretion, airway irritation, and cough, mediated by the efferent pathways of the autonomic nervous system [56]. Moreover, several studies have addressed the complexity of the interactions between greenspaces and human health and wellbeing. A review of field experiments conducted in 24 forests across Japan on the effects of *shinrin-yoku* (taking in the forest atmosphere, or “forest bathing”) showed that forest environments could lower concentrations of cortisol, decrease heart rate and blood pressure, increase parasympathetic nerve activity, and lower sympathetic activity compared with city settings [207]. Recently, Gladwell and colleagues [208] showed that autonomic balance can change with an increase in vagal activity by the simple act of viewing natural scenes. In their study, a slideshow containing natural scenes, compared with another that incorporated built or urban scenes lacking green space, was associated in an increased parasympathetic activity [208]. However, comparisons of our findings regarding to autonomic nervous system with those studies are limited by the different methodologies used to assess autonomic nervous system activity and environmental exposure. The differences found in autonomic nervous system response may be related to the effects of the type of natural settings (parks, gardens, sports fields, forests, tree corridors, or other green space types) and the time spent in each area [325]. In this study, we assess the effect of green areas in an urban environment, where green areas may be smaller and where children are expected to spend less time, as opposed to previous studies in Japan that

reported the effect of green areas outside the city, specifically, in forest areas [207]. Our study highlights the link between school neighbourhood and children health effects permitted to underline the positive effects of green areas on lung function and on autonomic nervous system activity.

School neighbourhood, obesity and body composition (study IV)

In this study, we found an association among school neighbourhood environment and both BMI z-scores and BMI defined by percentiles, and body fat percentage in schoolchildren. Built areas around schools were positively associated with children's obesity. Moreover, a positive trend was observed between green urban areas and obesity parameters, suggesting that greenspaces around school may promote a framework of lifestyle or behaviours conducive to achieving a healthy weight.

Similar to the effects on lung function, this study also suggest that built areas in school neighbourhood may play an important role in obesity development by interfering with several pathways. Obesity is a complex metabolic disease and is a result of the interplay of many factors at multiple levels ranging from biological to environmental factors [326]. Although there is growing evidence of the built environment effects on health, namely on obesity, the underlying pathways and mechanisms remain unclear. The most widely pathway by which urban environment impacts obesity may be the discouragement for physical activity [327, 328]. In urban areas, greenness may indicate the proximity to parks, playfields, or other open spaces that promote either physical activity or increased time spent outdoors [329], having a positive impact on reducing obesity and improving health [330, 331]. Additionally, psychosocial stress associated with urban environment, may be an important mechanism by which neighbourhood associate with obesity, leading to the activation and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system [332, 333], with consequent chronic elevation of glucocorticoid and catecholamine pathways [334]. It is then reasonable to assume that increased exposure to built environments increases the sympathetic nervous system activity and, consequently, a lower rate of thermogenesis, a positive energy balance and obesity [315, 335].

It has previously been suggested that greenness can also minimize the negative effects of air pollution in urban areas providing benefits to public health [336]. A set of mechanisms and responses in human body may be effected by environmental pollutants, increasing the risk of obesity by changing lipid homeostasis, altering the proliferation and differentiation of adipocytes or the development of neural circuits that regulate feeding behaviour, altering homeostatic

metabolic set points [316, 317, 326, 337]. In addition, weight gain has been associated with changes in the normal activity of ANS due to the exposure to different chemicals [314]. This changes in the ANS may suppress appetite, enable the body to mobilize fat stores for use and stimulate physical activity levels [314]. Furthermore, a change in balance between parasympathetic and sympathetic nervous system activity plays an important role as a mediator of sense of satiety after gastric distension, gut hormone release, in the modulation of satiety, and in the release of leptin and insulin, which regulate body weight and adiposity [315]. According to Bartness [338], lipid accumulation in obesity could be due either to a decrease in SNS activity or by an increase in parasympathetic activity.

On the other hand, changes of the human microbiome induced by lower exposures to green areas and higher exposures to air pollutants may affect metabolic and physiological homeostasis that would lead to obesity [339]. Claus *et al.* [339] suggested that gastrointestinal microbiome may affect obesity by altering the absorption, disposition, metabolism and excretion of environmental chemical parameters and reciprocally these pollutants can also interfere with the composition and metabolic activity of human microbiome. Thus, urban green spaces not only provide balance for ecosystems but also promote physical activity, psychological well-being, and public health in urban populations [199].

Similar to our study, previous studies assessed the effects of green and built areas within an urban context, but in living neighbourhoods. Living in neighbourhoods characterized by higher greenspace was found to be associated with more walking and cycling for transport and leisure, with moderate to vigorous physical activity [340] and with reduced obesity and overweight among children [329, 341-343] and adults [344-348]. Our study considers neighbourhood around schools only; however, several studies reported the impact of the school environment on planning decisions that support changes in behaviours related to diet and physical activity, active commuting to school (accessible schools with low traffic, sidewalks) and in the decrease of the automobile dependence in childhood which carries over into adolescence and adulthood [240, 349, 350]. Although several studies have found a significant increase in the percentage of overweight and obesity with an increase in the percentage of neighbourhood greenness [327, 351], the methodology-specific factors, such as definition of green spaces, conceptualization of built environment and analytical tools, and on individual and environmental characteristics [261, 327, 352], may be linked to the different reported patterns of results between neighbourhood greenness and obesity. Wilhelmsen *et al.* [327] investigated the relationship between green areas around schools and BMI, however Norwegian green areas are typically farmland, woods, and mountains.

The school neighbourhood should not only be considered a physical space, but also a source of possibilities and constraints for children to engage in certain activities and change behaviours. Thus, our results support the relevance of health-oriented urban policies to provide access to health, safe and green urban spaces in which children can walk and cycle to kindergartens and schools, play and undertake physical activity, meeting the goals proposed by the United Nations [353].

6.4. Influence of an indoor pool environment in skin and gut microbiome

Swimming pool environment, skin and gut microbiome in swimmers (study V)

Exposure to indoor swimming pool environment seems to be associated with a significant difference in diversity and composition of skin and gut microbiome in elite swimmers compared to non-water competitive athletes. The skin and gut microbial communities were different between both groups, but with overlap in some taxa, with a higher alpha diversity (species richness and evenness) in the skin samples from non-water athletes, and a distinctive community, when comparing beta diversity (community composition similarity), were observed. However, the richness and evenness of microbial gut communities were similar in both groups.

To our knowledge, this study is the first one to use 16S rRNA gene sequencing of skin and stool samples from competitive athletes (swimmers and non-water athletes), assessing the influence of the indoor pool environment in diversity and composition of human microbiome. Swimming pools are an important recreational environment, being an adequate sport to practice physical activity and to gain health benefits. However, besides the health benefits, the highly intensive training coupled to disinfection by-products (DBPs) exposure have been associated with several unwanted health effects [354, 355]. Several studies have reported the effect of these products in airways epithelium damage and hyperresponsiveness, showing an association between exposure to indoor swimming pool environment and a higher risk of asthma in children [356-359]. Similar, Carlsen *et al.* [360] also reported that environmental factors associated with swimming pool exposure, including chlorine in pool water, may be related with the increased prevalence of exercise-induced asthma. Our results pointed to a decrease in bacterial diversity and composition of the skin and gut microbiome of athletes exposed to swimming pool environment, underlying another effect of daily and prolonged exposure to this environment, which has been associated with asthma development.

Similar to other body sites location, including gut, skin microbiome have essential roles in the protection against pathogens and in the education of immune system [361]. Based on sequencing

surveys in healthy individuals, the composition of microbial communities was found to be dependent on the physiology of the skin site, with changes in abundance of bacteria associated with moist, dry and sebaceous microenvironments [362]. According to Byrd *et al.* [362], dry areas, such as the volar forearm, were dominated by *Corynebacterium*, *Streptococcus* and *Staphylococcus* species, and we found similar results in non-water athletes' samples. However, our results suggested that the exposure to swimming pool environments, characterized by higher levels of humidity and higher concentrations of DBPs, may be associated with a higher relative abundance of *Enhydrobacter* species in competitive swimmers' samples, which are preferentially abundant in moist areas [362], and also with a lower composition and diversity in skin microbiome among this group. Although longitudinal studies have reported that skin microbial communities are largely stable over the time, namely at sebaceous sites [363], the daily and prolonged exposure to an extreme environment, such as an indoor swimming pool, may be associated with significant long-term effects in skin microbiome, and consequently to dysbiosis. This dysbiosis may also modify the local environment of the skin and the inter-species interaction, inhibiting or enhancing the growth of other microorganisms, and has been associated with many common skin diseases [362] and allergy [230]. Grice *et al.* [364] also reported that skin sites with a greater diversity of microorganisms, including the volar forearm, tend to be less stable over time, suggesting that environmental factors may drive changes in community membership and structure. Although several studies have reported the effect of natural environment on the composition of the skin microbiome [210, 219, 222], there are no studies assessing the effect of indoor environment. In this study, we also found significant differences between swimmers and non-water athletes' skin samples, being the alpha and beta diversity different between both groups; skin microbiome of swimmers was less diverse and had a lower relative abundance.

In addition, each individual is provided with a gut microbiome profile, which is also shaped by environmental factors, playing important functions in host nutrient metabolism, maintenance of intestinal epithelium integrity, immunomodulation, and protection against pathogens [365]. Recent studies highlight that there is not a unique optimal gut microbiome composition since it is different for each individual [365]; however, several studies reported the existence of a dominant gut microbial phyla, which include *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia*, with *Firmicutes* and *Bacteroidetes* representing 90% of gut microbiome [366, 367]. In our study, there is also a higher relative abundance of *Firmicutes* (*Faecalibacterium*, *Roseburia*) and *Bacteroidetes* (*Bacteroides*, *Prevotella*) in all athletes. Furthermore, comparing both groups, we have found a difference in the relative abundance of some genera composition; swimmers have a higher relative abundance of other *Firmicutes*,

including *Lachnospiracea* and *Blautia* genera, and non-water athletes have also a higher relative abundance of *Actinobacteria* (*Bifidobacterium*). Research in this area has focused mostly on the interactions of the microbiome with human health, with few studies evaluating the effect of indoor environment on microbiome composition and diversity. In addition, antibiotics and other pharmaceutical products have an obvious impact on gut microbiome, namely on both pathogenic and beneficial microbiome, leading to perturbation of its composition and diversity [185]. No studies have investigated to date the influence of an extreme environment exposure, such as swimming pools, and changes in athletes' microbiome. Our study suggested that exposure to indoor swimming pool environment may be associated with a significant difference in diversity and composition of gut microbiome in elite swimmers. Similarly, recent experimental studies demonstrated, in mice, that DBPs were associated with changes in relative abundance of gut *Bacteroidetes,* a reduced *Firmicutes*/*Bacteroidetes* ratio [187] and with decreased levels of *Clostridium spp*, *Enterobacteriaceae*, and *Staphylococcus* [188]. In gut samples from competitive swimmers there were also a change in the proportion between *Firmicutes* and *Bacteroidetes*, being the relative abundance of Bacteroidetes higher in this group compared to non-water athletes, and a lower abundance of *Clostridiales* genus were also found among swimmers' samples. Nevertheless, no significant differences were observed in richness and evenness of gut microbiome between the two groups, which may be related to the lower variability of gut microbiome over time. Similar results were also reported in other studies, showing that gut community structure is resilience to perturbation and stable over time [279, 368, 369]. However, other preclinical studies demonstrated that the gut microbiome composition may change over a short time, being influenced by several factors, including environmental exposures [370]. As showed by studies [368, 371, 372], the gut microbiome may be more stable over time once established, suggesting that the changes in swimmers gut microbiome composition and diversity may occur gradually, mainly by water ingestion.

This observed changes in diversity and composition on microbiome may be due to the effect of several environmental chemicals, which have been showed to alter the composition and/or the metabolic activity of microbiome and, consequently, contributing to reshape athletes' microbiome [177, 183, 185]. Exposure to different chemicals in swimming pools, through ingestion, inhalation or dermal contact, may also alter chemical exposures by damaging the metabolic capacity of the microbiome or changing the environment that supports microbiome-induced chemical metabolism [177]. Therefore, disruptions in the microbiome may in turn induce effects on host physiological responses and health [174, 189].

Our results suggest that a specific indoor environment may play a role in reshaping human microbiome. These results emphasize the influence of a specific training environment in the composition and diversity of microbiome, suggesting that some unwanted health effects of specific indoor environment may be also underlined by changes on microbiome. Moreover, this study highlights the growing recognition that human beings are holobionts, whose dynamic bidirectional interactions respond and change to environmental exposures, influencing human health.

6.5. Limitations and Strengths

Regarding the systematic review (**study I**), the results were limited by the number of individual VOC that were assessed by each study and by the low number of studies that reported both indoor and outdoor VOC concentrations by season and setting. The included studies also used different methodologies for assessing and analysing VOC, making it difficult to directly compare the observed results. A further limitation was the combined analysis of the results without considering tobacco smoke exposure. However, few studies have reported the results considering exposure to tobacco smoke or even report whether occupants smoked indoors. Nevertheless, the systematic review (**study I**) provides an overview of exposure to VOC in four major indoor environments, including schools, houses, offices and other important indoor settings, over the last 20 years. Additionally, the analysis by season and results of the indoor/outdoor ratio support the importance of sampling VOC throughout the year and also suggested that indoor sources played a major role in the occupant's exposure to VOC.

The cross-sectional design (**studies II** - **V**) does not allow the establishment of causal relationship or the analysis of cumulative exposure to different environments. In **study II**, no on-site monitoring data regarding the levels of other indoor air pollutants, such as other chemical compounds, PM, moulds or allergens, were considered in the analysis. Additionally, **study II** considers classroom exposure only. Nevertheless, following their own home, children spend most of their day at school, especially indoors, and usually stay in the same classroom through primary school years. Thus, indoor air quality in schools has an important role in the assessment of the health effects of EDCs exposure. In addition, Rudel *et al.* [4] and Oziol *et al.* [311] reported that concentration of EDCs is frequently higher indoors, due to the greater number of sources indoor, and also to a more limited degradation indoors than outdoors. Most EDCs considered in this study were synthetic organic chemicals used in a wide range of materials and goods and can be found in many products in schools, including materials used in building construction and furnishing, along with

cleaning products and paints [129]. Moreover, indoor VOC concentrations were measured only in winter, as during this period VOC concentrations are usually high indoors, probably due to the lower air renewal in winter and the persistence of the compounds due to reduced photochemical degradation and gradual accumulation [373]. Although several studies identified a significant seasonal cycle in VOC concentrations [373], our findings showed that even low-level exposure to EDCs may increase the risk of asthma, respiratory symptoms or obesity in schoolchildren. Nevertheless, it will be important to assess the effect of seasonality on EDCs concentrations to understand the extent of exposure as well as the potential health effects. Despite the sampling of VOC at a single time point (limiting the temporal analysis) and the short period of exposure assessment, the one-week sampling period and the use of passive samplers allowed the assessment of average indoor air concentrations and the detection of relatively low concentrations of EDCs, as suggested by recent reports [374, 375]. In addition, EDCs were measured in indoor air, which has been identified as an important source of chemical exposure [131]. Over the past decade, it has become clear that humans, particularly children, are exposed to EDCs via particles in indoor environments [134-136]. Urinary levels of EDCs were not measured; however, several studies have observed correlations between urinary levels of EDCs metabolites and indoor air concentrations, indicating that indoor air is a significant route of exposure [376, 377].

In **studies III** and **IV**, no on-site monitoring data regarding air pollution levels were measured, and we did not address the quality of green spaces, vegetation types or biodiversity, accessibility of greenspaces, and also the availability of food stores. Nevertheless, several studies on urban environmental effects reported that land use could be used as a proxy of urban-related air pollution, such as traffic, without outdoor air monitoring [378, 379]. Rosenlund *et al.* [380] also found a reasonable agreement between land-use and traffic emissions. In our studies, neighbourhood land use patterns and walkability around schools were quantified numerically by objective measures, yielding accurate land cover and avoiding bias related to participants' perception of their neighbourhoods. Additionally, **study III** considers only the walkability around schools; several studies have also reported the impact of walkability around schools in planning school neighbourhoods, including accessible schools with low traffic and sidewalks, in decisions that support the active commuting to school [240], and the decrease of automobile dependence [240]. In addition, assessing the walkability around individual's home may not necessarily reflect the facilities that they use or environments in which they are active [381].

All questions regarding asthma symptoms and clinical assessment considered in **studies II, III** and **IV** were performed under the assumption that the children did not have colds or flu, thus

reducing the effect of co-exposure factors and the probability of chance findings. Although several confounders have been considered, there may be other unknown or unmeasured confounders. School environment (**studies III** and **IV**) and exposure to EDCs (**study II**) may also be associated with other important confounders related to asthma, respiratory symptoms, obesity or body composition and it is possible that those may increase the risk of indoor exposures and built areas compared to healthy ones. Indicators of asthma severity, such as number of asthma attacks, attendance in emergency service and hospitalization due to asthma in the last 12 months, and asthma medication use were not considered. Thus, it will be important to assess the effect of long-term exposure to school environments to understand the extent of health effects.

Another potential limitation of **studies II**, **III** and **IV**, as in other cross-sectional studies, is the selection bias. However, in **study III** no significant differences were found between the children not included in each study and those included, being expected that our associations were most likely not biased. In **studies II** and **IV** children not included were similar to those included, except for in the BMI z-scores and for the prevalence of underfat, which may result in an underestimated association between exposure to EDCs (**study II**) and exposure to school neighbourhood environment (**study IV**) and BMI and body composition, respectively. Additionally, physical activity, which was considered as a confounder in **study IV**, was defined based on a single question using self-reported data, which may not reflect how active the children really were and may lead to some misclassification bias. However, compared to direct measures, self-reported methods may be able to represent the type of activity that is undertaken and appear to estimate greater amounts of higher intensity physical activity [382], which would underestimated the observed association with school neighbourhood and obesity.

The effect of schools' neighbourhoods in **studies III** and **IV** were measured using a robust statistical tool that allowed a multilevel approach, considering the complex relationship among the different levels of variables. Our results are also limited by low ICCs to estimate the percent of total variance in outcomes between neighbourhoods generated by the variables of the multilevel analysis. However, even low ICCs may coexist with important fixed effects of contextual variables. Public health is full of examples of risk factors that explain very little inter-individual variance but are considered important predictors of health outcomes. Thus, as Duncan and colleagues [383] have stated, even variables with low ICC are considered important predictors of health outcomes and are compatible with important policy effects of neighbourhood characteristics on health. Since ICCs represent the proportion of the variance at the school level rather than individual, they may indicate to what extent school interventions and policies influence outcome-relevant individual predictors [384]. Our results suggest that the school neighbourhood explains an important portion

of the variance for all outcomes suggesting that school-level changes may have an important impact on children health outcomes. Furthermore, higher ICCs observed in **study III** suggest that the effect on lung function and autonomic nervous system activity in children may be predicted by school neighbourhood as well as characteristics of the children. The robust statistical tool used in **studies III** and **IV** allow modelling the effects of individual-level and neighbourhood-level variables on the outcomes of interest. Multilevel analysis takes account the hierarchical structure of our data, considering the complex relationship among the school neighbourhood and children lung function, BMI and body composition. Furthermore, the multilevel analysis has been well described as an appropriate tool for assess neighbourhood level effects on individual health [55].

Studies II, III and **IV** comprise a comprehensive clinical assessment on a large number of participants, including an assessment of autonomic status that allowed us to assess the children's ability to respond to stress and to assess neurogenic pathway of the autonomic nervous system. In **study II** we also measured the concentrations of several EDCs in a substantial number of classrooms. All the clinical assessments were performed in each primary school, in the children's normal environment, which would help reduce the co-effect of stress in the schoolchildren. Furthermore, the assessment week was arranged with the school principal and teachers to avoid examinations and special days. Even assuming an increase in stress levels, the effect of stress is expected to be independent of environmental exposure. Moreover, different asthma definitions have been considered in studies **II** and **IV**, including respiratory symptoms, lung function and reported medical diagnosis of asthma, allowing the identification of different characteristics among children. Our definitions included the combination of different parameters: medical diagnosis of asthma and self-reported asthma symptoms based on ISAAC questionnaire and airway reversibility "Medical diagnosis of asthma and symptoms or BD+". Silva *et al.* [385] recently reported that a standardized definition of asthma should include questionnaire information and airway reversibility, as these measures are likely to consider different expressions of the disease in children. Although **study III** assessed the effect of schools' neighbourhoods on lung function, asthma is characterized by airflow obstruction [386] with changes in lung function parameters [387]. Regular assessment of lung function, namely FEV1, might also help to identify children at risk for developing a progressive decline in airflow [388]. Data on symptoms in previous 12 months (**studies II-IV**) and symptoms in the last 3 months (**study II**) were also evaluated allowing the identification of children with long-term and recent symptoms, respectively. In **studies III** and **IV** different criteria were also used to define BMI and to determine the effect of environmental exposure on obesity. However, the establishment of different cut-offs is generally statistical rather than based on risk or the degree of body fatness. As a result, different definitions often do not

give the same results. In these studies, we assessed the effect of environmental exposure (indoor exposure to EDCs and the effect of school's neighbourhood) on BMI considering that the reference values resulted in similar but not identical estimates. Additionally, BMI was calculated based on measured height and weight avoiding self-perceptions of weight categories by parents, since most of parents underestimated their children's overweight/obese status [389]. **Study IV** also considered the effect of school neighbourhood in body fat, free fat mass, and total body water. Recent studies highlighted the usefulness of body composition parameters in obtaining body fat, free fat mass, and total body water separately and as potential indicators of body adiposity [390, 391]. Although BMI is highly correlated and widely used as a surrogate measure of body fatness [40-42], it is a measure of excess weight adjusted for height, rather than excess body fat and does not distinguish overweight due to excess fat mass from overweight due to excess lean mass [392]. Studying several different measures of obesity may also provide some insight into the possible mechanisms involved in health effects of school neighbourhoods.

Study V only considers the effect of exposure to swimming pool environment on skin and gut microbiome composition and diversity. However, swimming practice is one of the most popular, practiced and recommended physical activities in children, highlighting the importance of assessing the effect of exposure to swimming pool environment on composition and diversity of swimmers' microbiome. In addition, disinfection products, which are necessary to maintain hygienic conditions and to prevent microbial proliferation that might lead to infectious diseases [393], may also induce changes in human microbiome, playing also a role in the risk associated with exposure to environmental pollutants. The sample size is also a limitation in **study V**; however, this is the first study assessing the effect of an extreme environment such as swimming pool on human microbiome. In humans, many factors can contribute to variations on bacterial communities, such as diet, metabolism, use of antibiotics, age, and health status [174, 394], but our study did not consider the effect of these factors in the assessment of exposure effect to swimming pool environment in skin and gut microbiome. However, our aim was to assess the influence of an indoor swimming pool environment on athletes' microbiome rather than the interaction environment-microbiome-host. Moreover, no significant differences were found between swimmers and non-water athletes' characteristics, and athletes have not reported antibiotic use in last months, being expected that the effects of swimming pool environment were not associated with the characteristics of the athletes that were evaluated. Physical activity has also been recognized as being associated with human microbiome, although the impact of exercise has been variable across studies. Bai *et al.* [395] suggested associations between the frequency of physical activity and gut microbiome composition among children and adolescents,

with daily exercise increasing the diversity of gut microbial composition. In 2014, Clarke *et al.* [396] explored the impact of exercise on the gut microbiome in professional athletes from an international rugby union squad, and reported a higher diversity in athletes, representing more than 20 distinct phyla, compared to controls with high or low BMI. In the present study, we evaluate the effect of an extreme indoor environment on microbiome, comparing two distinct groups of athletes who participate in exercise at a competitive level and over an extended period. O'Donovan *et al.* [397], in 2019, reported no significant differences on microbial diversity based on sports classification groups (SCGs). Samples from elite athletes within the same SCG generally cluster together from both a compositional and functional potential perspective. Authors also found no significant differences between any nutrients or food groups based on SCGs [397], suggesting that the differences in alpha and beta diversity that we have found between swimmers and non-water competitive athletes may be associated with exposure to swimming pool training environment.

In our study, the community of microbiome was assessed using pyrosequenced 16S rRNA gene V3-V4 amplicon data, providing more detailed information on the composition and diversity of the microbiome, and a basis to further investigate the impact of the microbiome on human health. Culture-based approaches are still being employed in some studies [398, 399] despite the incomplete information of the vast diversity found in the human microbiome, namely in the identification of anaerobic bacteria, such as members of the genus Bacteroides and of the phyla Actinobacteria and Firmicutes [400]. The 16S sequences identified are clustered into OTUs according to sequence similarity by using specific PCR primers. This approach allows the description in terms of which OTUs are present, their relative abundance, and/or their phylogenetic relationship [400]. However, 16S rRNA method is limited to bacteria, as parasites, fungi and viruses do not have 16rRNA genes, and are also likely to interact with ecosystem and influence human microbiome; it is also unable to distinguish between 16S rRNA genes that are derived from living versus dead organisms [400, 401]. In addition, the in-depth analysis of samples from different anatomical sites, but related to the same individuals, is an important strength of this study, providing a unique opportunity to study how environmental factors may affect the composition of the human microbiome. Blank and control samples have been used and run in parallel to athletes' samples to identify possible extraction/sequencing contaminants or amplification artefacts. Although contamination of samples collected from low biomass anatomical location, such as skin [183], is a persistent challenge in DNA sequence-based studies [305], all procedures were performed to minimize this contamination.

6.6. Implications for practice and future research

Evidence suggests that environment play a crucial role in asthma and allergic diseases development. The results in the present study imply that both environments levels – indoor environment and neighbourhood could influence the development of asthma, respiratory symptoms, as well as, the development of obesity in schoolchildren. Furthermore, the dynamic and complex interaction between individual and environment may be mediated by the autonomic nervous system. These cross-sectional studies suggest a mechanism other than the Th2 inflammatory pathway linking the environment and asthma, which should be clarified in further prospective studies, in order to get one step closer to assessing causality and investigating the changes over time. Additionally, future research should focus on assessing the cumulative effect on different exposures, namely exposure to different environments including homes, and pollutants, over time. The quality of greenspaces, vegetation types or biodiversity, accessibility of greenspaces and the diversity of the surrounding microbiome should be considered in future studies to understand the interaction between nature, biodiversity and microbiome with our immune system and its health implications.

In addition, a diversity of anthropogenic chemicals used in different domestic applications are resulting in higher concentrations in indoor environments, and their effects on human health are a global concern. However, it is still unclear how indoor environmental chemicals and human microbiome interact and whether these interactions affect human health, namely the development of asthma and obesity in children. Thus, further comprehensive analysis of chemical–human microbiome interactions are still needed to understand how the environment can shape the human microbiome, and the mechanisms by which the microbiome is involved in human health and disease. The association between dysbiosis and several chronic diseases such as asthma, allergy and obesity, underlie extensive interest in identifying factors causing dysbiosis, and in developing strategies to assess the impact of the environment on microbiome composition and diversity for health benefit. Further longitudinal studies are also needed to characterize the human microbiome over time, and consequently its impact on health. Future studies will also need to consider the characteristics of the microbiome from different body sites (e.g. nose and oral samples), as well as the effect of exposure to swimming pool training environment at different moments (e.g. before and 2 hours after swimming training), and the capacity of swimmers to restore their microbiome to a “healthy” state.

Given the number and range of VOC concentrations observed indoors it is important to assess indoor air pollutants, namely VOC identified as endocrine disrupting-chemicals, in order to

determine the potentially attributable health risk and identify the most appropriate risk management strategies. In this context it is important to consider sensitive subpopulations when assessing risk characterization and hazard. Schools are important settings for the implementation of programmes to improve indoor air quality and behaviours that contribute to minimize the effects of EDCs exposure and promoting health and healthy lifestyles. This thesis may contribute to action plans aimed at improving indoor air quality and decreasing EDCs exposure in schools, thereby improving children's health. Exploring the effects of individual or co-exposure to EDCs is crucial for planning and making recommendations to decision makers to prevent the use of materials that could be indoor sources of these compounds, create monitoring programmes to characterize spatiotemporal trends, and create healthier indoor environments, with the potential to decrease the risk of asthma and obesity in childhood. Our study meets the goals of the WHO and the United Nations Environment Programme (UNEP), demonstrating the importance of understanding EDCs, their effects on human health, and policies and scientific evidence for health development and reduce exposures to EDCs. This study also highlights the need of some interventions to reduce indoor EDCs concentrations in schools and consequently the risks of exposure, such as avoiding redecoration, new furniture, air fresheners and perfumed items and increasing ventilation. Further studies are also needed to evaluate the effectiveness of interventions to reduce indoor VOC concentrations.

This thesis further supports the positive health effects of green areas in school neighbourhoods, contributing to the implementation of urban planning policies and practices that may promote a healthy lifestyle and reconnection with nature. Exploring the effects of schools' neighbourhoods is crucial for planning, defining guidelines, and making recommendations to cities planners and decision makers in order to create healthier and sustainable urban environments, with potential to protect citizens against the development of asthma and allergic diseases. Considering the goals of the WHO European Healthy Cities Network, our results demonstrated the need of integrated policies and scientific evidence for health development, public health and urban regeneration to promote and protect human health. Finally, our results support the promotion of urban environment changes, such as introducing or improving existing green spaces (parks, green corridors, urban gardens or green exercise programmes), which would provide opportunities for health improvement and social interactions, thus adding to the additional benefits of green urban areas to the local economies, sustainability and self-sufficiency of cities.

7. Conclusions

As stated before, this study shows that where we are and where we live plays an important role in who we are. This thesis highlights the specific role of indoor environment and of the school neighbourhood as determinants of paediatric asthma, showing that an exposure to emerging indoor pollutants - the endocrine-disrupting compounds – inside the school, and the characteristics of built areas around schools imposed by the growing urbanization and Western lifestyles, may affect children health, namely by increasing the risk of asthma, respiratory symptoms and obesity. Additionally, this thesis underlines the role of the exposure to the swimming pool indoor environment, as an extreme environment, in the composition and diversity of skin and gut microbiome. Based on the results presented in this thesis, the following conclusions can be drawn:

1) Most volatile organic compounds identified indoors are linked to indoor sources, including materials, consumers products, and specific activities and behaviours of the occupants. A seasonal trend in air volatile organic compounds concentration and specific organic compounds was also observed in all the four indoor environments.
2) Both individual exposure and co-exposure to low concentrations of volatile organic endocrine-disrupting compounds in classrooms may affect child health, namely the risk of asthma, the presence of respiratory symptoms in the previous 3 months and obesity.
3) Classroom exposure to individual or combined endocrine-disrupting compounds is associated with changes in the autonomic nervous system, specifically parasympathetic dysautonomia; in fact, endocrine-disrupting compounds exposure may increase parasympathetic activity, resulting in a subsequent increase in the risk of asthma and obesity.
4) Neighbourhoods and walkability around schools may also be determinants of paediatric asthma, by specifically influencing both lung function and the autonomic nervous system activity. Built areas around schools adversely affect lung function but not eosinophilic airway inflammation, underlying the role of autonomic nervous system balance in the interaction between the environment and lung function.
5) Green areas in school neighbourhoods have a positive health effects, namely on the body mass index and body fat percentage of schoolchildren. Thus, promoting healthier cities by the creation of green areas around schools constitutes a valuable opportunity for decreasing children’s exposure to detrimental factors in urban settings and reduce associated health expenses.

6) Indoor swimming training environment may play a role shaping human microbiome, driving skin and gut dysbiosis. The results may suggest that adverse health effects associated with swimming pool attendance, namely asthma, may be underline by changes in microbiome.

8. References

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Annex I: Scientific papers

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A Systematic Review of Evidence and Implications of Spatial and Seasonal Variations of Volatile Organic Compounds (VOC) in Indoor Human Environments

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A Systematic Review of Evidence and Implications of Spatial and Seasonal Variations of Volatile Organic Compounds (VOC) in Indoor Human Environments

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ABSTRACT

Many volatile organic compounds (VOC) are classified as known or possible human carcinogens, irritants, and toxicants, and VOC exposure has been associated with asthma and other respiratory symptoms/diseases. This review summarizes recent quantitative data regarding VOC in four categories of indoor environments (schools, housing, offices, and other indoor) and compares the types and concentration levels of individual VOC that were detected, measured, and reported according to season (cold and warm). The influence of outdoor air on concentrations of indoor VOC was also assessed as ratios of indoor versus outdoor. Papers published from 2000 onward were reviewed and 1383 potentially relevant studies were identified. From these, 177 were removed after duplication, 1176 were excluded for not meeting the review criteria, and 40 were included in this review. On average, higher mean concentrations of indoor VOC were found in housing environments, in offices, and in the cold season. Volatile organic compounds are commonly present in indoor air and specific compounds, and their concentrations vary among indoor environments and seasons, indicating corresponding differences in sources (indoors and outdoors). Actions and policies to reduce VOC exposures, such as improved product labeling and consumer education, are recommended.

Many volatile organic compounds (VOC) are classified as known or possible human carcinogens, irritants, and toxicants (U.S. Environmental Protection Agency, 2012a). In addition, several studies linked VOC exposure to asthma and other respiratory symptoms/diseases (Nurmatov et al. 2015). Valcke and Haddad (2015) demonstrated that human responsiveness to VOC not only was dependent upon chemical concentrations but also needed to consider sensitive subpopulations such as neonates, toddlers, and pregnant women. It is widely recognized that indoor concentrations of many VOC are higher than levels in outdoor air (Annesi-Maesano et al. 2013; Edwards et al. 2001b; Guo et al. 2004; Madureira et al. 2009; 2015a, 2015b). Most exposures to VOC occur indoors, as a result of numerous indoor emission sources, low ventilation rates, and time spent indoors. Important indoor VOC sources include construction materials, furnishing, paints, glues, heating appliances, tobacco smoke, cooking, cleaning products, and pesticides (Annesi-Maesano et al. 2013; Edwards et al. 2001a; Jurvelin 2003; Zhang et al. 2002). Outdoor air also contributes to indoor VOC levels, mainly because of their transport to indoors such as vehicle emissions and proximity to industrial activities the major ambient sources (U.S. Environmental Protection Agency, 2012b; Miller, Xu, and Luginaah 2009).

Based on health outcomes, the wide variety of sources including indoors and outdoors, the type and levels of VOC detected in different indoor environments, and time spent indoors highlight the need to summarize and compare data on VOC types and their levels in various microenvironments within and between indoor and outdoor air, and consequently, enhance available information on exposure to VOC indoors. Beyond the spatial variations (indoor/outdoor) and differences related to each microenvironment, such as homes, schools, offices, hospitals, and

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Supplemental data for this article can be accessed here.

stores, the season when samplings occur also influences the concentration of VOC indoors. Several studies demonstrated a significant seasonal cycle (Kim, Chun, and Jo 2015; Pegas et al. 2011b; Rehwagen, Schlink, and Herbarth 2003), probably due to (1) lower mobility of air in the wintertime, (2) longer persistence of the compounds due to reduced photochemical degradation (Fuselli et al. 2010), and (3) gradual accumulation (Pegas et al. 2011b). The large number of VOC combined with the time that people spend indoors justifies considering indoor air pollution among one of the most significant environmental health risks and emphasizes the importance of characterization of different indoor environments in order to understand the contribution of each on human exposure to VOC.

The purpose of this study was to review, summarize, and compare existing quantitative data concerning spatial (four major human environments: schools, housing, offices, and other indoor) and seasonal variation (cold and warm) of VOC concentrations that were detected, measured, and reported both indoors and outdoors. These data may be useful to many investigators and stakeholders interested in this environmental topic, namely, those responsible for adopting appropriate risk management strategies.

Methods

Search Strategy

Following the 2009 "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) guidelines (Moher et al. 2015), original published studies from 2000 to 2015 were searched through PubMed, Scopus, and Web of Science. The search included the combined terms "volatile organic compounds AND indoor air AND outdoor air." Additionally, a cross-reference check was performed to search for additional potential studies. All studies that fulfilled the following inclusion criteria were considered: published in English; VOC measured in both indoor and outdoor environments; and VOC measured in at least one season. The exclusion criteria were studies reporting modeled or estimated exposure.

Data extraction

Two researchers independently screened titles and abstracts of identified studies, and reviewed the full text of studies meeting the inclusion and exclusion criteria. Disagreements were resolved through discussion between the two researchers. For each publication, the compounds and collected information on VOC concentration by outdoor and indoor environments, season, and data on exposure to tobacco smoke were identified.

Definitions and analysis

Studies that reported mean concentration values for different VOC were considered in data analyses, which was stratified by space (outdoor and indoor environments) and season (cold—winter and fall; warm—summer and spring). All studies that defined the sampling period month(s) and the season (winter, spring, summer and/or fall) or season periods (cold and/or warm) and whose results were presented in accordance with this definition were included in this study and categorized according to the preceding definition—warm and cold season.

Indoor environments comprised four categories: (1) schools, (2) housing such as homes and hotels, (3) offices, and (4) other indoor. This last category included shopping malls, hospitals and dental clinics, restaurants, photocopy centers, and museums. All eligible studies are described in the supplementary file stratified by space and season and with the information on exposure to tobacco smoke, when applicable.

No restrictions were made in the selection of studies according to analytical method that were used for identification and quantification of VOC. In the included studies, the most reported method was gas chromatography coupled with mass spectrometry (GC-MS), which was used in 18 studies, followed by gas chromatography coupled with flame ionization detector (GC-FID) (11 studies), whereas only 6 studies used gas chromatography coupled with mass selective detector (GC-MSD) and only 1 used gas chromatography coupled with an electron capture detector (GC-ECD). Some studies (three) reported the use of high-performance liquid chromatography (HPLC).

Distribution of quantitative variables was determined using the Shapiro–Wilk test, and the Mann–Whitney or Kruskal–Wallis test was used to test comparisons between VOC concentrations, indoor and outdoor, and seasons for the four indoor environments. All of the analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 17.0, and the level of statistical significance was set at .05.

Results

Individual VOC in different indoor environments

Initially, 1393 papers were retrieved from the search, including overlapping publications across the three databases. After removal of duplicates (177), 1123 papers were excluded by title and abstract screening. Another 53 papers were removed after assessment of the full text, as these did not meet inclusion criteria (Table 1). Figure 1 shows the number of studies identified and included/excluded. Ultimately, 40 papers were included in this review and are described in detail in the supplementary file. Twelve studies were conducted at schools, 21 in housing environments, 4 in offices, and 7 in other indoor environments, between 1996 and 2014 (Table 2).

At schools, 33 VOC were measured and reported, of which 18 were measured in both seasons, with 12 exclusively in the warm and only 3 exclusively in the cold season. Higher mean concentrations for specific VOC, with measurements in both seasons (10 of 18), were found during the cold season (Figure 2). The concentrations ranged between nondetectable and 160 μg/m^3; the highest level was noted for *m/p*-xylene and the lowest for various specific VOC with both values during cold season (supplementary file). A greater number of specific VOC were identified in housing environments (81), mostly measured in both seasons (74), with 6 measured in the warm and 1 in the cold season (tetrachlorocarbon).

In contrast to schools, the highest mean concentrations for specific VOC were identified in the warm season; however, the highest levels detected in the cold season were for toluene (293 μg/m^3) (Figure 3). The concentrations ranged between nondetectable and 293 μg/m^3, being higher for toluene (cold season) and lower for various specific VOC (supplementary file).

In offices, 20 VOC were measured in both seasons (14), with 4 in the cold and the remainder in the warm season (2). Similar to housing environments,

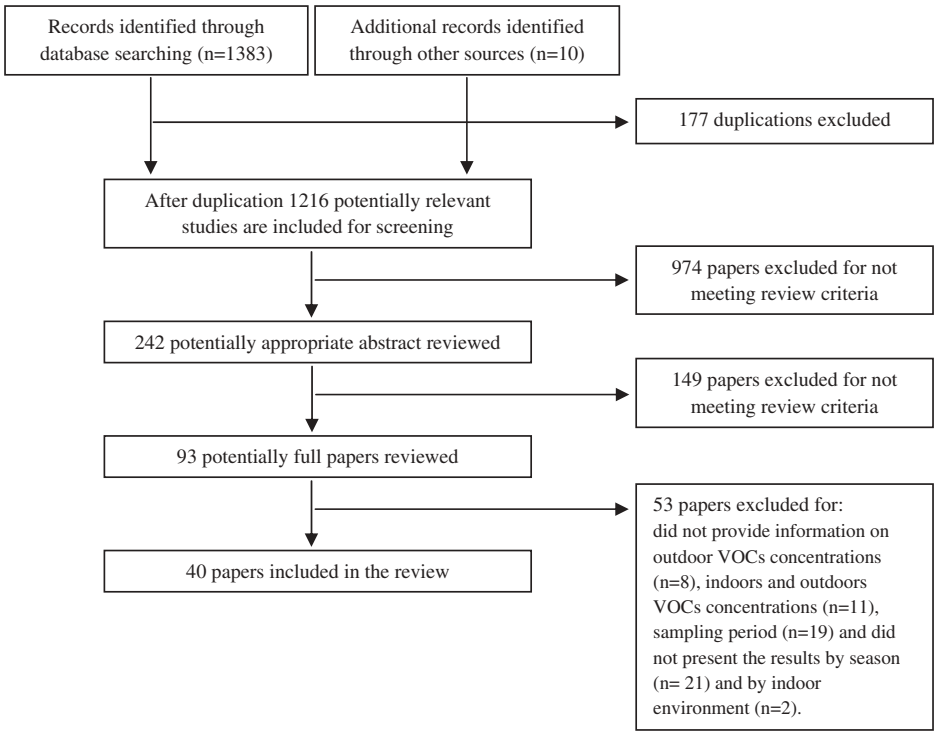

Figure 1. Systematic review flowchart.

Table 1. Excluded studies.

Reference	Year	Country	Indoor environment (n)	Season	Analytical method	Reasons for exclusion
Batterman et al., 2006	–	USA	Homes (1)	–	GC-MS	Did not provide information about sampling period
Batterman et al., 2007	–	USA	Homes (15)	–	GC-MS	
Caselli et al., 2009	–	Italy	Newspaper stands (16)	–	GC-MS	
de Gennaro et al., 2013	–	Italy	8 schools	–	TD-GC-MS	
Elkilani and Bouhamra, 2001	–	Kuwait	Homes (10)	–	GC	
Geiss et al., 2011	–	Italy	Car showrooms	–	GC-FID	
Guo et al., 2003	–	Hong Kong	Restaurants (4), schools (6), offices (6), shopping malls (6) and homes (6)	–	GC-MS	
Lerner et al., 2012	–	Argentina	Enterprise (laboratories, sewing workrooms, electromechanical repair and car painting centers, food shops and photocopy center)	–	GC-FID	
Pandit et al., 2001	–	India	Homes (3)	–	GC-FID	
Phillips et al., 2005	–	USA	Homes (42)	–	GC-MS	
Shin and Jo, 2013	–	Korea	Homes (25)	–	GC-MS	
Shin and Jo, 2014	–	Korea	Homes (107)	–	GC-FID GC-MS	
Srivastava et al., 2000	–	India	Offices (1)	–	GC-FID	
Yoon et al., 2011	–	Korea	Schools (17)	–	GC-MSD	
Yoon et al., 2011	–	Korea	17 preschools	–	GC-MSD GC-NPD	
Batterman et al., 2012	2004, 2005, 2010	USA	Homes (288)	Winter, summer, fall, spring	GC-MS	Did not provide information by season
Canha et al., 2015	2010	France	Schools (17)	–	GC-FID	
Cheng et al., 2015	2008, 2009	Australia	Homes (40)	–	GC-MS GC-FID HPLC	
Du et al., 2015	2011-2012	USA	Homes (74)	–	GC-MS	
Duan et al., 2014	2011, 2012	China	50 homes	Heating and non-heating season	GC-MSD GC-FID	
Edwards et al., 2001	1996-1997	Finland	201 microenvironments: homes and workplace	Fall 1996 to winter 1997	GC-FID GC-MS	
Eklund et al., 2008	2003-2005	USA	Shopping center (1)	–	GC-MS	
Fuselli et al., 2010	2007-2009	Italy	Offices (1)	–	GC-MS	
Gallego et al., 2008	2000, 2001	Spain	7 public buildings; 54 homes	Spring-summer and winter of 2000; and summer and winter of 2001	GC-FID	
Hinwood et al., 2006	2000	Australia	27 microenvironments: homes, offices, restaurants and nightclubs	–	GC-MS	
Jia et al., 2010	2005, 2006	USA	10 commercial or industrial buildings	–	GC-MS	
Jia et al., 2012	2004, 2005	USA	Homes (162)	Summer, winter, fall and spring	GC-MS	
Jung et al., 2011	2006, 2007	USA	Homes (15)	Summer and winter	GC-MS	
Lee et al., 2002a	–	Hong Kong	Restaurants (4), schools (10), offices (10), shopping malls (9), homes (6)	–	GC-MS	

(*Continued*)

Table 1. (Continued).

Reference	Year	Country	Indoor environment (n)	Season	Analytical method	Reasons for exclusion
Lu et al., 2007	2003-2004	Taiwan	Offices (86)	–	Photo-ionization detector (PID) for real-time monitoring of VOCs	
Mishra et al., 2015	2010-2012	Australia	25 schools	–	GC-MS	
Payne-Sturges et al., 2004	2000-2001	USA	Homes (37)	–	GC-MS	
Rehwagen et al., 2003	1994-2001	Germany	Homes (1499 indoor and 222 outdoor measurements)	–	GC-FID/ECD	
Sax et al., 2006	1999, 2000	USA	Homes (121)	Winter, summer, fall	GC-MSD	
Serrano-Trespalacios et al., 2004	1998, 1999	Mexico	Homes (30)	–	GC-MS	
Sexton et al., 2004	1999	USA	Homes	Spring, summer and fall	GC-MS	
Shinohara et al., 2013	2011-2012	Japan	Homes (19)	–	GC-MS	
Topp et al., 2004	1995-1997, 1996-1998	Germany	Homes (631)	–	GC-FID	
Chin et al., 2014	2010	USA	Homes (126)	Spring, summer, fall and winter	GC-MS	Did not provide information on outdoor concentrations
Delgado-Saborit et al., 2011	2005-2007	UK	Homes (155), workplaces (40)	–	GC-MS	
Jo and Kim, 2010	2009	Korea	Schools (1)	Summer	GC-MS	
Sohn et al., 2009	2004	Korea	Schools (55)	Summer, autumn and winter	GC-FID	
de Blas et al., 2012	2008	Spain	1 school	Winter	GC-MS GC-FID	Did not provide information by indoor and outdoor
Dodson et al., 2009	2000, 2004, 2005	USA	Homes (95)	–	GC-MS HPLC	
Massolo et al., 2010	2000-2002	Argentine	Kindergartens (92) and homes (92)	Winter	GC-MS	Did not provide information by indoor environment
Sakai et al., 2009	2004-2007	Japan	67 rooms from 56 buildings [Offices (53), shops (7), classrooms (3), library (2), conference hall (2)]	Summer and winter	GC-MS	
Walgraeve et al., 2011	2007-2009	Belgium	Homes (6)	–	GC-MS	Did not provide information by indoor and outdoor Did not provide information by season
Tham et al., 2004	–	Singapore	Offices (1)	–	GC	Did not provide information on outdoor concentrations Did not provide information about sampling period

(*Continued*)

Table 1. (Continued).

Reference	Year	Country	Indoor environment (n)	Season	Analytical method	Reasons for exclusion
Ongwandee et al., 2011	2009	Thailand	Offices (17)	–	GC-MS	Did not provide quantitative results by indoor and outdoor
Sexton et al., 2007	1999	USA	70 microenvironments: homes, work/school, other locations (grocery, stores, restaurants, shopping malls)	Spring, summer and fall	GC-MS	
Xiong et al., 2015	2003-2007	USA	Homes (17)	–	GC-MS	Did not provide information by season

GC-MS: Gas chromatography coupled with mass spectrometry; GC-FID: Gas chromatography coupled with flame ionization detector; GC-MSD: Gas chromatography coupled with mass selective detector; GC-ECD: Gas chromatography coupled with an electron capture detector; GC-NPD: Gas chromatography coupled with nitrogen phosphorus detector; TD-GC-MS: thermal desorption-gGas chromatography coupled with mass spectrometry

Table 2. Included studies.

Reference	Year	Country	Indoor environment (n)	Season	Analytical method	Seasonal variation
Adgate et al., 2004a	2000	USA	Homes (113) and schools (113)	Winter and spring	GC-MS	In schools and in homes, most VOCs were more frequently detectable and at higher concentrations in winter compared with spring
Adgate et al., 2004b	1997	USA	Homes (284)	Warm season	GC-MS	–
Bae et al., 2004	2001-2002	Korea	Shopping malls (32)	Cold season	GC-MS	–
Baez et al., 2003	1996, 1997, 1998	Mexico	Homes (2), Museums (2) and Offices (2)	Warm and cold season	HPLC	–
Chan et al., 2009	2006	China	Hotels (8)	Winter	GC-MS	–
Feng et al., 2004	2002	China	Hotels (4)	Warm season	HPLC	–
Fischer et al., 2000	1995	Netherlands	Homes ()	Cold season	GC	–
Godoi et al., 2009	2005	Brazil	Schools (2)	Cold season	GC-MS	–
Godwin and Batterman, 2007	2003	USA	Schools (29)	Warm season	GC-MS	–
Gokhale et al., 2008	2005	Germany	Homes (7)	Warm season	–	–
Guo et al., 2013	2007	China	Homes (59)	Warm season	GC-MS	–
Jia et al., 2008	2004, 2005	USA	Homes (159)	Summer, winter, fall and spring	GC-MS	Seasonal effects on indoor levels were inconsistent
Jo et al., 2003	2011	Korea	Homes (86)	Spring	GC-FID	–
Jo et al., 2004	2000	Korea	Homes (443)	Cold season	GC-FID	–
Kinney et al., 2002	1999	USA	Homes (46)	Winter and summer	GC-MS	There was a tendency for I/O ratios to be lower (closer to 1) in summer than in winter
Klinmalee et al., 2009	2005, 2006	Thailand	Schools (1)	Cold season	GC-FID	–
Kumar et al., 2014a	2011-2012	India	Library (1)	Winter and summer	GC-FID	Mean concentrations of TVOC, toluene were higher in winter
Kumar et al., 2014b	2011	India	Homes (27) Hostels (45)	Warm season	GC-FID	–
Larroque et al., 2006	2015	France	Schools (2)	Warm season	GC-MS	–
Lee et al., 2001	2000	Hong Kong	Restaurants (4)	Warm season	GC-MS	–
Lee et al., 2002b	1999	Hong Kong	Homes (6)	Warm season	GC-MS	–
Lu et al., 2006	2004	China	Hospitals (4)	Warm season	GC-MSD	–
Ohura et al., 2006	2001	Japan	Homes (46)	Winter and summer	GC-ECD	The concentrations of aromatic hydrocarbons and volatile organic halogenated compounds substantially increased in winter

(*Continued*)

Table 2. (Continued).

Reference	Year	Country	Indoor environment (n)	Season	Analytical method	Seasonal variation
Ohura et al., 2009	2006, 2007	Japan and China	Homes (57 in Japan, 14 in China)	Summer and winter	GC-MSD GC-FID	In Japan, the concentrations of VOCs indoors were higher in winter. In China, higher concentrations were observed in summer.
Pegas et al., 2011a	2009	Portugal	Schools (14)	Warm season	GC-FID	–
Pegas et al., 2011b	2009, 2010	Portugal	Schools (14)	Winter, spring, autumn	GC-FID HPLC	For VOC, highest concentrations occurred during the coldest months. Formaldehyde concentrations in spring were higher than those in colder months.
Pekey and Arslanbas, 2008	2006, 2007	Turkey	Homes (15), schools (3), offices (10)	Winter and summer	GC-FID	There is a general increase of nearly all the compounds measured from summer to winter. For nearly all compounds, indoor VOC concentrations were significantly greater in winter than in summer.
Rios et al., 2009	2003	Brazil	Offices (2 buildings)	Winter	GC-FID, GC-MS	–
Roda et al., 2011	2003	France	Schools (28)	Cold season and hot season	GC-MS	No seasonal differences was identified
Sakai et al., 2004	1998	Japan and Sweden	Homes (37 in Japan, 27 in Sweden)	Winter	GC-ECD	–
Santarsiero et al., 2009	–	Italy	Dental clinic (1)	Warm season	GC-FID HPLC	–
Sarkhosh et al., 2012	2010, 2011	Iran	Photocopy centres (4)	Winter and spring	GC-MSD	The indoor VOC concentrations were higher in winter than in spring
Sax et al., 2004	1999 and 2000	USA	Homes (87)	Winter, summer and fall	GC-MSD	Mean concentrations of acetaldehyde, hexaldehyde, propionaldehyde, styrene, toluene, trichloroethylene, benzene, ethylbenzene, o-xylene, m/p-xylene, tetrachloroethylene, 1,1,1-trichloroethane were significantly higher in winter. Mean concentrations of formaldehyde were significantly higher in summer.
Sofuoglu et al., 2011		Turkey	Schools (3)	Fall, winter and spring	GC-MS	–
Son et al., 2003	2001	Korea	Homes (60)	Warm season	GC-MS	–
Tang et al., 2005	2002	China	Shopping malls (1)	Winter	GC-MSD	–
Tovalin-Ahumada and Whitehead, 2007	2002	Mexico	Office (33)	Warm season	GC-MS	–
Uchiyama et al., 2015	2011-2014	Japan	Homes (602)	Winter and summer	GC-MS	Almost all compounds were present at higher levels in summer than in winter
Ullrich et al., 2002	2001-2002	Germany	Schools (44)	Winter	GC-MS	–
Zhou et al., 2011	2008	China	Homes (10) Offices (6)	Summer	GC-MSD	–

GC-MS: Gas chromatography coupled with mass spectrometry; GC-FID: Gas chromatography coupled with flame ionization detector; GC-MSD: Gas chromatography coupled with mass selective detector; GC-ECD: Gas chromatography coupled with an electron capture detector; VOC: total volatile organic compounds;

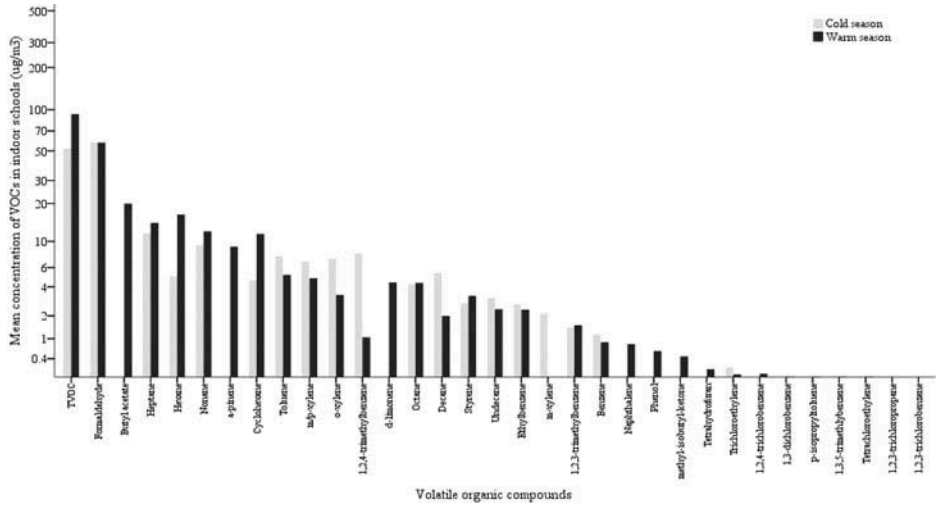

Figure 2. Comparison of VOCs mean concentrations measured in schools in cold and warm season.

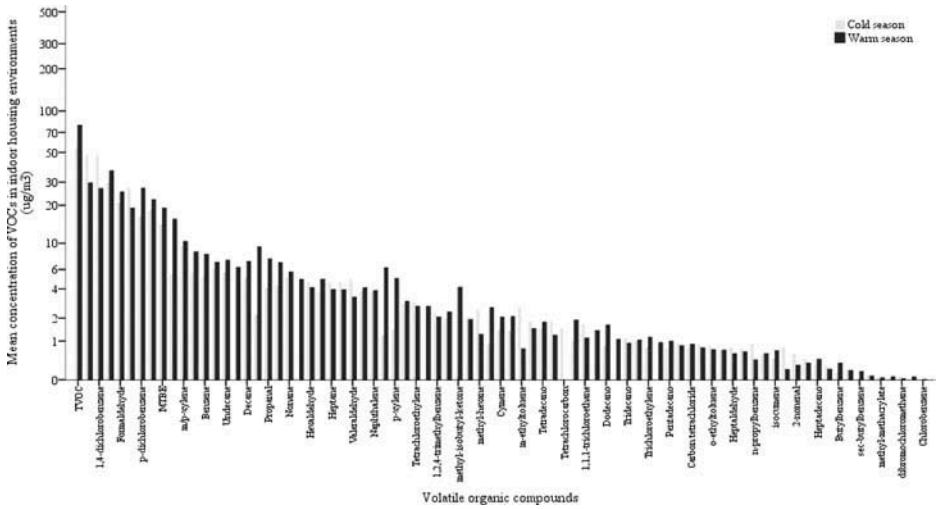

Figure 3. Comparison of VOCs mean concentrations measured in housing environments in cold season and warm season.

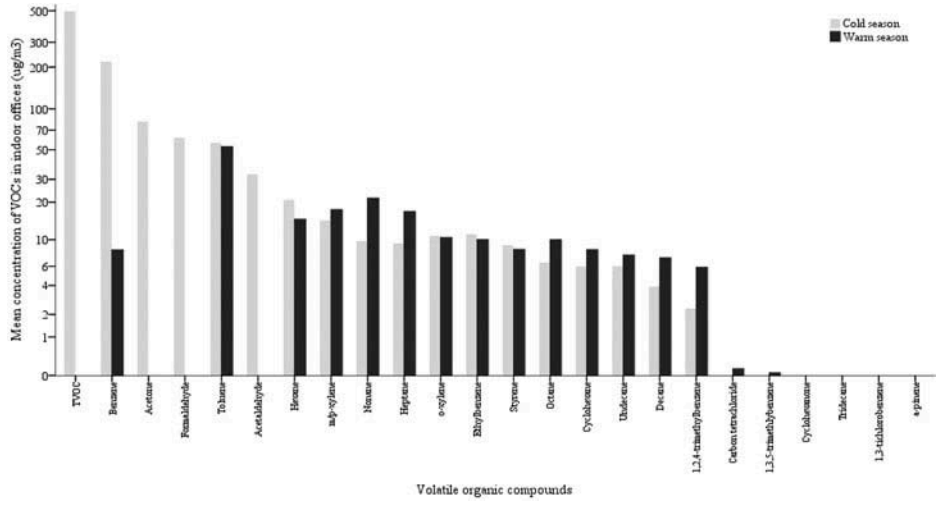

Figure 4. Comparison of VOCs mean concentrations measured in offices in cold season and warm season.

concentrations of most specific VOC, with measurements in both seasons, were higher in the warm season (Figure 4). The levels ranged between 0.05 μg/m^3 and 532 μg/m^3, higher for benzene in the cold (404 μg/m^3) and lower for 1,2,3-trimethylbenzene in the warm season (supplementary file).

In other indoor environments, including commercial spaces, hospitals, restaurants, dental settings, photocopy centers, and museums considered in the analysis, 26 VOC were measured and reported, with 11 present in both seasons, 13 in the cold and only 2 in the warm season (benzaldehyde and carbon tetrachloride). For most VOC the concentration was higher in the cold compared with the warm season (Figure 5). The levels ranged between 7.32 × 10^{-4} μg/m^3 and 185.1 μg/m^3, which were observed in the cold season (benzene and toluene, respectively) (supplementary file).

There were six VOC common to the four previously described indoor environments and determined in both seasons. The highest concentrations were observed in cold season and in offices (benzene [334 μg/m^3], *o*-xylene [10.8 μg/m^3], and styrene [9 μg/m^3]), in other indoor environments (*m/p*-xylene [38 μg/m^3]), and in housing environments (toluene [171 μg/m^3]) (Figures 2–5). Only for ethylbenzene were there found higher concentrations in the warm season (20 μg/m^3) in offices. When comparing all individual VOC for each different indoor environment, no marked differences were noted between VOC concentrations and seasons. When considering the VOC that were common to the four indoor environments (schools, housing, offices, and other indoor), significant differences were also not observed for the seasons.

The geographical assessment was analyzed by dividing data into continents: Europe, Asia, and America. In the cold season, higher mean concentrations of benzene indoors were found in offices in America; for the remaining five VOC higher mean concentrations indoors were observed in Asia (ethylbenzene, toluene, and *m/p*-xylene in other indoor environments, *o*-xylene in offices, and styrene in housing). In the warm season, for most indoor VOC (benzene, ethylbenzene, toluene, and *m/p*-xylene, and styrene) higher mean concentrations were detected in Asian schools; for *o*-xylene higher mean concentrations were found in Asian offices. However, no significant differences were observed for the continents (Figures 1S and 2S, supplementary file).

Seasonal variations in indoor/outdoor ratio

The indoor/outdoor (I/O) ratio was estimated for each environment by season (Figures 6–9) in order to determine the origin of possible VOC sources. In schools there were 31 measured compounds common to both indoor and outdoor environments and the values ranged between 0.51 (1,2,4-trimethylbenzene) and 23 in the cold season (methyl-isobutyl-ketone). Based on 17 specific VOC and comparing cold and warm seasons, the I/O ratio was higher than unity for the majority of

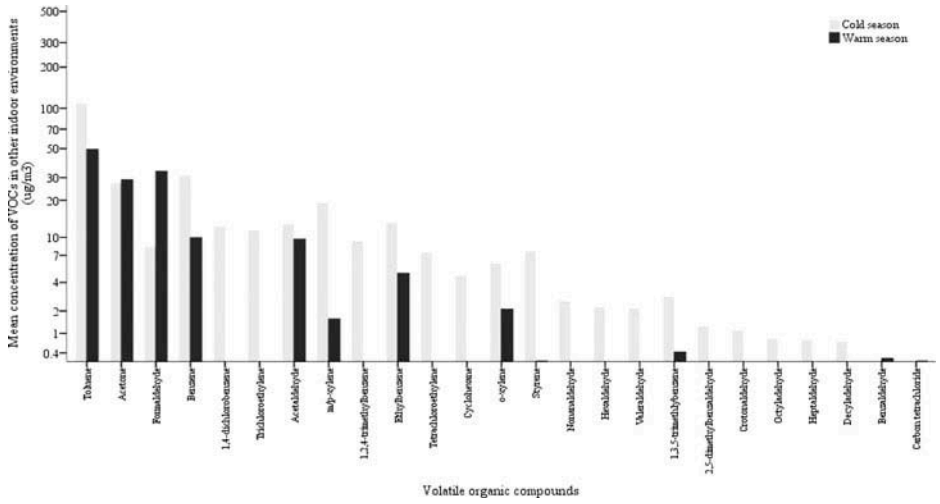

Figure 5. Comparison of VOCs mean concentrations measured in other indoor environments in cold season and warm season.

VOC in both seasons (10 of 17), but mostly higher in the cold season; only 3 VOC (benzene, trichloroethylene and hexane) displayed values lower than 1. For the remaining VOC with information just for one season (1 in the cold and 13 in the warm season) the I/O values ranged from 1 (1,2,3-trichlorobenzene and 1,2,3-trichloropropane) to 23 (methylisobutyl ketone) (Figure 6).

In the housing environments a greater number of VOC (71) with both indoor and outdoor concentrations were identified. There were in total 61 VOC reported in both seasons, and for most of these the I/O was higher than 1 (57) and higher in the warm season. Four VOC presented in one of the seasons an I/O ratio lower than 1 (*o*-xylene, tetrachloroethylene, and *p*-xylene in the warm season [0.93, 0.91, and 0.31, respectively] and carbon tetrachloride in the cold season [0.87]). For VOC identified in only one season (10), 9 of them presented an I/O higher than 1 (Figure 7).

There were 19 measured VOC common to both indoor and outdoor office environments. Fourteen of them had values of I/O for both seasons and mostly higher than 1 (11 compounds all in the warm season). Only four VOC presented I/O values for warm or cold seasons, of which two had an I/O higher indoors (Figure 8).

In other indoor environments (Figure 9) and for VOC with I/O for both seasons (8), only 2 presented values lower than 1 (formaldehyde in the cold season [0.79] and *p*-xylene in the warm season [0.76]). Higher I/O values were observed in the cold season

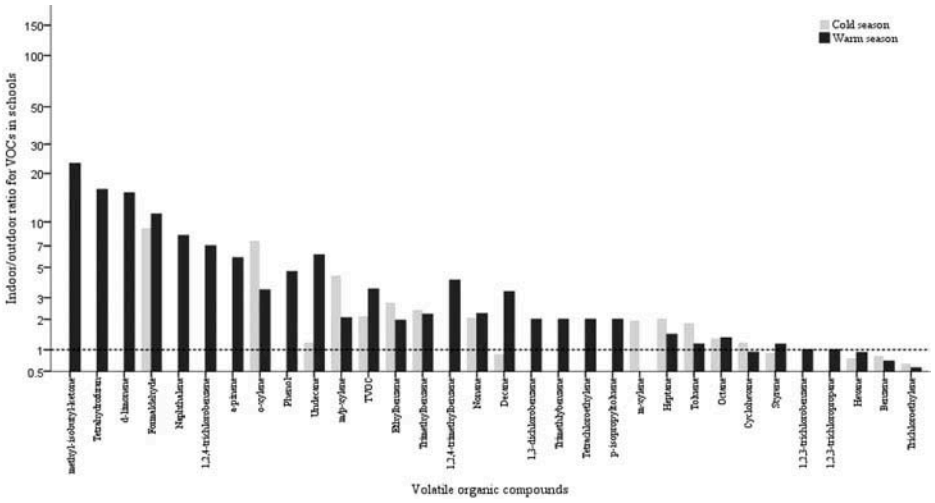

Figure 6. Comparison of indoor/outdoor ratio of VOCs mean concentrations measured in schools in cold season and warm season.

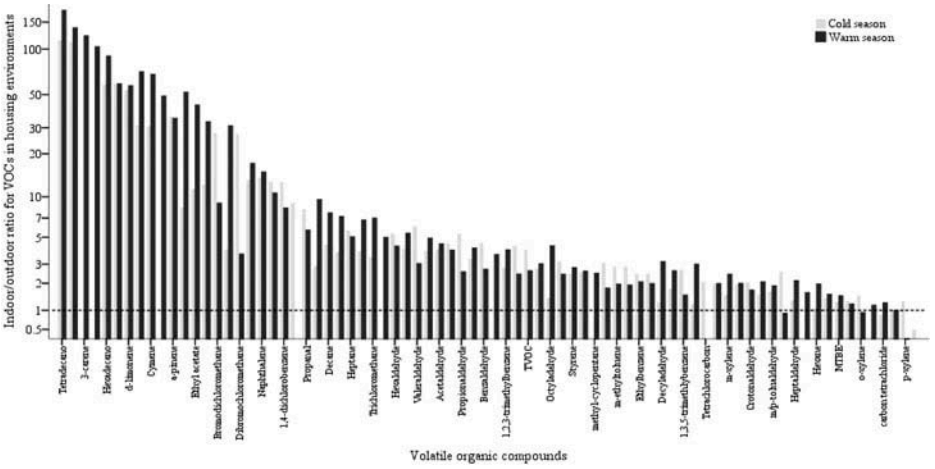

Figure 7. Comparison of indoor/outdoor ratio of VOCs mean concentrations measured in housing environments in cold season and warm season.

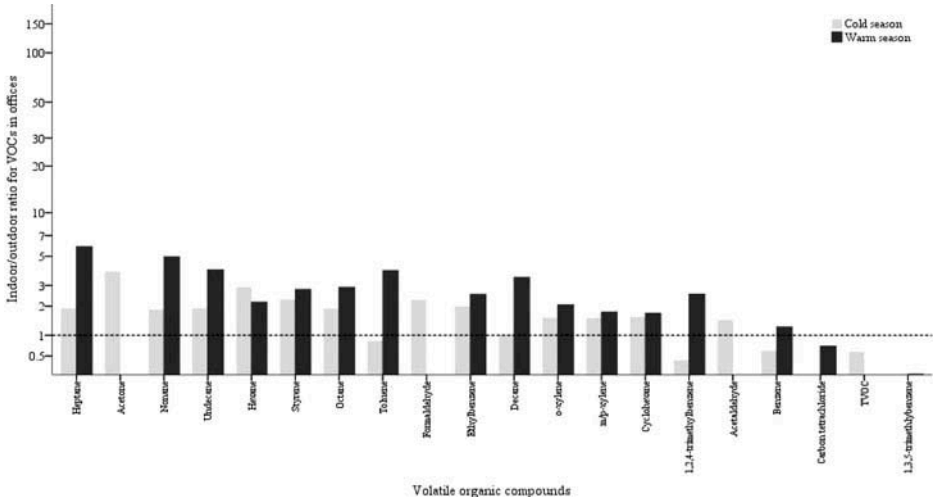

Figure 8. Comparison of indoor/outdoor ratio of VOCs mean concentrations measured in offices in cold season and warm season.

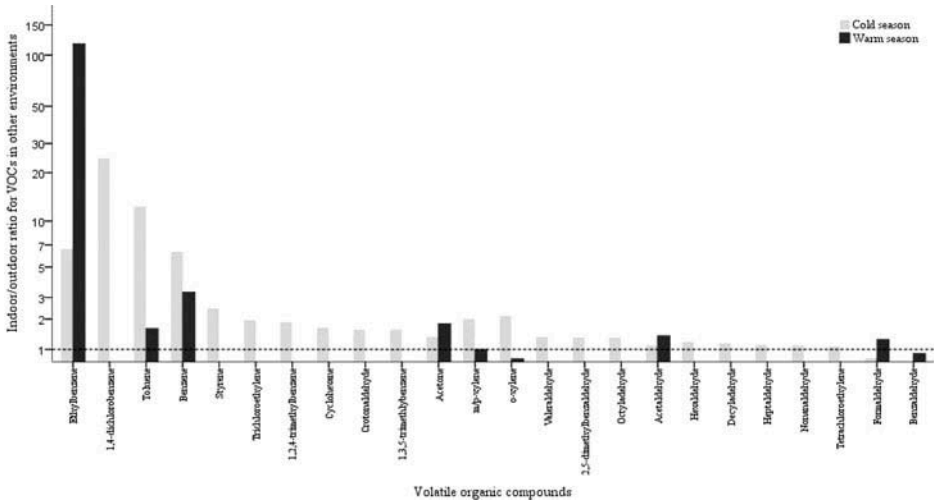

Figure 9. Comparison of indoor/outdoor ratio of VOCs mean concentrations measured in other indoor environments in cold season and warm season.

for most VOC. From the VOC with measurements in a single season (16 compounds) only I/O for formaldehyde was lower than 1 (0.89 in the warm season).

In housing environments, unlike the other three indoor environments, there was a significant difference between VOC levels indoors and outdoors considering the analysis by season (Figure 7). Concentrations of formaldehyde, 1,2,3-trimethylbenzene, and acetaldehyde were significantly higher indoors than outdoors for both seasons. Indoor concentrations of ethylbenzene and 1,3,5-trimethylbenzene were significantly higher only in the cold season. Higher concentrations of toluene, styrene, heptane, nonane, decane, and 1,2,4-trimethylbenzene were also observed indoors, but significantly elevated only in the warm season.

In the cold season, highest levels for outdoors were observed in Asia (styrene in schools, ethylbenzene and *o*-xylene in offices, and *m*/*p*-xylene in other environments) and America (benzene and toluene in offices); in the warm season, higher concentrations for all six VOC were observed in Asian schools (Figures 3S and 4S, supplementary file). However, no marked differences were noted for the continents for both seasons.

Discussion

This review summarized and compared existing data on type and levels of individual VOC detected, measured, and reported both indoors and outdoors by season in four major indoor environments (schools,

housing, offices, and other indoor). As observed, VOC are commonly found in indoor air, and the specific compounds and their concentrations vary between indoor environments and season. In general, the collected data indicated that levels of indoor VOC were higher in housing environments and offices. A higher number of compounds were also identified at housing environments. This might be due to several indoor sources in both environments, and activities and behaviors of occupants in the respective buildings, especially in housing environments. Unlike offices where occupational exposure limits need to be respected, no regulatory limit or public health objective has been set for residential indoor spaces, which may also contribute to higher VOC concentrations. According to Symanski et al. (2009), housing environments are affected by one or more potential sources of VOC, including the use of consumer products (household cleaners, air freshness, deodorizers, personal care products, waxes, and pesticides), tobacco smoke, cooking, and heating appliances.

The VOC identified in each environment, especially indoors, and their concentrations varied considerably among the studies. This variation may be attributed to differences in sampling methods and geographic settings, as well as fluctuations in air exchange rates, occupants' behaviors, and outdoor air concentrations. The large range of levels among the studies reviewed supports the use of concentration distributions rather than a single measure of distribution to characterize concentrations in indoor air.

In the four indoor environments considered in the current review, the mean concentration of most of these compounds was higher in the cold season, showing that there is a time variation of VOC composition and level during different seasons and providing information regarding the influence on indoor air quality from occupants and their behaviors and from climatic conditions. It might be due to lower ventilation, because during the cold season, most of the time windows or doors are closed, and lower air exchange rates contribute to a gradual accumulation of VOC (De Blas et al. 2012; Madureira et al. 2016).

As observed, geographical differences (like local sources, culturally related human behavior, and climate) may affect the concentrations of VOC outdoors and indoors. Although no significant differences were found between the VOC concentrations by continents and for both seasons, weather patterns in each region/country may exert an important impact on the outdoor and indoor concentrations, due to changing human behavior related to weather circumstances, opening windows during warm or cold days, and heating habits (e.g., wood vs. gas heating) (Ballesta et al. 2006; Ilgen et al. 2001). Sarigiannis et al. (2011) also reported a variation in VOC concentrations indoors in different geographical locations in Europe. Sarigiannis et al. (2011) found higher concentrations of formaldehyde in northern Europe than in the south, especially in homes, which was explained by different ventilation schemes between north and south, due to the differing climatic conditions, resulting in lower air exchange between indoor and outdoor environment, as well as with furniture and building materials in the European north. For benzene and toluene the same study demonstrated a greater influence of outdoor sources (such as transport) on indoor air concentrations in the south than in the north, because of the higher outdoor-to-indoor air penetration observed due to the different climates (warmer in the south).

The I/O ratios were larger than 1 for nearly all VOC identified in different environments, suggesting that indoor sources and poor ventilation provide a greater contribution to the levels of VOC indoors. However, for some VOC, an I/O close to or below 1 was found, indicating that indoor concentrations were affected by outdoor air. Outdoor emission sources, such as traffic and industries (which strongly depend on the degree of urbanization), and outdoor-to-indoor air penetration also supply an important contribution to VOC and levels observed indoors.

The outdoors VOC concentrations for the different continents may be explained, in addition to different traffic patterns and industries, by meteorological conditions. For Europe, Cocheo et al. (2000) noted an increase in annual average outdoor VOC concentrations from north to south, which was attributed to differences in prevailing meteorological conditions, such as local wind speeds. Lower wind speeds have been associated with less dispersion and higher outdoor concentrations in the region (Miller, Xu, and Luginaah 2009; Sari et al. 2014).

Some interventions need to be made to reduce indoor VOC concentrations and consequently the risks of exposure, such as avoiding redecoration, new furniture, air fresheners, and perfumed items, and increasing ventilation (Annesi-Maesano et al. 2013; Dales et al. 2004) and banning tobacco smoke at home (Brajenovic, Karaconji, and Bulog 2015). Low-VOC-emission materials and consumer products, decrease in number of students in classrooms, and avoiding use of air cleaners, incense, and candles (Lim et al. 2014; Pegas et al. 2011b) might also be considered as interventions in the different indoor environments. Further studies are also needed to evaluate the effectiveness of interventions to reduce the VOC concentrations.

The main limitations of this review are (i) number of assessed individual VOC; (ii) distribution of samples collected in each environment; (iii) amount of studies that reported both indoor and outdoor VOC concentrations in various seasons; and (iv) different methodologies used for sampling VOC. A further limitation of our review is the combined analysis of the results without considering tobacco smoke exposure. However, and regarding housing, offices, and other indoor environments, few studies presented results by exposure to tobacco smoke and some did not report whether the occupants smoked indoors.

The overview of the results indicated that for some VOC a wide variability is observed, due to the different source characteristics. Thus, their presence and concentrations are limited to each indoor environment, season, and geographical location, where socioeconomic, regulatory, or consumer behavior greatly affects indoor air quality. For some VOC, strong outdoor emission sources and outdoor-to-indoor air penetration might have a more significant contribution than indoor sources, making source apportionment even more complicated (Sarigiannis et al. 2011).

This review provides an overview of VOC concentrations and compounds in 4 major indoor environments (schools, housing, offices and other indoor) over the last 20 years. Comparisons of results by season and ratios I/O support the importance of sampling VOC throughout the year and also confirm that indoor sources (in both seasons) play a major role in the exposure to VOC.

Conclusions

An overview of the results indicated that for some contaminants a wide variation was observed due to different source characteristics. This review confirmed that most VOC identified indoors are linked to indoor sources and to specific activities and behaviors of the occupants. A seasonal cycle in VOC concentration and compounds was also identified in all indoor environments. Given the number and range of VOC concentrations observed indoors, it is important to assess indoor air pollutants, namely, VOC, in order to determine the potentially attributable health risk and to identify the most appropriate risk management strategies. In this context it is important to consider sensitive subpopulations when assessing risk characterization and hazard (Valcke and Haddad 2015). It is noteworthy that actions and policies to reduce VOC exposures, such as improved product labeling and consumer education, are recommended.

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ORIGINAL ARTICLE

Asthma and Lower Airway Disease

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Exposure to indoor endocrine-disrupting chemicals and childhood asthma and obesity

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Abstract

Background: Indoor air contaminants may act as endocrine-disrupting chemicals (EDCs). However, to what extent these contaminants affect health is poorly known. We aimed to assess the association between EDCs exposure and asthma, respiratory symptoms and obesity in schoolchildren.

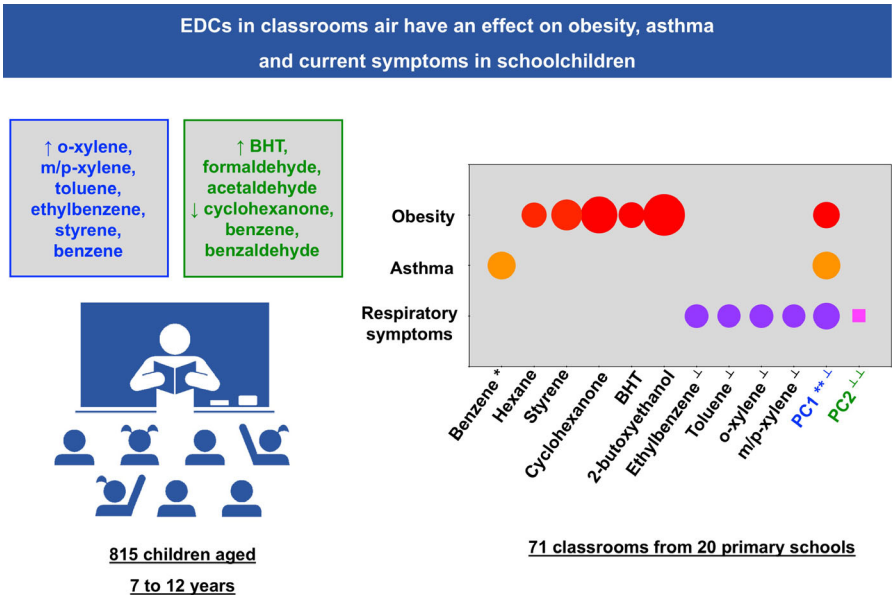
Methods: Data from a cross-sectional analysis of 815 participants from 20 schools in Porto, Portugal, were analysed. Symptoms were assessed, asthma was defined on lung function, and airway reversibility and body mass index (BMI) were calculated. The concentrations of 13 volatile organic compounds and 2 aldehydes identified as EDCs were measured in 71 classrooms throughout 1 week. Principal component analysis (PCA) was used to assess the effect of co-exposure. Associations were estimated by regression coefficients using linear and logistic regression models.

Results: Increased individual and combined EDCs levels were found in classrooms having more children with asthma and obesity. Higher levels of hexane, styrene, cyclohexanone, butylated hydroxytoluene and 2-butoxyethanol were associated with obesity, and higher levels of cyclohexanone were associated with increased child BMI. Toluene, o-xylene, m/p-xylene and ethylbenzene were significantly associated with nasal obstruction. A positive association was found between PC1 and the risk of obese asthma (OR = 1.43, 95% CI 1.01, 1.98) and between PC2 and overweight (OR = 1.51, 95% CI 1.28, 1.79). PC1 and PC2 were also associated with nasal obstruction, and PC2 was associated with breathing difficulties and lean body mass, although EDCs concentrations were low.

Conclusions: Our findings further support the role of EDCs in asthma and obesity development. Moreover, even low levels of indoor exposure may influence the risk of asthma, respiratory symptoms and obesity.

KEYWORDS
asthma, children, endocrine-disrupting chemicals, indoor air, obesity

Abbreviations: ACV, average constriction velocity; ADV, average dilation velocity; ATS, American Thoracic Society; BHT, butylated hydroxytoluene; BMI, body mass index; CDC, Centres for Disease Control and Prevention; CI, confidence interval; EBC, exhaled breath condensate; EDCs, endocrine-disrupting compounds; ERS, European Respiratory Society; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IOTF, International Obesity Task Force; MCV, maximum constriction velocity; NO, nitric oxide; PCA, principal component analysis; PC, principal component; SPT, skin prick test; T4CE, 4-chloroethylene; T75, total time taken by the pupil to recover 75% of its initial resting diameter after it reached the peak of constriction; TRPA1, transient receptor potential ankyrin; TRP, transient receptor potential; TRPV1, transient receptor potential vanilloid type 1; VOC, volatile organic compounds; WHO, World Health Organization.



GRAPHICAL ABSTRACT

Exposure to low levels of EDCs have an effect on asthma, current symptoms and obesity in school-age children. Individual or combined EDCs also associate with ANS changes, that may possibly mediate the interaction between EDCs and childhood asthma and obesity. Our findings may contribute to action plans to reduce exposures to EDCs and to promote a healthy indoor school environment.

ANS: autonomic nervous system; BHT: butylated hydroxytoluene; EDCs: endocrine-disrupting chemicals;

The circles represent the Odds ratio (OR) values, being the size proportional do the OR.

Circles: OR <1; square: OR >1.

*Positive bronchodilatation; **Obese asthma; ⊥ Nasal obstruction; ⊥⊥ Breathing difficulties

1 | INTRODUCTION

Growing urbanization and the rapid development of new building materials and consumer products have resulted in a changing pattern in the production and use of chemical compounds and consequently in an increase in of these compounds indoors.[1] Some chemicals used in building materials, furnishings and consumer products have been shown to be endocrine-disrupting chemicals (EDCs), making them potentially important indoor contaminants/pollutants and suggesting that indoor exposure may be a greater contributor to overall EDCs exposure.[1,2] Furthermore, people spend most of their time indoors, where EDCs concentrations can be more relevant than the outdoor concentrations to assessments of human EDCs exposure.[3,4] This observation led to the recognition that changes in modifiable environmental exposures are likely to play a critical role in the increasing prevalence of noncommunicable diseases such as asthma and obesity among children in developed countries,[5,6] an increase that cannot be explained only by genetic changes.[7]

Despite the widespread presence of these chemicals indoors, the understanding of the extent as well as the health effects of the exposure is limited by insufficient data on exposure patterns and the action of EDCs over the lifespan.[8] According to the World Health Organization (WHO), approximately 800 chemicals are known or suspected to be capable of interfering with various mechanisms of the endocrine system, such as receptor binding and/or hormone synthesis, potentially inducing adverse health effects in exposed individuals or populations and possibly playing a substantial role in many endocrine disorders.[8,9]

In recent decades, growing evidence has suggested that EDCs exposure is implicated in the development of several human diseases and that multiple mechanisms might be involved.[9,10] Exposure to EDCs has been associated with an increase in oxidative stress and changes in cytokines production. For instance, EDCs may have a role in T_H2 cells modulation, changing IgE production and eosinophilic responses, and in the hypomethylation of the promoter region of TNF-α.[11,12] Some EDCs can also disrupt or interfere with lipid homeostasis, promoting adipogenesis and lipid accumulation, thus leading to weight gain and obesity.[13,14] Furthermore, exposure to EDCs may also interfere with the normal activity of the autonomic nervous system (ANS), which is partly responsible for the development of asthma [15,16] and weight changes.[17] Furthermore, the dose and timing of EDCs exposure have important implications in adverse health effects.[18]

Childhood is a critical window of susceptibility to EDCs exposure, as exposure in childhood can induce long-lasting effects persisting into adulthood and affecting future generations.[8,18] This highlights the need for a better understanding of the effect of EDCs exposure on the health of children, who may be especially susceptible to low-concentration effects, considering the large amount of

time spent indoors. Therefore, we aimed to assess the association between indoor EDCs exposure in classrooms and asthma, respiratory symptoms and obesity in school-age children.

2 | METHODS

The present study included participants from a cross-sectional study. Twenty of 53 primary schools within the city of Porto, Portugal, were selected, corresponding to a total of 71 assessed classrooms (see the Methods section in the Online Repository). The University Health Ethics Committee approved the study, and informed consent was obtained from children's legal guardians.

2.1 | Participants and assessments

A self-administered ISAAC-based questionnaire[19] covering information on social, demographic and behavioural characteristics and including questions regarding the respiratory/allergic health and current symptoms (previous 3 months) of children was filled out by the children's caregivers and reviewed by a research nurse (see the Methods section in the Online Repository). Children were considered to have current symptoms in the previous 3 months if there was a positive answer to the question "During the past 3 months, has your child had any of the following symptoms?": "Itching on the face or neck," "Eye irritation (redness, dryness, itching)," "Swollen eyes," "Runny nose," "Nasal obstruction," "Dry throat," "Sore throat," "Irritative cough" and "Breathing difficulties."

Anthropometry and clinical assessments were performed in the primary schools by a research nurse. Weight (Kg), body fat percentage, body fat mass (Kg) and total body water (TBW, %) were measured using a digital scale (Tanita™ BC-418 Segmental Body Analyzer), and height in centimetres (cm) was measured with a portable stadiometer. Body fat percentage was classified in accordance with sex-specific centile curves for body fat in children.[20] Body mass index (BMI) was calculated using the ratio of weight/height^2 (Kg/m^2) and classified according to the age- and sex-specific percentiles defined by the US Centers for Disease Control and Prevention (CDC),[21] World Health Organization (WHO)[22] and International Obesity Task Force (IOTF).[23]

Lung function and airway reversibility were assessed by spirometry according to ATS/ERS guidelines[24] using a portable spirometer (MIR Spiro bank, A23-04003237) before and 15 minutes after the inhalation of 400 μg of salbutamol. Different asthma and asthma-like conditions were considered: (a) *Medical diagnosis of asthma*: self-reported medical diagnosis; (b) *Positive bronchodilation (BD+)*: at least a 12% and over 200 mL increase in FEV1 after bronchodilation; (c) *Medical diagnosis of asthma and symptoms or BD+*: at least a 12% and over 200 mL increase in forced expiratory volume in 1 second (FEV1) after bronchodilation or a self-reported medical diagnosis with reported symptoms (wheezing, dyspnoea or dry cough) in the past 12 months; iv) *Medical diagnosis of asthma or BD+:* at least a 12% and over 200 mL increase in FEV1 after bronchodilation or a self-reported medical diagnosis (see Table S1 in the Online Repository).

Airway inflammation was assessed by measuring the fractional exhaled nitric oxide level using a NObreath analyzer (Bedfont Scientific, Ltd.) in accordance with the ATS guidelines.[25] The pH of the exhaled breath condensate (EBC) was measured before and 10 minutes after deaeration with oxygen using a calibrated pH meter (pHenomenal® pH 1100 H) to an accuracy of ± 0.005 pH units. (see the Methods section in the Online Repository).

Pupillometry was performed with a portable infrared PLR-200 pupillometer (NeurOptics PLR-200™ Pupillometer, NeurOptics Inc., CA). The children spent at least 15 minutes in a semi-dark and quiet room to allow pupil adjustment to the low level of light, after which they were instructed to focus on a small object 3 metres away with the eye that was not being tested, keeping their head straight and eyes wide open during targeting and measurement. The eye was briefly illuminated by light-emitting diodes with a single light stimulus having a peak wavelength of 180 nm. A pupillary light response curve for each eye was recorded for each child. Data were recorded for each child for the diameter of the pupil before (initial) and at constriction peak (minimal), in millimetres; for the relative constriction amplitude, as a percentage; for the maximum constriction velocity (MCV) and the average constriction and dilation velocities (ACV and ADV, respectively), all in mm/s; and the total time taken by the pupil to recover to 75% of the initial resting pupil size after reaching peak of constriction (T75), in seconds. Pupillometry is a simple non-invasive technique that can provide valuable data concerning the functioning of both branches of the autonomic nervous system. The pupil diameter, ACV, MCV and constriction amplitude are related to parasympathetic activity, while ADV and T75 are measures of sympathetic activity.[26] All pupillary data reported were from one eye, since no side-to-side difference was observed in the pupil responses.

In total, 1602 children (7-12 years old) in the 3rd and/or 4th grades were invited to participate. Of those, 686 did not return the signed informed consent form, and 58 refused to perform clinical tests. These children had a significantly higher BMI z-score than the included children but no differences in gender, age, parental education, or the prevalence of asthma and respiratory symptoms. Among the 858 included children, 13 were excluded due to poor quality data (participation rate of 52.7%). Thus, this study was based on data from 845 children (49.2% girls) (Table 1). The prevalence of atopy, rhinitis and current rhinitis was 35.4%, 13.4% and 31.9%, respectively. The prevalence of asthma ranged between 6.5% (medical diagnosis of asthma criteria) and 12.3% (medical diagnosis of asthma or BD+ criteria). The prevalence of overweight or obesity was nearly 30% (Table 1).

2.2 | Indoor air exposure

The indoor levels of volatile organic compounds (VOC) and aldehydes (formaldehyde and acetaldehyde) were measured in each classroom over 1 week, from early Monday until late Friday, during regular daily activities and under representative conditions of

TABLE 1 Characteristics of the participants

Characteristics	Total n = 845	Girls n = 416	Boys n = 429
Age (y [mean ± SD])	9.0 (8.0-9.0)	9.0 (8.0-9.0)	9.0 (8.0-9.0)
Parental education (n [%])			
0-9 y	219 (32.1)	97 (29.6)	122 (34.5)
10-12 y	201 (29.5)	103 (31.4)	98 (27.7)
≥13 y	262 (38.4))	128 (39.0)	134 (37.9)
Asthma (n [%]) defined by[a]			
Medical diagnosis	53 (6.5)	27 (6.7)	26 (6.3)
Positive bronchodilatation (BD+)	57 (6.7)	35 (8.4)	22 (5.1)
Medical diagnosis and symptoms or BD+	80 (9.5)	**49 (11.8)**	**31 (7.2)**
Medical diagnosis or BD+	104 (12.3)	60 (14.4)	44 (10.3)
Symptoms in previous 3 mo (n [%])			
Itching on the face or neck	54 (7.1)	22 (5.9)	32 (8.4)
Eye irritation	97 (12.6)	41 (10.8)	56 (14.4)
Swollen eyes	58 (7.8)	29 (7.9)	29 (7.7)
Runny nose	345 (45.5)	181 (48.0)	164 (42.0)
Nasal obstruction	404 (53.1)	199 (53.1)	205 (53.1)
Dry throat	154 (20.5)	81 (21.7)	73 (19.4)
Sore throat	302 (39.7)	**168 (44.6)**	**134 (31.2)**
Irritative cough	232 (30.5)	119 (31.7)	113 (29.4)
Breathing difficulties	82 (10.8)	44 (11.7)	38 (9.9)
Wheezing symptoms (n [%])	78 (9.2%)	38 (9.1)	40 (9.3)
Wheezing previous year (n [%])	64 (31.4)	31 (25.6)	35 (24.8)
More than 4 wheezing episodes previous year (n [%])	16 (7.8)	9 (8.8)	7 (6.9)
Cough symptoms (n [%])	97 (11.5)	50 (12.0)	47 (11.0)
Asthma symptoms (n [%])[b]	119 (14.1)	59 (14.2)	60 (14.0)
Atopy (n [%])[c]	296 (35.4)	145 (35.3)	151 (35.6)
Rhinitis (n [%])	101 (13.4)	**40 (10.8)**	**61 (16.0)**
Current rhinitis (n [%])	89 (31.9)	**36 (26.1)**	**53 (37.6)**
Body fat percentage (%)	21.4 (18.2-26.2)	**23.2 (20.2-27.6)**	**19.5 (16.7-23.8)**
Body fat mass (Kg)	6.6 (5.0-9.6)	**7.4 (5.6-10.4)**	**6.0 (4.6-8.4)**
Total body water (%)	17.9 (16.0-20.4)	**17.5 (15.7-20.1)**	**18.2 (16.4-20.7)**
BMI (n [%])			
CDC			
Underweight	39 (4.6)	21 (5.0)	18 (4.2)
Normal weight	575 (68.0)	281 (67.5)	194 (68.5)
Overweight	128 (15.1)	67 (16.1)	61 (14.2)
Obese	103 (12.2)	47 (11.3)	56 (13.1)
WHO			
Underweight	12 (1.4)	5 (1.2)	7 (1.6)
Normal weight	541 (64.0)	260 (62.5)	281 (65.5)
Overweight	172 (20.4)	98 (23.6)	74 (17.2)
Obese	120 (14.2)	53 (12.7)	67 (15.6)
IOTF			
Underweight	75 (8.9)	42 (10.1)	33 (7.7)
Normal weight	539 (63.8)	253 (60.8)	286 (66.7)

(Continues)

TABLE 1 (Continued)

Characteristics	Total n = 845	Girls n = 416	Boys n = 429
Overweight	168 (19.9)	88 (21.2)	80 (18.6)
Obese	63 (7.5)	33 (7.9)	30 (7.0)
Percentage of body fat			
Underfat	8 (1.0)	3 (0.7)	5 (1.2)
Normal	556 (66.7)	286 (70.1)	270 (63.5)
Overfat	134 (16.1)	63 (15.4)	71 (16.7)
Obese	135 (16.2)	56 (13.7)	79 (18.6)
EBC pH (n [%])			
<7.4	527 (62.4)	268 (64.4)	259 (60.4)
≥7.4	274 (32.3)	122 (29.3)	151 (35.2)
Exhaled NO (ppb)	11.0 (6.0-20.0)	**10.0 (6.0-17.5)**	**12.0 (6.0-21.0)**
Pupillometry			
Baseline pupil diameter (mm)	5.3 (4.7-5.9)	5.3 (4.6-5.9)	5.4 (4.8-5.9)
Final pupil diameter (mm)	3.4 (3.0-3.8)	3.4 (2.9-3.8)	3.4 (3.0-3.8)
ACV (mm/s)	4.0 (3.6-4.4)	4.0 (3.5-4.4)	4.0 (3.6-4.4)
MCV (mm/s)	5.3 (4.7-5.9)	**5.2 (4.6-5.8)**	**5.4 (4.8-6.0)**
Constriction amplitude (%)	35.0 (32.0-38.0)	**35.0 (32.0-38.0)**	**36.0 (33.0-39.0)**
ADV (mm/s)	1.1 (1.0-1.3)	1.1 (1.0-1.3)	1.2 (1.0-1.3)
T75 (s)	1.7 (1.2-2.1)	1.7 (1.1-2.1)	1.7 (1.2-2.2)

ACV, average constriction velocity; ADV, average dilation velocity; BD, bronchodilation; BMI, body mass index; CDC, US Centres for Disease Control; EBC, exhaled breath condensate; FEV_1, forced expiratory volume in the first second of forced vital capacity; IOTF, International Obesity Task Force; MCV, maximum constriction velocity; T75, the total time taken by the pupil to recover 75% of the initial resting pupil size after it reached the peak of constriction; WHO, World Health Organization.

Data reported as median (25th, 75th percentile) unless otherwise stated. Significant differences in bold.

[a]The following asthma definitions were adopted: (a) *Medical diagnosis of asthma*: self-reported medical diagnosis; (b) *Positive bronchodilatation (BD+)*: at least a 12% and over 200 mL increase in FEV1 after bronchodilation; (c) *Medical diagnosis of asthma and symptoms or BD+*: at least a 12% and over 200 mL increase in forced expiratory volume in 1 second (FEV1) after bronchodilation or self-reported medical diagnosis with reported symptoms (wheezing, dyspnoea or dry cough) occurring in the past 12 mo; (d) *Medical diagnosis of asthma or BD+*: at least a 12% and over 200 mL increase in FEV1 after bronchodilation or self-reported medical diagnosis.

[b]Reported symptoms (wheezing, dyspnoea or dry cough) occurring in the past 12 mo.

[c]defined by a positive skin prick test to at least one of the allergens.

occupancy and use of the classrooms in winter. Previous studies revealed that the indoor VOC concentration is usually higher during winter than during summer[27]; however, no measurements were performed in spring/summer. VOC were collected passively onto thermally desorbed adsorbents (see the Methods section in the Online Repository). Formaldehyde and acetaldehyde were sampled by Radiello® passive devices (RAD 165, Sigma Aldrich), and concentrations were determined using isocratic reverse phase high-performance liquid chromatography (HPLC) (Agilent Technologies, 1220 Infinity LC) with a UV detector operated at 360 nm, according to the ISO 16000-4 standard[28] (see the Methods section in the Online Repository).

The concentrations of 13 VOC and 2 aldehydes identified as EDCs by *The Endocrine Disruption Exchange Research Institute* (TEDX)[29] were considered, including toluene, o-xylene, m/p-xylene, hexane, ethylbenzene, styrene, cyclohexanone, butylated hydroxytoluene (BHT), benzene, benzaldehyde, 4-chloroethylene (T4CE), 2-butoxyethanol, 2-ethyl-1-hexanol, formaldehyde and acetaldehyde. The median indoor air concentrations of these EDCs and their frequency in the classrooms are presented in Table S2 (Online Repository).

2.3 | Data analysis

The Kolmogorov-Smirnov test was used to check continuous variables for normality. The Mann-Whitney test was used to compare variables between the girls and the boys. Significant differences were reported with an α-value of less than 5% ($P < 0.05$). Principal component analysis (PCA) was used to identify major EDCs patterns based on the 15 individual compounds. Varimax rotation was performed to simplify the interpretation of the factor loading structure. The number of components was chosen based on eigenvalues greater than one. The PCA divided the EDCs into two principal components (PC1 and PC2), which were included in each PC if the correlation coefficient was equal to or greater than 0.30. Of the two factors, PC1 had a higher absolute correlation with toluene, o-xylene,

TABLE 2 EDCs and asthma, respiratory symptoms in the previous 3 mo and body mass index (BMI)

	Median (25th-75th percentile)			
	No	Yes	Spearman's rho	*P* value
Toluene (μg/m^3)				
Symptoms in previous 3 mo				
Nasal obstruction	8.8 (5.3; 11.8)	10.1 (5.7; 14.9)	-	0.006
Dry throat	8.9 (5.6; 13.4)	10.3 (6.0; 14.9)	-	0.027
Sore throat	8.9 (5.3; 13.4)	10.1 (5.7; 14.9)	-	0.015
BMI (WHO criteria)				
Underweight	-	6.0 (3.1; 12.4)	-	0.023
Normal weight	-	8.9 (5.5; 14.1)	-	
Overweight	-	9.1 (5.6; 14.1)	-	
Obese	-	10.6 (6.9; 14.7)	-	
o-xylene (μg/m^3)				
Medical diagnosis asthma or BD+[a]	2.2 (1.0; 3.5)	2.7 (1.3; 4.0)	-	0.016
Symptoms in previous 3 mo				
Nasal obstruction	2.0 (1.0; 3.1)	2.5 (1.1; 3.9)	-	0.008
m/p-xylene (μg/m^3)				
Medical diagnosis asthma or BD+[a]	3.8 (2.1; 6.2)	5.0 (2.3; 7.6)	-	0.015
Symptoms in previous 3 mo				
Nasal obstruction	3.7 (2.1; 6.1)	4.3 (2.2; 7.5)	-	0.016
Dry throat	3.8 (2.0; 6.7)	4.9 (2.6; 7.5)	-	0.030
Sore throat	3.8 (2.0; 6.2)	4.2 (2.2; 7.5)	-	0.042
BMI (WHO criteria)				
Underweight	-	2.9 (1.6; 8.2)	-	0.023
Normal weight	-	3.8 (2.1; 6.7)	-	
Overweight	-	4.2 (2.1; 6.1)	-	
Obese	-	5.0 (3.2; 7.4)	-	
z-score CDC	-	-	0.072	0.036
z-score WHO	-	-	0.069	0.045
Ethylbenzene (μg/m^3)				
Medical diagnosis asthma[a]	1.0 (0; 2.6)	1.7 (0.6; 4.0)	-	0.031
Medical diagnosis asthma or BD+[a]	1.0 (0; 2.5)	1.7 (0.6; 3.6)	-	0.002
Medical diagnosis asthma and symptoms or BD+[a]	1.0 (0.0; 2.5)	1.6 (0.4; 3.6)	-	0.024
Symptoms in previous 3 mo				
Nasal obstruction	1.0 (0; 2.5)	1.2 (0; 2.8)	-	0.021
Styrene (μg/m^3)				
BMI (percentage of body fat criteria)				
Underfat	-	0.1 (0; 0.6)	-	0.046
Normal	-	0.3 (0.03; 0.9)	-	
Overfat	-	0.6 (0.2; 1.2)	-	
Obese	-	0.4 (0.03; 0.9)	-	
z-score CDC	-	-	0.091	0.008
z-score WHO	-	-	0.090	0.009
Cyclohexanone (μg/m^3)				
Symptoms in previous 3 mo[b]				
Runny nose	0.7 (3.2)	1.3 (4.9)		0.006

(Continues)

TABLE 2 (Continued)

	Median (25th-75th percentile)			
	No	Yes	Spearman's rho	*P* value
BMI (percentage of body fat criteria)[b]				
Underfat	-	0.0 (0.0)	-	0.017
Normal	-	0.8 (3.6)	-	
Overfat	-	0.9 (4.2)	-	
Obese	-	2.0 (5.6)	-	
z-score CDC	-	-	0.078	0.023
z-score WHO	-	-	0.080	0.020
Butylated hydroxytoluene (μg/m^3)				
Symptoms in previous 3 mo				
Dry throat	2.1 (0; 5.1)	0.8 (0; 4.1)	-	0.005
Benzene (μg/m^3)				
Medical diagnosis asthma or BD+[a]	0.7 (0.4; 1.3)	1.0 (0.4; 1.5)	..	0.033
Symptoms in previous 3 mo				
Itching on the face or neck	0.7 (0.4; 1.4)	1.2 (0.4; 1.9)		0.027
4-chloroethylene (μg/m^3)				
BMI (CDC criteria)				
Underweight	-	0.3 (0.8)	-	0.020
Normal weight	-	0.4 (1.2)	-	
Overweight	-	0.5 (1.4)	-	
Obese	-	0.1 (0.6)	-	
2-butoxietanol (μg/m^3)				
Symptoms in previous 3 mo				
Eye irritation	9.0 (5.5; 61.5)	15.2 (6.6; 118.7)	-	0.031
2-ethyl-1-hexanol (μg/m^3)				
Medical diagnosis asthma[a]	3.4 (2.1; 5.6)	4.8 (3.4; 9.6)	-	0.003
Medical diagnosis asthma or BD+[a]	3.4 (2.1; 5.6)	4.5 (2.8; 6.8)	-	0.017
Symptoms in previous 3 mo				
Swollen eyes	3.4 (2.1; 5.3)	5.3 (2.4; 7.1)	-	0.012
Runny nose	3.3 (2.1; 5.3)	4.5 (2.4; 5.6)	-	0.006
Irritative cough	3.4 (2.0; 5.3)	4.6 (2.8; 5.6)	-	0.007
PCA1				
Medical diagnosis asthma or BD+[a]	−0.3 (−0.5; −0.03)	−0.2 (−0.4; −0.004)	-	0.023
Symptoms in previous 3 mo				
Nasal obstruction	−0.3 (−0.5; −0.04)	−0.2 (−0.4; −0.004)	-	0.017
PCA2				
Symptoms in previous 3 mo				
Nasal obstruction	−0.01 (−0.5; 0.7)	−0.1 (−0.7; 0.6)	-	0.036

BMI, body mass index; CDC, US Centers for Disease Control; WHO, World Health Organization.

[a]The following asthma definitions were adopted: (a) *Medical diagnosis of asthma*: self-reported medical diagnosis; (b) *Positive bronchodilatation (BD+)*: at least a 12% and over 200 mL increase in FEV1 after bronchodilation; (c) *Medical diagnosis of asthma and symptoms or BD+*: at least a 12% and over 200 mL increase in forced expiratory volume in 1 second (FEV1) after bronchodilation or self-reported medical diagnosis with reported symptoms (wheezing, dyspnoea or dry cough) occurring in the past 12 mo; (d) *Medical diagnosis of asthma or BD+*: at least a 12% and over 200 mL increase in FEV1 after bronchodilation or self-reported medical diagnosis.

[b]Data reported as mean (standard deviation).

m/p-xylene, ethylbenzene, styrene and benzene, while PC2 had a higher absolute correlation with cyclohexanone, BHT, benzene, benzaldehyde, formaldehyde and acetaldehyde (Table S3).

Generalized linear models and multinomial logistic regression models were used to measure the effect of individual or combined EDCs on asthma, the presence of respiratory symptoms in the

previous 3 months, obesity and body composition in the children. Model 0, crude; model 1, adjusted for age and sex; and model 2, additionally adjusted for the following variables: (a) BMI (according to the CDC criteria), atopy and parental education; (b) asthma (according to the medical diagnosis of asthma and symptoms or BD+ criteria), parental education, physical activity and energy intake; (c) asthma (medical diagnosis of asthma and symptoms or BD+) and parental education; and (d) asthma (medical diagnosis of asthma and symptoms or BD+), BMI (the CDC criteria), parental education, physical activity and energy intake. PCA and mixed effect models were analysed using RStudio software, version 1.0.

3 | RESULTS

Classrooms with higher levels of o-xylene, m/p-xylene, ethylbenzene, benzene, 2-ethyl-1-hexanol and higher PC1 scores had a higher number of children with asthma (Table 2). Higher levels of several EDCs were also found in classrooms with a higher number of children reporting respiratory symptoms in the previous 3 months. Runny nose was reported by children in classrooms with higher concentrations of cyclohexanone and 2-ethyl-1-hexanol (Table 2). Additionally, higher classroom concentrations of toluene, m/-xylene, styrene and cyclohexanone showed a tendency to be associated with an increased number of overweight and obese children (Table 2). The results showed that classrooms with higher concentrations of toluene, o-xylene, m/p-xylene, ethylbenzene, 2-ethyl-1-hexanol and higher PC1 scores but lower PC2 scores had significantly higher numbers of children reporting nasal obstruction symptoms (Table 2). Higher concentrations of toluene, o-xylene, m/p-xylene, ethylbenzene, cyclohexanone, benzene and benzaldehyde and higher PC1 scores were found in classrooms with a higher number of children with significantly lower values of parasympathetic activity parameters (see Table S4 in the Online Repository). However, higher levels of BHT, formaldehyde and acetaldehyde and higher PC2 scores were found in classrooms with a higher number of children with higher values of parasympathetic activity parameters. Furthermore, higher EBC pH values were also found in children exposed to significantly higher levels of ethylbenzene, styrene and formaldehyde (see Table S4 in the Online Repository).

After adjustment, the risk of positive bronchodilation by exposure to higher levels of benzene increased by 1.5-fold (OR = 1.49, 95% CI 1.01, 2.53). Toluene, o-xylene, m/p-xylene and ethylbenzene were significantly associated with nasal obstruction (OR = 1.01, 95% CI 1.00, 1.03; OR = 1.04, 95% CI 1.01, 1.08; OR = 1.01, 95% CI 1.00, 1.03; and OR = 1.04, 95% CI 1.01, 1.09, respectively). The levels of hexane, styrene, cyclohexanone, BHT and 2-butoxyethanol were also associated with obesity, and the level of cyclohexanone was associated with increased child BMI. Additionally, positive associations were found between cyclohexanone, BHT and acetaldehyde and body composition (Table 3). Furthermore, a positive association was found between PC1 and the risk of obese asthma (OR = 1.43, 95% CI 1.01, 1.98) and between PC2 and overweight (OR = 1.51, 95% CI 1.28, 1.79). In addition, PC1 and PC2 were associated with nasal obstruction, and PC2 was associated with breathing difficulties and lean body mass (Table 3), although the concentrations of the EDCs were relatively low. Higher concentrations of individual EDCs and higher PC1 and PC2 scores were also positively associated with asthma, and child BMI and respiratory symptoms; however, after adjustment, the effect size estimates were similar but not statistically significant (Table S5).

Children exposed to higher levels of m/p-xylene and benzaldehyde exhibited decreased baseline pupil diameters; additionally, children exposed to higher levels of toluene, ethylbenzene and benzene exhibited decreased ADV (Table 4). Toluene, cyclohexanone and benzene were also negatively associated with constriction amplitude. Moreover, a negative association was found between both formaldehyde and acetaldehyde and ACV and between benzaldehyde and the baseline and final pupil diameters (Table 4). Nevertheless, toluene, o-xylene, m/p-xylene, ethylbenzene and benzene were also positively associated with constriction velocity (MCV and ACV), and 2-butoxyethanol, formaldehyde and acetaldehyde were positively associated with constriction amplitude. Positive associations were found between T4CE and MCV and between cyclohexanone and ACV. In addition, PC1 was positively associated with both MCV and ACV (Table 4), and PC2 was positively associated with baseline pupil diameter and constriction amplitude. However, higher PC2 scores were associated with lower values of MCV and ACV (Table 4).

4 | DISCUSSION

Our findings suggest that exposure to endocrine-disrupting compounds in classrooms is associated with an increased risk of asthma and obesity and an increased body fat percentage, as well as with an increased prevalence of nasal obstruction symptoms in the previous 3 months. Significant associations between compounds grouped by principal component analysis and obese asthma, current respiratory symptoms, obesity and body composition were also observed, even for low levels of EDCs exposure. Furthermore, our results showed that exposure to individual or combined EDCs is associated with changes in the ANS, specifically parasympathetic dysautonomia assessed through pupillometry,[30,31] thus suggesting that EDCs may increase parasympathetic activity, resulting in a subsequent increase in the risk of asthma and obesity.

Surprisingly, our findings showed that even low-level exposures of EDCs may increase the risk of asthma, the presence of respiratory symptoms in the previous 3 months or obesity in schoolchildren. This result suggests that no safe exposure level exists, especially when children are constantly exposed to these compounds. Indeed, other studies have shown that exposure to lower concentrations of EDCs may cause even stronger effects than higher doses, especially considering that exposure is lifelong.[32-34] Similar to natural hormones, even low concentrations of EDCs (in the parts-per-trillion to parts-per-billion

TABLE 3 Association between EDCs and asthma, respiratory symptoms in the previous 3 mo, and body mass index (BMI)

	OR (95% CI)		
	Model 0	**Model 1**	**Model 2**
Toluene			
Symptoms in previous 3 mo[a]			
Nasal obstruction	1.01 (1.00; 1.02)	1.01 (1.00; 1.02)	1.01 (1.00-1.03)
o-xylene			
Symptoms in previous 3 mo[a]			
Nasal obstruction	1.03 (1.00; 1.07)	1.03 (1.00; 1.07)	1.04 (1.01; 1.08)
m/p-xylene			
Symptoms in previous 3 mo[a]			
Nasal obstruction	1.01 (1.00, 1.02)	1.01 (1.00; 1.02)	1.01 (1.00; 1.03)
Hexane			
BMI (WHO criteria)[b]			
Underweight	Reference	Reference	Reference
Normal weight	1.00 (0.99; 1.02)	1.55 (1.54; 1.56)	1.18 (1.17; 1.19)
Overweight	1.00 (0.99; 1.02)	1.55 (1.54; 1.56)	1.18 (1.17; 1.19)
Obese	1.00 (0.99; 1.02)	1.55 (1.54; 1.56)	1.18 (1.17; 1.19)
Ethylbenzene			
Symptoms in previous 3 mo[a]			
Nasal obstruction	1.03 (1.00, 1.08)	1.03 (1.01; 1.08)	1.04 (1.01; 1.09
Styrene			
BMI (WHO criteria)[b]			
Underweight	Reference	Reference	Reference
Normal weight	1.20 (0.69; 2.06)	1.59 (0.39; 6.52)	1.65 (1.45; 1.88)
Overweight	1.20 (0.67; 2.13)	1.53 (0.37; 6.34)	1.54 (1.30; 1.83)
Obese	1.34 (0.76; 2.37)	1.81 (0.44; 7.51)	1.78 (1.52; 2.10)
Cyclohexanone			
BMI (WHO criteria)[b]			
Underweight	Reference	Reference	Reference
Normal weight	1.02 (0.88; 1.20)	1.59 (0.01; 1.59E2)	2.45 (2.36; 2.55)
Overweight	1.04 (0.88; 1.22)	1.61 (0.01; 1.61E2)	2.54 (2.43; 2.65)
Obese	1.06 (0.90; 1.24)	1.61 (0.01; 1.61E2)	2.53 (2.41; 2.65)
z-score CDC[c]	0.02 (0.005, 0.04)	0.02 (0.001; 0.04)	0.03 (0.01; 0.05)
z-score WHO[c]	0.03 (0.007, 0.05)	0.02 (0.002; 0.04)	0.03 (0.01; 0.06)
Body composition[b,c]			
Body fat (%)	0.18 (0.08; 0.27)	0.17 (0.07; 0.26)	0.21 (0.10; 0.32)
Butylated hydroxytoluene			
BMI (WHO criteria)[b]			
Underweight	Reference	Reference	Reference
Normal weight	1.35 (0.43, 4.18)	1.36 (0.4; 4.26)	1.23 (1.18; 1.29)
Overweight	1.33 (0.43, 4.14)	1.35 (0.43; 4.22)	1.25 (1.18; 1.32)
Obese	1.33 (0.43, 4.13)	1.35 (0.43; 4.22)	1.23 (1.16; 1.31)
Body composition[b,c]			
Lean body mass	0.10 (0.02; 0.19)	0.09 (0.01; 0.17)	0.10 (0.01; 0.19)
Total body water	0.08 (0.01; 0.14)	0.06 (0.006; 0.12)	0.07 (0.01; 0.14)
Benzene			
Positive bronchodilatation[d,a]	1.67 (1.02, 2.66)	1.64 (1.00; 2.65)	1.49 (1.01; 2.53)

(Continues)

TABLE 3 (Continued)

	OR (95% CI)		
	Model 0	Model 1	Model 2
2-butoxyethanol			
BMI (Percentage of body fat criteria)[b]			
Underfat	Reference	Reference	Reference
Normal	1.81 (0.08, 43.1)	4.36 (4.34; 4.38)	3.90 (3.85; 3.95)
Overfat	1.81 (0.07, 43.1)	4.35 (4.33; 4.38)	3.90 (3.85; 3.96)
Obese	1.80 (0.07, 42.9)	4.33 (4.30; 4.36)	3.81 (3.72; 3.91)
Acetaldehyde			
Body composition[b,c]			
Lean body mass	0.07 (−0.04; 0.18)	0.10 (−7.66E-4; 0.21)	0.16 (0.02; 0.30)
PC1			
Obese asthma[a]	1.45 (1.04; 1.94)	1.38 (0.98; 1.86)	1.43 (1.01; 1.98)
Symptoms in previous 3 mo[a]			
Nasal obstruction	1.25 (1.09; 1.47)	1.24 (1.08; 1.45)	1.32 (1.13; 1.58)
BMI (WHO criteria)[b]			
Underweight	Reference	Reference	Reference
Normal weight	0.89 (0.57; 1.39)	0.90 (0.57; 1.41)	0.89 (0.80; 1.01)
Overweight	0.79 (0.49; 1.27)	0.79 (0.49; 1.27)	0.80 (0.69; 0.92)
Obese	0.92 (0.58; 1.47)	0.94 (0.59; 1.51)	0.85 (0.73; 0.98)
BMI (Percentage of body fat criteria)[b]			
Underfat	Reference	Reference	Reference
Normal	1.40 (0.46; 4.32)	1.39 (0.47; 4.14)	1.39 (1.23; 1.57)
Overfat	1.29 (0.42; 4.03)	1.27 (0.42; 3.84)	1.17 (0.97; 1.40)
Obese	1.34 (0.43; 4.15)	1.33 (0.44; 4.01)	1.29 (1.10; 1.51)
PC2			
Symptoms in previous 3 mo[a]			
Nasal obstruction	0.86 (0.74, 0.99)	0.85 (0.73; 0.99)	0.83 (0.71; 0.98)
Breathing difficulties	0.80 (0.63, 1.01)	0.78 (0.61; 1.00)	0.74 (0.55; 0.97)
BMI (WHO criteria)[b]			
Underweight	Reference	Reference	Reference
Normal weight	1.19 (0.66; 2.15)	1.18 (0.65; 2.13)	1.18 (1.04; 1.33)
Overweight	1.12 (0.61; 2.05)	1.15 (0.63; 2.11)	1.24 (1.06; 1.45)
Obese	1.08 (0.58; 1.98)	1.12 (0.60; 2.07)	1.10 (0.93; 1.30)
BMI (Percentage of body fat criteria)[b]			
Underfat	Reference	Reference	Reference
Normal	1.35 (0.65, 2.82)	1.33 (0.63; 2.78)	1.31 (1.15; 1.48)
Overfat	1.41 (0.67, 2.97)	1.37 (0.65; 2.92)	1.51 (1.28; 1.79)
Obese	1.16 (0.55, 2.45)	1.17 (0.55; 2.48)	1.10 (0.93; 1.30)
Body composition[b,c]			
Lean body mass	0.33 (0.03; 0.63)	0.33 (0.03; 0.63)	0.40 (0.04; 0.76)

BMI, body mass index; CDC, US Centres for Disease Control; CI, confidence interval; IOTF, International Obesity Task Force; WHO, World Health Organization.

Model 0 is null model; model 1 is adjusted for age and sex; model 2 is additionally adjusted for:

[a]BMI (according to CDC criteria), atopy and parental education.

[b]Asthma (according to medical diagnosis asthma and symptoms or BD+ criteria), parental education, physical activity, energy intake.

[c]Data reported as β (95% CI).

[d]The following asthma definitions were adopted: (a) *Medical diagnosis of asthma*: self-reported medical diagnosis; (b) *Positive bronchodilatation (BD+)*: at least a 12% and over 200 mL increase in FEV1 after bronchodilation; (c) *Medical diagnosis of asthma and symptoms or BD+*: at least a 12% and over 200 mL increase in forced expiratory volume in 1 second (FEV1) after bronchodilation or self-reported medical diagnosis with reported symptoms (wheezing, dyspnoea or dry cough) occurring in the past 12 mo; (d) *Medical diagnosis of asthma or BD+*: at least a 12% and over 200 mL increase in FEV1 after bronchodilation or self-reported medical diagnosis.

TABLE 4 Association between EDCs and autonomic nervous system activity

	OR (95% CI)[a]		
	Model 0	**Model 1**	**Model 2[b]**
Toluene			
MCV	−0.004 (−0.01; −7.79E-4)	−0.004 (−0.01; −4.17E-4)	0.005 (8.00E-4; 0.009)
ACV	−0.003 (−0.006; −7.14E-4)	−0.003 (−0.006; −4.70E-4)	0.004 (0.001; 0.007)
Constriction amplitude	−0.02 (−0.004; 6.51E-4)	0.009 (−0.04; 9.76E-4)	−0.02 (−0.04; −0.004)
ADV	−0.001 (−0.003; −2.18E-4)	−0.001 (−0.003; −1.51E-4)	−0.002 (−0.003; -2.00E-4)
o-xylene			
MCV	−0.01 (−0.02; −0.002)	−0.01 (−0.02; −0.003)	0.01 (0.002; 0.02)
ACV	−0.01 (−0.02; −0.003)	−0.01 (−0.02; −0.003)	0.01 (0.003; 0.02)
m/p-xylene			
Baseline pupil diameter	−0.004 (−0.007; −0.001)	−0.004 (−0.007; −0.001)	−0.003 (−0.006; −4.14E-5)
MCV	−0.005 (−0.008; −0.001)	−0.005 (−0.008; −0.002)	0.005 (0.001; 0.008)
ACV	−0.004 (−0.006; −0.001)	−0.004 (−0.006; −0.001)	0.004 (0.001; 0.006)
Ethylbenzene			
MCV	−0.01 (−0.02; −0.002)	−0.01 (−0.02; −0.002)	0.01 (0.002; 0.03)
ACV	−0.01 (−0.02; −0.003)	−0.01 (−0.02; −0.003)	0.01 (0.003; 0.02)
ADV	−0.004 (−0.008; −1.55E-4)	−0.004 (−0.009; −1.93E-4)	−0.005 (−0.01; −1.00E-4)
Cyclohexanone			
ACV	−0.01 (−0.02; 7.69E-4)	−0.01 (−0.002; 0.001)	0.01 (0.002; 0.03)
Constriction amplitude	−0.09 (−0.17; −0.002)	−0.08 (−0.17; 0.004)	−0.10 (−0.20; −0.01)
Benzene			
MCV	−0.15 (−0.24; −0.05)	−0.12 (−0.22; −0.02)	0.15 (0.04; 0.26)
ACV	−0.13 (−0.20; −0.06)	−0.12 (−0.19; −0.04)	0.14 (0.06; 0.22)
Constriction amplitude	−0.59 (−1.10; −0.09)	−0.50 (−1.10; 0.005)	−0.74 (−1.30; −0.18)
ADV	−0.001 (−0.003; 0.001)	−0.004 (−0.009; −1.93E-4)	−0.05 (−0.09; −0.007)
Benzaldehyde			
Baseline pupil diameter	−0.01 (−0.02; −0.005)	−0.01 (−0.02; −0.003)	−0.01 (−0.02; −0.004)
Final pupil diameter	−0.007 (−0.01; −0.002)	−0.006 (−0.01; −0.001)	−0.008 (−0.01; −0.03)
4-chloroethylene			
MCV	−0.06 (−0.12; 0.001)	−0.07 (−0.14; −0.01)	0.07 (0.005; 0.14)
2-butoxyethanol			
Constriction amplitude	0.01 (0.002; 0.02)	0.01 (0.003; 0.02)	0.02 (0.005; 0.03)
Formaldehyde			
ACV	0.01 (0.003; 0.02)	0.01 (0.003; 0.02)	−0.010 (−0.02; −0.003)
Constriction amplitude	0.08 (0.03; 0.13)	0.08 (0.03; 0.13)	0.10 (0.05; 0.16)
Acetaldehyde			
ACV	0.03 (0.01; 0.05)	0.04 (0.02; 0.05)	−0.03 (−0.05; −0.010)
Constriction amplitude	0.14 (0.003; 0.28)	0.17 (0.03; 0.31)	0.16 (0.004; 0.31)
PC1			
MCV	−0.09 (−0.16; −0.02)	−0.09 (−0.16; −0.02)	0.10 (0.02; 0.18)
ACV	−0.07 (−0.12; −0.02)	−0.07 (−0.12; −0.02)	0.08 (0.03; 0.14)
PC2			
Baseline pupil diameter	0.11 (0.04; 0.17)	0.09 (0.02; 0.16)	0.09 (0.01; 0.16)
MCV	0.09 (0.02; 0.17)	0.09 (0.01; 0.17)	−0.10 (−0.19; −0.02)
ACV	0.11 (0.06; 0.17)	0.11 (0.06; 0.17)	−0.12 (−0.18; −0.06)
Constriction amplitude	0.70 (0.32; 1.09)	0.72 (0.33; 1.12)	0.86 (0.44; 1.29)

ACV, average constriction velocity; ADV, average dilation velocity; CI, confidence interval; MCV, maximum constriction velocity.
Model 0 is null model; model 1 is adjusted for age and sex; model 2 is additionally adjusted for:
[a]Data reported as β (95% CI).
[b]asthma (according to medical diagnosis asthma and symptoms or BD+ criteria) and parental education.

range) may have effects that influence human metabolism. These studies showed that very low levels of individual or combined EDCs were associated with reduced intelligence, disrupting reproductive system and cause other health problems.[32-34] Thus, the statement by Paracelsus that "the dose makes the poison" may not apply to EDCs exposure because of potential low-dose effects, which cannot be predicted by the effects of exposure to higher concentrations.[34]

Our findings may contribute to action plans aimed at improving indoor air quality and decreasing EDCs exposure, thereby improving children's health. This study assessed the effect of individual exposure and co-exposure to VOC identified as EDCs; however, most previous studies have focused primarily on the effects of exposure to individual semi-volatile organic compounds such as phthalates and bisphenol on respiratory health[35-37] and obesity in children[38,39] and on compounds that interact with the oestrogen, androgen and thyroid hormone systems.[8,40] Our results suggested that exposure to individual or combined EDCs may interfere with other systems, increasing the risk of asthma, the presence of respiratory symptoms in the previous 3 months and obesity in schoolchildren, and these results contribute to the understanding of the potential health risk of co-exposure. Additionally, exposure to combined EDCs (PC1) may also increase the risk of obese asthma, suggesting that children with obese asthma may be more sensitive than healthy children to EDCs.

Individual EDCs, such as toluene, hexane, styrene, o-,m/p-xylene, ethylbenzene, cyclohexanone, BHT, benzene and 2-butoxyethanol, and combined EDCs obtained in our study by principal component analysis were significantly associated with different health effects, suggesting that different EDCs may target different organs and systems. Exposure to EDCs may change cytokine production, T_H1 and T_H2 balance and activate the immune system.[41,42] Kuo et al[43] showed that human bronchial epithelial cells treated with different EDCs increased bronchial smooth-muscle cell proliferation and migration by increasing the secretion of IL-8 and regulated on activation and normal T-cell expression and secreted (RANTES), suggesting a possible role for EDCs in asthma airway remodelling. In addition, EDCs may mediate changes through alterations in DNA methylation, which are proposed to play a role in the development of asthma.[44] Higher exposure to EDCs is related to lower methylation of TNF-α 5′ CGI, which is associated with airway inflammation, hyperresponsiveness, the regulation of immune cells and a higher risk of asthma in children.[44] Similar to other air pollutants, EDCs may also stimulate airway C-fibre sensory nerves, which express transient receptor potential (TRP) cation channels; when exposed to irritants, TRP channels release neuropeptides locally, resulting in cough, airway irritation, mucous secretion and bronchoconstriction mediated by the efferent pathways of the autonomic nervous system.[45,46] Nassi et al[47] and Cantero-Recasens et al[48] reported the role of activation and/or increased expression of transient receptor potential vanilloid 1 (TRPV1) and ankyrin 1 (TRPA1) channels in the pathogenesis of asthma, providing evidence for the role of ANS in the regulation of airway function.

In obesity, EDCs inappropriately regulate lipid metabolism and adipogenesis by targeting peroxisome proliferator-activated receptors (PPARα, δ and γ) and the retinoic X receptor (RXR), which are essential for the control of intracellular lipid homeostasis as well as the proliferation and differentiation of adipocytes, and the aryl hydrocarbon receptor in adipocytes, thus increasing adipogenesis.[38,49] Obesogenic effects of EDCs may also be mediated by the methylation of PPARγ and RXRα, changing the regulation of adipogenesis and increasing triglycerides accumulation, glucose metabolism, and insulin sensitivity in mature adipocytes.[50,51] Additionally, Baillie-Hamilton[52] reported that exposure to different chemicals, such as EDCs, is also associated with weight gain, possibly by interfering with the normal activity of the ANS. These changes in the ANS may suppress appetite, enable the body to mobilize fat stores for use and stimulate physical activity levels.[52] The ANS plays an important role in the communication between the gastrointestinal system and the central nervous system, as a mediator of the sense of satiety after gastric distension, in the release of gut hormones, in the modulation of satiety and in the release of leptin and insulin, which regulate body weight and adiposity.[53]

Different mechanisms, such as mechanical, immunological, genetic, epigenetic, hormonal and environmental pathways, may mediate the association between obesity and asthma.[54] Recently, the microbiome has gained interest as an important factor in the development of both obesity and asthma.[55] In addition, our results described a possible noninflammatory mechanism underlying obese asthma from the disruption of the ANS by leptin due to exposure to indoor EDCs. These EDCs can mimic, block or interfere with the production of leptin,[56] a consequence that may also be involved in increasing the risk of asthma and obesity.[57] The lack of leptin signalling may affect airway smooth muscles by disrupting the activity of the parasympathetic nervous system. Additionally, leptin resistance in obesity also leads to increased parasympathetic signalling, with subsequent bronchoconstriction.[58,59]

This study has a few limitations. The cross-sectional design does not allow the establishment of causal relationships or the analysis of cumulative exposure to different indoor and outdoor environments. Furthermore, no on-site monitoring data regarding the levels of other indoor air pollutants, such as other chemical compounds, particulate matter, moulds or allergens, were considered in the analysis. The effect of sore throat as a potential co-exposure was not considered, since no significant associations were found between this symptom and asthma, the presence of other respiratory symptoms or EDCs exposure. Additionally, all questions regarding asthma symptoms and clinical assessment were performed under the assumption that the children did not have colds or flu, thus reducing the effect of co-exposure factors and the probability of chance findings. Additionally, our study considers classroom exposure only. However, after home, children spend most of their day at school, especially indoors, and usually stay in the same classroom through primary school years, being expected that indoor air quality in schools has an important role in the assessment of the effects of EDCs exposure. In addition, schools are important settings for the implementation of programmes that promote efforts to improve indoor air quality and behaviours that contribute to minimizing the effects of EDCs and promoting health and healthy lifestyles. Rudel et al[1] and Oziol et al[60] reported that EDCs concentrations are often highest

indoors because many sources are indoors and can influence outdoor EDCs concentrations and because of limited degradation indoors than outdoors. Most EDCs considered in this study were synthetic organic chemicals used in a wide range of materials and goods and can be found in many products in schools, including materials used in building construction and furnishing, along with cleaning products.[61] Moreover, indoor VOC concentrations were measured only in winter because during this period, VOC concentrations are usually high indoors, probably due to the lower mobility of air in winter and the prolonged persistence of the compounds due to reduced photochemical degradation and gradual accumulation.[27] Although several studies identified a significant seasonal cycle in VOC concentrations,[27] our findings showed that even low-level exposure to EDCs, which may be expected in summer or spring, may increase the risk of asthma, respiratory symptoms or obesity in schoolchildren. Nevertheless, it will be important to assess the effect of seasonality on EDCs concentrations to understand the extent of exposure as well as the health effects.

Our study also has important strengths. To our knowledge, this study is the first community-based study evaluating the effect of EDCs on asthma, current respiratory symptoms, obesity, body composition and ANS activity in school-age children. Additionally, we performed a comprehensive clinical assessment on a large number of participants, and we measured the concentrations of several EDCs in a substantial number of classrooms. In addition, the anthropometry and clinical assessments were performed in each primary school, in the children's normal environment, which would help reduce the co-effect of stress in the schoolchildren. Furthermore, the assessment week was arranged with the school principal and teachers to avoid examinations and special days. Even assuming an increase in stress levels, the effect of stress is expected to be independent of EDCs exposure. The children not included in the study were similar to those included, except for in the BMI z-scores, which may result in an underestimated association between exposure to EDCs and obesity. Despite the sampling of VOC at a single time point (limiting the temporal analysis) and the short period of exposure assessment, the 1-week sampling period and the use of passive samplers allowed the assessment of average indoor air concentrations and the detection of relatively low concentrations of EDCs, as suggested by recent reports,[62,63] and the levels are representative of the prevailing conditions/activities in each classroom and of the exposure to EDCs. We evaluated the effects of EDCs either alone or in combination, since EDCs can be released as mixtures and interact within or between classes, and combinations of low doses of EDCs that are individually inactive may cause a biological effect.[32,33] According to Kortenkamp[64] and Oziol et al,[60] it is important to understand the effects of co-exposure to EDCs, which may interact additively, synergistically or antagonistically, with potentially unknown intrinsic effects. The EDCs represented by PC1 (positive loadings on toluene, o-xylene, m/p-xylene, ethylbenzene, styrene and benzene) and PC2 (positive loadings on BHT, formaldehyde and acetaldehyde and negative loadings on cyclohexanone, benzene and benzaldehyde) showed that compounds may have different and even opposite effects, suggesting that diverse mechanisms may be involved. In addition, EDCs were measured in indoor air, which has been identified as an important source of chemical exposure.[8] Over the past decade, it has become clear that humans, particularly children, are exposed to EDCs via particles in indoor environments.[65-67] Urinary levels of EDCs were not measured; however, several studies have observed correlations between urinary levels of EDCs metabolites and indoor air concentrations, indicating that indoor air is a significant route of exposure.[68,69] Furthermore, different criteria were used to define BMI and to determine the effect of EDCs exposure on obesity. However, the establishment of different cut-offs is generally statistical rather than based on risk or the degree of body fatness. As a result, different definitions often do not give the same results. In this study, we assessed the effect of EDCs on BMI considering that the reference values resulted in similar but not identical estimates. Exposure to EDCs may also be associated with other important confounders related to asthma, respiratory symptoms and obesity, and those confounders may increase the risk of exposure compared to that of healthy children. Although several confounders were considered, there may be other unknown or unmeasured confounders.

Exploring the effects of individual or co-exposure to EDCs is crucial for planning and making recommendations to decision-makers to prevent the use of materials that could be indoor sources of these compounds, create monitoring programmes to characterize spatiotemporal trends and create healthier indoor environments, with the potential to decrease the risk of asthma and obesity in childhood. Thus, our results meet the goals of the WHO and the United Nations Environment Programme (UNEP), demonstrating the importance of understanding EDCs, their effects on human health, and policies and scientific evidence for health development and reduce exposures to EDCs.[32]

In conclusion, the present study suggests that both individual exposure and co-exposure to low concentrations of EDCs in classrooms may affect child health, specifically on asthma, obesity and the presence of respiratory symptoms in the previous 3 months. These results also highlight the negative health effects of EDCs, contributing to the implementation of recommendations to minimize exposure and promote a healthy indoor environment.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

All authors were involved in investigation and gave constructive criticism of the study manuscript. IP and AM were involved on study conceptualization and interpretation. IP, MS and AM contributed to the statistical analysis. IP and AM wrote the manuscript with input from all authors. EOF and AM obtained funding.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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School environment associates with lung function and autonomic nervous system activity in children: a cross-sectional study

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Children are in contact with local environments, which may affect respiratory symptoms and allergic sensitization. We aimed to assess the effect of the environment and the walkability surrounding schools on lung function, airway inflammation and autonomic nervous system activity. Data on 701 children from 20 primary schools were analysed. Lung function, airway inflammation and pH from exhaled breath condensate were measured. Pupillometry was performed to evaluate autonomic activity. Land use composition and walkability index were quantified within a 500 m buffer zone around schools. The proportion of effects explained by the school environment was measured by mixed-effect models. We found that green school areas tended to be associated with higher lung volumes (FVC, FEV1 and FEF25–75%) compared with built areas. FVC was significantly lower in-built than in green areas. After adjustment, the school environment explained 23%, 34% and 99.9% of the school effect on FVC, FEV1, and FEF25–75%, respectively. The walkability of school neighbourhoods was negatively associated with both pupil constriction amplitude and redilatation time, explaining −16% to 18% of parasympathetic and 8% to 29% of sympathetic activity. Our findings suggest that the environment surrounding schools has an effect on the lung function of its students. This effect may be partially mediated by the autonomic nervous system.

Urbanization is one of the leading global trends of the 21st century, with significant changes in living standards, lifestyles, social behaviour, and health. Steady urbanization has increased the relevance of understanding the relationships between the environment and human health and wellbeing. While an increased standard of living offers many opportunities, unhealthy diets, physical inactivity, and exposure to urban air pollution are unfortunate side effects of urbanization[1].

Over the past decades, urbanization and the Western lifestyle have been linked to the rising prevalence of inflammatory disorders, including asthma and allergic diseases. Epidemiological studies have demonstrated that several urban factors, such as traffic-related air pollution, residential proximity to roads and heavy traffic, and household characteristics, are associated with reduced lung function[2,3] and increased risk of asthma-related

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symptoms[4,5]. However, the pathways whereby they influence lung function and the development of asthma are complex and interactive. One of the possible mechanisms is the induction of a persistent inflammatory state mediated by the immune system[6]. Airway inflammation is an important factor in the pathogenesis and pathophysiology of asthma. The dysregulation of endogenous immune processes, particularly by the autonomic nervous system, are, in part, responsible for the development and chronicity of asthma[7].

The human airways are innervated by efferent and afferent autonomic nerves, which regulate many aspects of airway physiology, including airway smooth muscle tone, mucus secretion, microvascular permeability, and the recruitment and activation of inflammatory cells[8,9]. The parasympathetic nervous system is the dominant neuronal pathway in the control of smooth muscle tone and secretion in airways[8]. In asthmatics patients, increased basal parasympathetic tone is observed[10,11]. This results in constricted airways and an enhanced bronchoconstriction response to different inhaled agents that are known to stimulate airway C-fibre sensory nerves[12,13]. In turn, their activation due to environmental exposure may lead to the release of neuropeptides locally by transient receptor potential (TRP) cation channels, resulting in cough, airway irritation, mucous secretion, and bronchoconstriction mediated by the efferent pathways of the autonomic nervous system[14,15]. Nevertheless, these mechanisms are associated not only with urban factors but also with individual determinants and behaviours, such as physical activity, diet, and obesity[16,17].

The complexity of the interactions among urbanization, environmental change and human health and wellbeing requires an integrated approach. Therefore, to be effective in promoting health and healthy behaviour, public health interventions should address not only individual characteristics but also the physical and social environment[1]. A few studies have focused on the relationship between the surrounding greenness levels in children's living environment and their health[18–20]. However, since children spend a large proportion of their time at school, the school environment has recently garnered attention as a potential contributor to child health[21]. Thus, the aim of the present study was to evaluate the effect of school neighbourhoods and their walkability on lung function, airway inflammation and autonomic nervous system activity in children.

Results

An increased proportion of built areas in the school neighbourhood was associated with significantly lower values of FVC (model 0: $\beta = -5.13$, 95% CI −9.36, −0.91; model 2: $\beta = -4.98$, 95% CI −10.3, −0.35), while green areas showed a tendency to be associated with higher values of FVC, FEV_1 and $FEF_{25–75\%}$ (Supplementary Table S1). The highest ICCs were observed for FEV_1 and FVC (0.40% and 0.04%, respectively), indicating that approximately 1% of the total variation in these parameters was found between schools. After adjustment for age, sex, asthma, WHO z-score for BMI and family history of asthma or allergy, the neighbouring environment explained 98%, 96%, and >99.9% of the effect of school on FVC, FEV_1, and $FEF_{25–75\%}$, respectively (model 5, Table 1; Supplementary Fig. S1). No associations were observed between school neighbourhood and EBC pH (Supplementary Fig. S2) or exhaled NO (Supplementary Fig. S3).

No significant associations were observed between green or built areas and pupillometry parameters. Still, a positive trend was found between built areas and pupillometry parasympathetic parameters (ACV, MCV and constriction amplitude; Supplementary Fig. S4a). After adjustment, estimates of ICCs for pupillometry suggested that between 0% and 22% of the total variance was at the school level. The neighbouring environment explained 6% of the effect of school on MCV, 8% of its effect on ADV, 11% of its effect on constriction amplitude and 13% of its effect on T75 (model 5, Table 1; Supplementary Fig. S4b).

Neighbourhood walkability explained >99.9% of the school effect on FVC, FEV_1 and $FEF_{25–75\%}$. Regarding autonomic nervous system response, neighbourhood walkability explained 11% and 18% of the parasympathetic outcomes (constriction amplitude and MCV, respectively) and 7% and 29% of the pupillometry sympathetic parameters (ADV and T75, respectively) (Table 2; Supplementary Table S2). Lung function and exhaled NO decreased nonsignificantly with neighbourhood walkability (Supplementary Figs S5 and S7), while a positive association was observed for exhaled breath condensate pH level (Fig. S6). After adjustment for age, sex, asthma and WHO z-score for BMI, a significant negative association between walkability around schools and constriction amplitude ($\beta = -1.62$, 95% CI −2.87, −0.37) and T75 ($\beta = -0.19$, 95% CI −0.36, −0.02) was observed. Additionally, walkability showed a tendency to be associated with lower values of ACV, MCV and baseline pupil diameter (Supplementary Fig. S8a,b).

Discussion

We report for the first time an association among school neighbourhood environments, lung function, and autonomic function in children. Built areas around schools were inversely associated with children's lung function, specifically forced vital capacity, in both crude and adjusted mixed-effect models. Moreover, a non-significant relationship between schools surrounding greenness and lung function parameters was observed. On the basis of our results, it is plausible that effects of environment on lung function may be partly neurogenically mediated, as schools' neighbourhood walkability explained up to 14% and 30% of the effect of school on parasympathetic and sympathetic activity, respectively.

Our study has a few limitations. The cross-sectional design does not allow the establishment of causal relations or the analysis of cumulative exposure to different neighbourhoods. Furthermore, no on-site monitoring data regarding air pollution levels were measured, and we did not address the quality of green spaces, vegetation types or biodiversity. Nevertheless, several studies on urban environmental effects reported that land use could be used as an indicator of urban-related air pollution, such as traffic, without outdoor air monitoring[22,23]. Additionally, the use of an exposure metric based on urban land use thereby incorporates traffic-related emissions, but also includes other urban factors[22,23]. Rosenlund, *et al.*[24] also found a reasonable agreement between land-use and traffic emissions. Nevertheless, neighbourhood land use patterns and walkability around schools were quantified numerically, avoiding bias related to participants' perception of their neighbourhoods. Walkability is an objective

Outcome	β (95% CI)		School		
	PC1	PC2	ICC	Variance	Explained variation*
FVC					
Model 0	2.17 (−1.98; 6.33)	−5.13 (−9.36; −0.91)	—	—	—
Model 1	—	—	1.78%	4.48	Reference
Model 5[a]	3.66 (−3.01; 10.3)	−1.33 (−7.87; 5.02)	0.04%	0.08	98.2%
FEV_1					
Model 0	2.78 (−1.07; 6.63)	−3.11 (−7.02; 0.81)	—	—	—
Model 1	—	—	2.13%	4.53	Reference
Model 5[a]	1.54 (−4.58; 7.65)	1.07 (−4.93; 7.07)	0.40%	0.16	96.5%
$FEF_{25\%-75\%}$					
Model 0	5.05 (−1.27; 11.4)	−0.50 (−6.94; 5.93)	—	—	—
Model 1	—	—	0.37%	2.14	Reference
Model 5[a]	−5.19 (−16.2; 5.77)	4.86 (−5.89; 15.6)	7.10E-7%	3.71E-6	>99.9%
EBC pH					
Model 0	0.02 (−0.20; 0.24)	−0.05 (−0.17; 0.17)	—	—	—
Model 1	—	—	2.04%	4.33	Reference
Model 5[a]	0.12 (−0.53; 0.77)	−0.002 (−0.65; 0.64)	9.32%	0.09	97.8%
Exhaled NO					
Model 0	0.20 (−0.02; 0.41)	−0.14 (−0.35; 0.07)	—	—	—
Model 1	—	—	3.98%	0.03	Reference
Model 5[b]	−0.12 (−0.68; 0.44)	−0.37 (−0.93; 0.18)	6.76%	0.05	−54.6%
Baseline pupil diameter					
Model 0	−0.09 (−0.32; 0.14)	−0.03 (−0.26; 0.19)	—	—	—
Model 1	—	—	20.3%	0.158	Reference
Model 5[c]	−0.12 (−0.84; 0.60)	0.02 (−0.70; 0.75)	22.1%	0.178	−12.5%
Final pupil diameter					
Model 0	−0.08 (−0.24; 0.08)	−0.10 (−0.26; 0.06)	—	—	—
Model 1	—	—	14.1%	0.053	Reference
Model 5[c]	−0.10 (−0.51; 0.31)	−0.07 (−0.48; 0.35)	15.1%	0.059	−10.3%
ACV					
Model 0	−0.11 (−0.29; 0.07)	0.17 (−0.01; 0.35)	—	—	—
Model 1	—	—	15.7%	0.075	Reference
Model 5[c]	−0.05 (−0.56; 0.45)	0.23 (−0.28; 0.74)	16.7%	0.081	−8.24%
MCV					
Model 0	−0.06 (−0.32; 0.19)	0.26 (0.01; 0.51)	—	—	—
Model 1	—	—	12.1%	0.122	Reference
Model 5[c]	0.03 (−0.61; 0.68)	0.26 (−0.39; 0.91)	12.7%	0.118	−6.12%
Constriction amplitude					
Model 0	−0.25 (−1.56; 1.04)	1.17 (−0.12; 2.45)	—	—	—
Model 1	—	—	8.38%	2.07	Reference
Model 5[c]	−0.07 (−2.80; 2.65)	1.50 (−1.26; 4.27)	7.52%	1.85	10.9%
ADV					
Model 0	−0.02 (−0.10; 0.07)	0.06 (−0.03; 0.14)	—	—	—
Model 1	—	—	8.57E-8%	8.10E-11	Reference
Model 5[c]	−0.02 (−0.13; 0.09)	0.06 (−0.05; 0.17)	7.82E-8%	7.48E-11	7.60%
T75					
Model 0	0.21 (0.01; 0.42)	−0.13 (−0.33; 0.06)	—	—	—
Model 1	—	—	4.53%	0.023	Reference
Model 5[c]	0.31 (−5.07E-4; 0.63)	−0.10 (−0.42; 0.22)	3.89%	0.020	13.0%

Table 1. Multilevel model analysis of the association between individual and neighbouring environment and lung function, pH, exhaled NO and pupillometry parameters explained by school. *corresponds to the proportion of between-schools variance that could be explained by exposure and individual characteristics; PC1: discontinuous dense urban fabric, discontinuous medium density urban land, green urban areas, and water bodies; PC2: construction sites, land without current use, and railways; 95% CI: 95% confidence interval; ICC: intra-class correlation coefficient; FVC: forced vital capacity; FEV_1: forced expiratory volume in the first

second of FVC; $FEF_{25–75}$: forced expiratory flow in the middle portion of FVC; EBC: Exhaled breath condensate; ACV: Average constriction velocity; MCV: Maximum constriction velocity; ADV: Average dilation velocity; T75: the total time taken by the pupil to recover 75% of its initial resting diameter after it reached the peak of constriction. Model 0 only included the PC1 and PC2 score; [a]Model 1 is null model, baseline model without any exposure variable; Model 5[a] is additionally adjusted for age, sex, asthma, WHO z-score for BMI and family history of asthma or allergy; Model 5[b] is additionally adjusted for age, sex, asthma, atopy, WHO z-score for BMI and family history of asthma or allergy; Model 5[c] is additionally adjusted for age, sex, asthma, and WHO z-score for BMI.

measure of built environments and represents how friendly a neighbourhood area is to walking and bicycling; this measure is shaped by different urban-design features such as residential density, pedestrian-friendly design, street connectivity and diversity of neighbourhood land use[25]. Living in neighbourhoods characterized by higher walkability was found to be associated with more walking and cycling for transport and leisure and with moderate to vigorous physical activity[26] and reduced obesity and overweight[27]. Walkable urban areas may offer health benefits, but may also come with health costs when exposure to air pollution is considered[28]. Our study considers only the walkability around schools, however, several studies have reported the impact of walkability around schools in planning school neighbourhoods (accessible schools with low traffic, sidewalks), in decisions that support the active commuting to school[29], and also in the decrease of automobile dependence in childhood that carries over into adolescence and adulthood[29]. In addition, assessing the walkability around individual's home may not necessarily reflect the facilities that they use or environments in which they are active[30]. Moreover, indicators of asthma severity, such as number of asthma attacks, attendance in emergency service and hospitalization due to asthma in the last 12 months, and asthma medication use were not considered. However, time-dependent exposure to the effect of school neighbourhoods is expected to be associated with severe exacerbation of asthma in asthmatic children. Nevertheless, it will be important to assess the effect of long-term exposure to school neighbourhoods to understand the extent of health effects. The potential selection bias is also a limitation; however, no significant differences were found between the children not included in the study and those included, being expected that our associations were most likely not biased. Additionally, we measured the effect of schools' neighbourhoods using a robust statistical tool that allowed a multilevel approach, considering the complex relationship among the different levels of variables. Our results are also limited by low intraclass correlation coefficients (ICCs) to estimate the percent of total variance in outcomes between neighbourhoods generated by the variables of the multilevel analysis. However, even low ICCs may coexist with important fixed effects of contextual variables. Public health is full of examples of risk factors that explain very little inter-individual variance but are considered important predictors of health outcomes. Thus, as Duncan and colleagues[31] have stated, even variables with low ICCs are considered important predictors of health outcomes and are compatible with important policy effects of neighbourhood characteristics on health. Since ICCs represent the proportion of the variance at the school level rather than individual, they may indicate to what extent school interventions and policies influence outcome-relevant individual predictors[32]. Our results suggest that the school neighbourhood explains an important portion of the variance for all outcomes suggesting that school-level changes may have an important impact on children health outcomes. Furthermore, higher ICCs suggest that the effect on lung function and autonomic nervous system activity in children may be predicted by school neighbourhood as well as characteristics of the children.

Our study has also important strengths. To our knowledge, this is the first community-based study evaluating the effect of schools' neighbourhoods on lung function, airway reversibility and inflammation, and autonomic nervous system activity. Additionally, we performed a comprehensive clinical assessment with a large number of participants, including an assessment of autonomic status that allowed us to assess the children's ability to respond to stress. Different studies have demonstrated that subjects with increased bronchial hyperresponsiveness have higher vagal tone, proposing that increased parasympathetic activity could predispose individuals to increased bronchomotor tone[33,34]. However, according to the European Respiratory Society (ERS) and American Thoracic Society (ATS) guidelines, bronchial responsiveness tests are suitable for adults and older children. Young children have a short concentration span and relatively poor cooperation on these pulmonary function tests[35]. Although this study assessed the effect of schools' neighbourhoods on lung function, asthma is characterized by airflow obstruction[36] with changes in lung function parameters[37]. Regular assessment of lung function, namely FEV1, might help to identify children at risk for developing a progressive decline in airflow[38]. Furthermore, airway obstruction in children is often triggered by environmental factors. Previous studies have reported associations between exposure to urban areas and adverse respiratory health effects, especially in children, with the ESCAPE meta-analysis of data for 5921 children from five European birth cohorts reporting that annual exposures to NO_2, NO_x, PM_{10}, and $PM_{2.5}$ were associated with reduced lung function[2]. The negative impact of exposure to urban environment has also been further reinforced by Mudway, *et al.*[3], in which exposure to urban air, particularly to NO_x and NO_2, was inversely associated with lung function, and by Gauderman, *et al.*[39], which showed that reductions in pollution delivered significant improvements in FEV1 and FVC. Taken together, our findings may contribute support for plans of action aiming to improve urbanization plans in cities and thereby improve respiratory health in children. This study assessed the effects of green and built areas within an urban context, while most previous studies of environmental impacts on asthma and allergies have reported differences between urban and rural environments. Our results suggested that the presence of urban green areas has a positive effect on lung function. Our findings suggest that autonomic nervous system may play a role in mediating the interaction between the environment and the individual (Fig. 1).

Several studies have addressed the use of pupillometry to measure autonomic nervous system activity, using different indices from the constriction (parasympathetic) and dilation (sympathetic) phases in response to light[40,41]. Autonomic balance can change with an increase in vagal activity by the simple act of viewing natural

Outcome	Walkabilityβ (95% CI)	School		
		ICC	Variance	Explained variation*
FVC				
Model 0	−0.58 (−2.79; 1.63)	—	—	—
Model 1	—	1.78%	4.48	Reference
Model 5[a]	−2.62 (−6.00; 0.77)	1.32E-6%	2.51E-6	>99.9%
FEV_1				
Model 0	−1.02 (−3.07; 1.02)	—	—	—
Model 1	—	2.09%	4.53	Reference
Model 5[a]	−2.63 (−5.71; 0.46)	4.47E-7%	7.09E-7	>99.9%
$FEF_{25\%-75\%}$				
Model 0	−1.27 (−4.74; 1.98)	—	—	—
Model 1	—	0.37%	2.14	Reference
Model 5[a]	−0.72 (−6.33; 4.89)	5.27E-7	2.77E-6	>99.9%
EBC pH				
Model 0	0.09 (−0.03; 0.21)	—	—	—
Model 1	—	3.48%	0.03	Reference
Model 5[a]	−0.004 (−0.34; 0.33)	8.52%	0.08	>−99.9%
Exhaled NO				
Model 0	−0.05 (−0.17; 0.07)	—	—	—
Model 1	—	3.98%	0.03	Reference
Model 5[b]	−0.07 (−0.38; 0.23)	8.07%	0.06	−90.6%
Baseline pupil diameter				
Model 0	−0.22 (−0.34; −0.09)	—	—	—
Model 1	—	20.4%	0.158	Reference
Model 5[c]	−0.18 (−0.54; 0.17)	22.1%	0.178	−12.5%
Final pupil diameter				
Model 0	−0.04 (−0.13; 0.05)	—	—	—
Model 1	—	14.1%	0.053	Reference
Model 5[c]	−0.05 (−0.26; 0.16)	15.1%	0.059	−10.3%
ACV				
Model 0	−0.22 (−0.32; −0.12)	—	—	—
Model 1	—	14.7%	0.070	Reference
Model 5[c]	−0.21 (−0.46; 0.03)	16.7%	0.081	−16.4%
MCV				
Model 0	−0.31 (−0.45; −0.17)	—	—	—
Model 1	—	12.3%	0.112	Reference
Model 5[c]	−0.29 (−0.60; 0.02)	10.1%	0.091	18.0%
Constriction amplitude				
Model 0	−1.94 (−2.65; −1.23)	—	—	—
Model 1	—	8.38%	2.073	Reference
Model 5[c]	−1.62 (−2.87; −0.37)	7.52%	1.847	10.9%
ADV				
Model 0	0.01 (−0.04; 0.06)	—	—	—
Model 1	—	8.57E-8%	8.10E-11	Reference
Model 5[c]	0.005 (−0.06; 0.07)	7.82E-8%	7.48E-11	7.60%
T75				
Model 0	−0.17 (−0.29; −0.06)	—	—	—
Model 1	—	4.53%	0.023	Reference
Model 5[c]	−0.19 (−0.36; −0.02)	3.19%	0.016	29.3%

Table 2. Multilevel model analysis of the association between individual and walkability and lung function, pH, exhaled NO and pupillometry parameters explained by school. *corresponds to the proportion of between-schools variance that could be explained by exposure and individual characteristics; 95% CI: 95% confidence interval; ICC: intra-class correlation coefficient; FVC: forced vital capacity; FEV_1: forced expiratory volume in the first second of FVC; FEF_{25-75}: forced expiratory flow in the middle portion of FVC; EBC: Exhaled breath condensate; ACV: Average constriction velocity; MCV: Maximum constriction velocity; ADV: Average dilation velocity; T75: the total time taken by the pupil to recover 75% of its initial resting diameter after it reached the peak of constriction. Model 0 only included the PC1 and PC2 score; [a] Model 1 is null model, baseline model without any exposure variable; Model 5[a] is additionally adjusted for age, sex, asthma, WHO z-score for BMI and family history of asthma or allergy; Model 5[a] is additionally adjusted for age, sex, asthma, atopy, WHO z-score for BMI and family history of asthma or allergy; Model 5[c] is additionally adjusted for age, sex, asthma, and WHO z-score for BMI.

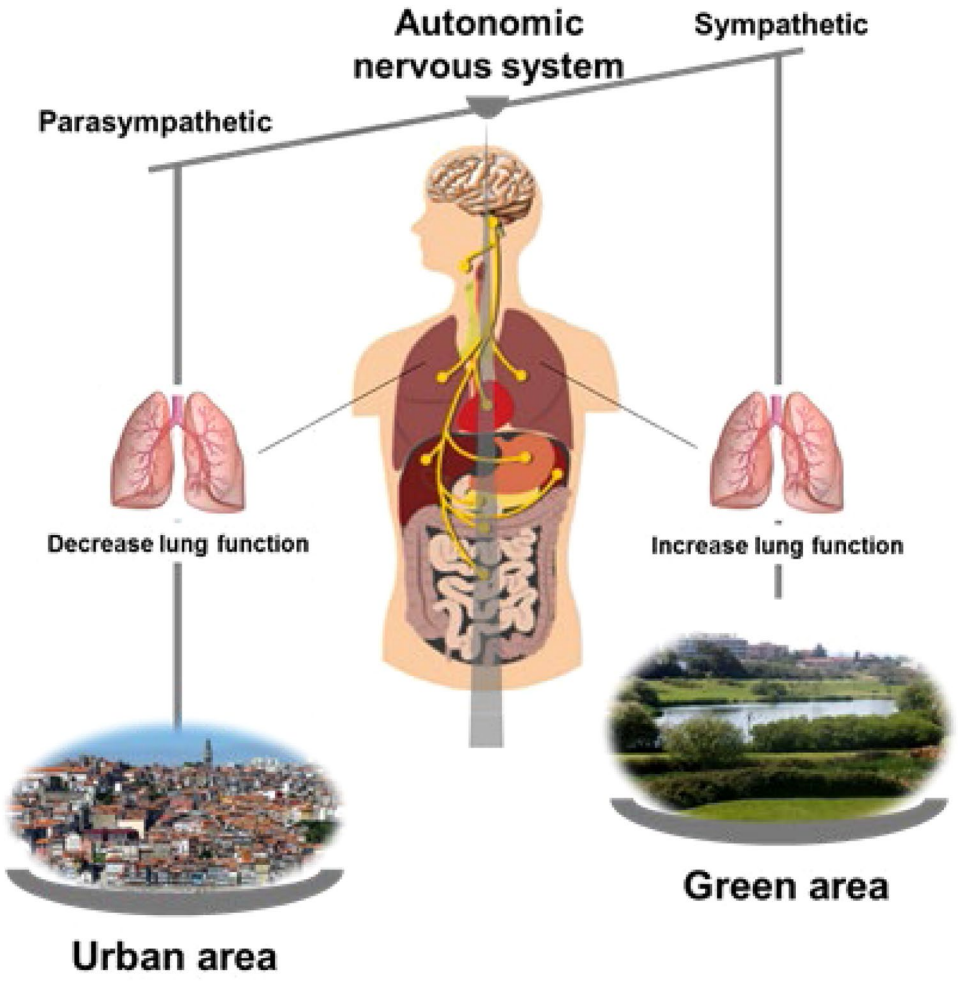

Figure 1. Environment-lung function interaction: a hypothesis focused on autonomic nervous system activity.

scenes, as has been recently shown by Gladwell and colleagues[42]. In their study, a slideshow containing natural scenes, compared with another that incorporated built or urban scenes lacking green space, induced changes in autonomic control via increases in vagal modulation[42]. Additionally, a review of field experiments conducted in 24 forests across Japan on the effects of shinrin-yoku (taking in the forest atmosphere, or "forest bathing") showed that forest environments could lower concentrations of cortisol, decrease heart rate and blood pressure, increase parasympathetic nerve activity, and lower sympathetic activity compared with city settings[43]. However, comparisons of our findings with those of other studies are limited by the different methodologies used to assess autonomic nervous system activity and environmental exposure. The differences found in autonomic nervous system response may be related to the effects of the type of natural settings (parks, gardens, sports fields, forests, tree corridors, or other green space types) and the time spent in each area[44]. In this study, we assess the effect of green areas in an urban environment, where green areas may be smaller and where children are expected to spend less time, as opposed to previous studies in Japan that reported the effect of green areas outside the city, specifically, in forest areas[43]. Thus, urban green areas may have a different effect on autonomic nervous system activity. In addition, several animal studies also highlight the role of autonomic nervous system balance in the interaction between the environment and the individual[45,46].

In our study, built areas around schools adversely affect lung function but not eosinophilic airway inflammation. While the effect of outdoor air pollution on asthma and related symptoms is already recognized, the underlying mechanisms remain unclear[47]. Air pollutants, such as particulate matter, ozone, and nitrogen dioxide, can activate the transient receptor potential (TRP) cation channels on airway C-fibre sensory nerves, namely, TRP vanilloid type 1 (TRPV1) and ankyrin (TRPA1), and cause several responses, such as bronchoconstriction, mucus secretion, airway irritation, and cough, mediated by the efferent pathways of the autonomic nervous system[15]. Akopian *et al*. and Geppetti *et al*. described the association between environmental pollutants and the expression of TRP channels in pulmonary disease, providing evidence for the role of autonomic nervous system activity in the regulation of airway function[15,48]. Therefore, exposure to air pollution is expected to be higher in built areas around schools' neighbourhood than in green areas and may be associated with an activation and/or increased expression of TRPV1 and TRPA1. This may, in turn, result in increased parasympathetic activity with subsequent decreased lung function.

Recent studies have shown evidence of beneficial associations between greenness and health outcomes. Urban green spaces not only provide balance for ecosystems but also promote physical activity, psychological well-being, and public health in urban populations[49]. Greenness may influence health by promoting physical activity and opportunities for social interactions, decreasing the risk of many chronic diseases and psychophysiological stress and reducing air pollution levels, noise, and heat exposure[50]. In children, exposure to green areas has been associated with reduced obesity and sedentary behaviours[50,51]. Ruokolainen and colleagues have shown the amount of forest and agricultural land around homes to be inversely associated with the risk of atopy in children[52]. These findings provide support for a role of natural environmental on the regulation of the T_H1, T_H2 immune response mediated by the children commensal microbiota[52,53]. Furthermore, in children living in greener areas of Vancouver, as measured by the normalized differential vegetation index, had a slightly reduced risk of incidence of asthma (aOR = 0.96; 95% CI 0.93–0.99)[54]. Similarly, lower asthma prevalence in areas with greater tree density in New York City has been reported[55]. Nevertheless, no individual-level studies are available to compare with our findings; however, these associations are similar to the reported results of previous studies on the association between greenness and asthma. Although several studies reported the role of greenness as a buffer against exposure to air pollution and the positive effect of greenspaces in urban context[50,56], air pollution can also affect plant health and functions and limit pollutant dispersion and thus increase local pollutant concentration[57,58].

Exploring the effects of schools' neighbourhoods is crucial for planning, defining guidelines, and making recommendations to cities planners and decision makers in order to create healthier and sustainable urban environments, with potential to protect citizens against the development of asthma and allergic diseases. Thus, our results meet the goals of the WHO European Healthy Cities Network, demonstrating the importance of policies and scientific evidence for health development, public health and urban regeneration to promote and protect human health. Furthermore, this study may contribute to changes in urban environments, such as introducing or improving existing green spaces (parks, green corridors, urban gardens or green exercise programmes), which would provide opportunities for health improvement and social interactions, thus adding to the additional benefits of green urban areas to the local economies, sustainability and self-sufficiency of cities.

The present study demonstrates that the neighbourhoods around schools may have an effect on child health, specifically on lung function and on autonomic nervous system activity. The effects on lung function may be potentially mediated by an increase in parasympathetic activity. These results also underline the positive health effects of green areas in school neighbourhoods, contributing to the implementation of urban planning policies and practices that may promote a healthy lifestyle and reconnection with nature.

Methods

The present study included participants from a cross-sectional study assembled in Porto, Portugal. The 20 schools with the highest number of students were selected from a total of 53 primary schools, corresponding to a total of 71 assessed classrooms (see the methods section in the Online Repository). The evaluations included a questionnaire and a physical and clinical assessment of children. The University Health Ethics Committee approved the study, and informed consent was obtained from the children's legal guardians. All research was performed in accordance with the Declaration of Helsinki.

Questionnaire. The evaluation included a self-administered ISAAC-based questionnaire filled out by parents, covering information on social, demographic and behavioural characteristics and questions regarding the respiratory/allergic health of the children (ever had and over the past 12 months) (see the methods section in the Online Repository).

Physical and clinical assessment. A physical and clinical assessment was also performed at each primary school by a trained health professional. Spirometry with bronchodilation, exhaled level of nitric oxide, exhaled breath condensate (EBC), pupillometry, skin prick test (SPT), weight, and height were measured for all participants (physical and clinical assessment methods are detailed in the Supplementary Material).

Pupillary measurements were taken with a portable infrared PLR-200 pupillometer (NeurOptics PLR-200™ Pupillometer, NeurOptics Inc., CA). Children spent at least 15 min in a semi-dark and quiet room to allow pupillary adjustment to the low level of light, after which they were instructed to focus with the eye that was not being tested on a small object three metres away, keeping their head straight and eyes wide open during targeting and measurement. Light-emitting diodes briefly illuminated the eye once with a peak wavelength of 180 nm. One pupil light response curve for each eye was recorded for each child. Data on the diameter (millimetres) of the pupil before the light stimulus (initial) and at constriction peak (minimal), relative constriction amplitude (%), maximum constriction velocity (MCV), average constriction (ACV) and dilation (ADV) velocities (mm/s), and total time (seconds) taken by the pupil to recover 75% of its initial resting diameter after it reached the peak of constriction (T75) were recorded for each child. Pupillometry is a simple, noninvasive technique that can provide valuable data concerning the functioning of both branches of the autonomic nervous system. Pupil diameter, ACV, MCV, and constriction amplitude are related to parasympathetic activity, while ADV and T75 are measures of sympathetic activity[59] (Supplementary Material).

Urban land use. The land use near each school was calculated on the basis of the European Urban Atlas using a geographical information system (GIS). The Urban Atlas (https://www.eea.europa.eu/data-and-maps/data/copernicus-land-monitoring-service-urban-atlas) city information is currently the most up-to-date, harmonized database for the European Union countries, offering a high-resolution land-use map of cities (population $\geq$ 100,000), mapped using a total of 20 land use classes (Supplementary Table S3)[60]. A circular buffer of 500 metres around each participant's primary school address was created (Fig. 2). This buffer was based on reasonable walking distances described by Browson and colleagues[61], corresponding to approximately 6 minutes' walking distance for children.

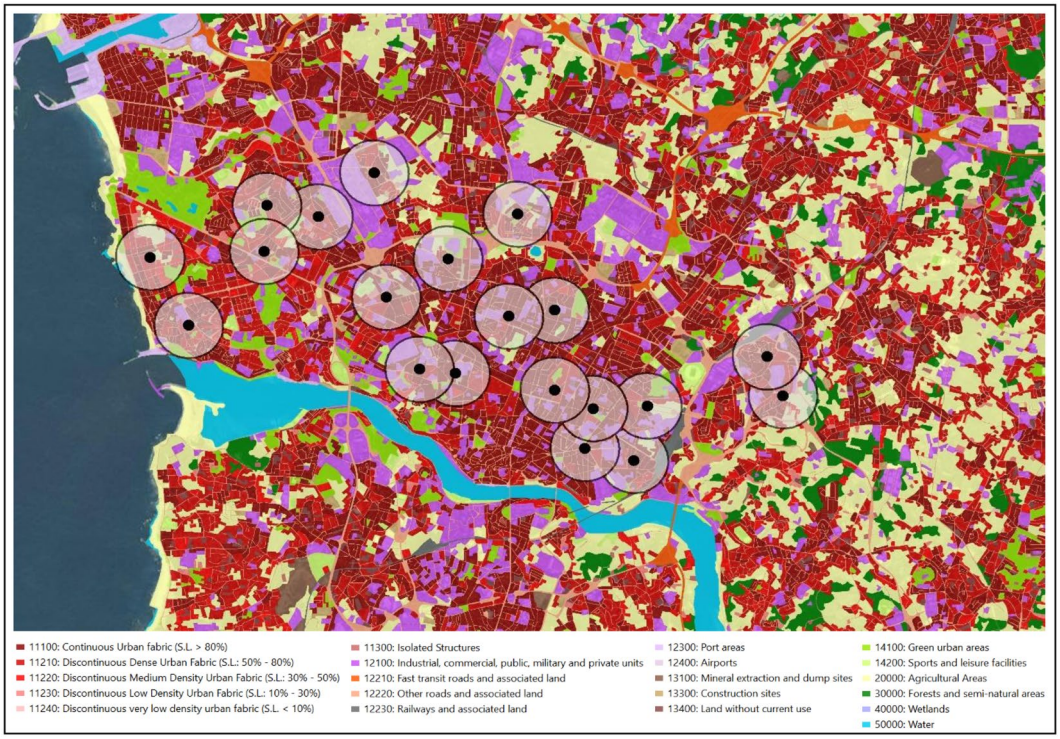

Figure 2. Neighbourhood land use around the 20 evaluated primary schools in Porto. Each school was represented by a point and a circular buffer of 500 metres. For this assessment, we used the ArcGIS 10.4 Network Analyst tool (Environmental Systems Research Institute, ESRI, Redlands, CA, USA).

Walkability. The term walkability has been used to conceptualise a combination of built environment factors such as street connectivity, residential density, net area retail and land use mix, that are conducive to walking (i.e. walking to destinations, including work, school, shopping)[25]. Walkability is an indicator of how user-friendly a neighbourhood area is for walking and biking[25].

The walkability index was calculated on the basis street connectivity, residential density, and land use mix (expressed as an index of entropy), within the 500-metre buffer. This calculation has been previously described and determined across Porto neighbourhoods by Ribeiro and colleagues[62]. Briefly, the street connectivity was calculated from the density of street junctions within the primary school's neighbourhood. Residential density in each neighbourhood was obtained by calculating the density (number/area) of households. Land use mix expresses the diversity of land-use types in each neighbourhood (commercial, residential, recreational/leisure, business/industrial, educational and others).

After these three components were calculated for each neighbourhood (connectivity, residential density, and land use mix), the raw values were normalized using z-scores. The walkability index was calculated according to the following formula:

$$\text{Walkability} = (2_{*}\text{z–connectivity}) + (\text{z–residential density}) + (\text{z–land use mix})$$

This formula is an adapted version of the formula of Frank and colleagues[25]. Next, the values were normalized between zero (least walkable) and one (most walkable). Primary schools' neighbourhoods were characterized according to tertiles (from low to high) of neighbourhood walkability (Fig. 3).

Participants. In total, 1602 children (7–12 years old), all in the 3rd and/or 4th grades, were invited to participate. Among them, 686 did not return the signed informed consent form and 58 refused to undergo clinical tests. Among the remaining 858 children, 146 were excluded owing to poor-quality data. Thus, this study was based on data from 701 children (50.9% girls). Of those, almost 9.4% reported wheezing symptoms, and 12% reported cough symptoms. The prevalence of asthma, rhinitis, current rhinitis, and atopy were 10.7%, 13.0%, 30.4%, and 35.5%, respectively (Table 3).

Data analysis. The Kolmogorov-Smirnov test was used to check continuous variables for normality. The Mann-Whitney test was used to compare variables between girls and boys. Significant differences were defined according to an α-value of 5% ($p < 0.05$).

Principal component analysis (PCA) was used to identify major neighbourhood patterns based on 20 land use classes. Varimax rotation was performed to simplify the interpretation of the factor loading structure. A fixed number of factors were extracted, and two principal components were selected on that basis. The PCA divided neighbourhood land use around schools into two principal components (PC1, PC2) (Supplementary Table S4). Between the two factors, PC1 had higher absolute correlation with discontinuous dense urban fabric, discontinuous medium-density urban land, green urban areas, and water bodies while PC2 had higher absolute correlation with

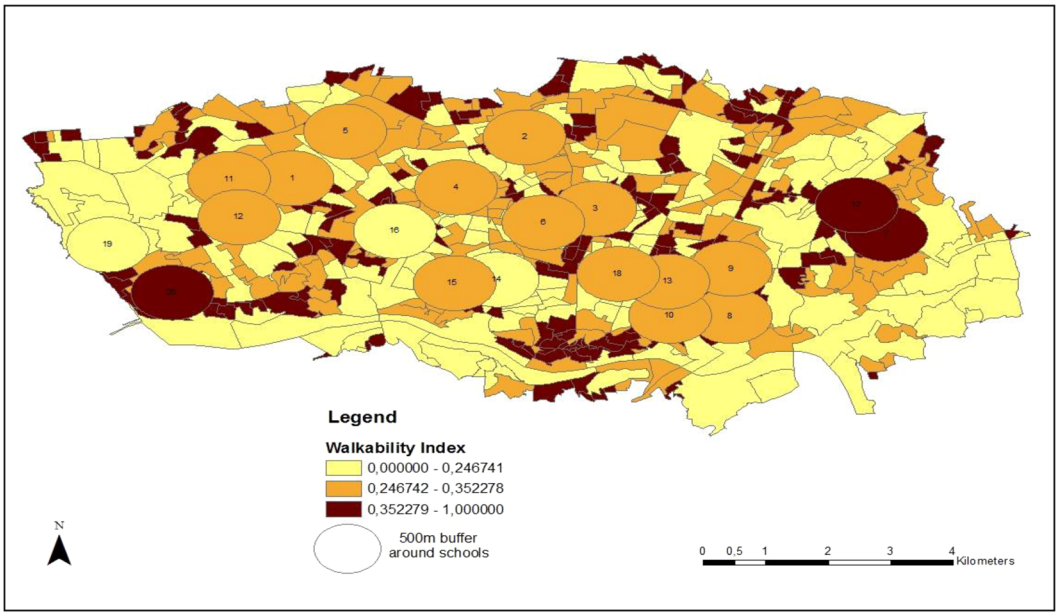

Figure 3. Neighbourhood walkability index around each primary school. Each school, represented by a number and a circular buffer of 500 metres, was characterized according to tertiles of neighbourhood walkability. For this assessment, we used the ArcGIS 10.4 Network Analyst tool (Environmental Systems Research Institute, ESRI, Redlands, CA, USA).

Characteristics	Total n = 701	Girls n = 357	Boys n = 344	*p* value*
Age [years (mean ± SD)]	9 ± 0.8	9 ± 0.8	9 ± 0.8	0.574
Wheezing symptoms [n (%)]	66 (9.4)	34 (9.5)	32 (9.3)	0.920
Cough symptoms [n (%)]	82 (11.7)	45 (12.6)	37 (10.8)	0.446
Asthma [n (%)]**	75 (10.7)	47 (13.2)	28 (8.1)	0.037
Rhinitis [n (%)]	81 (13.0)	36 (11.3)	45 (14.8)	0.233
Current rhinitis [n (%)]	69 (30.4)	32 (26.8)	37 (34.3)	0.611
Atopy [n (%)]	245 (355)	128 (36.4)	117 (34.5)	0.580
BMI [n (%)]				0.978
Underweight	33 (4.7)	17 (4.8)	16 (4.7)	
Normal weight	478 (68.2)	242 (67.8)	236 (68.6)	
Overweight	108 (15.4)	57 (16.0)	51 (14.8)	
Obese	82 (11.7)	41 (11.5)	41 (11.9)	
EBC pH	6.9 (0.9)	6.8 (0.9)	6.9 (0.9)	0.105
Exhaled NO (ppb)	17.1 (20.1)	14.6 (15.5)	19.6 (23.7)	0.010
Lung function				
FVC (%)	106.8 (15.7)	113.4 (14.9)	99.7 (13.2)	< 0.0001
FEV_1 (%)	102.8 (14.6)	109.3 (13.8)	95.8 (11.8)	< 0.0001
FEF_{25-75} (%)	98.1 (24.3)	98.6 (24.3)	97.6 (24.2)	0.486
Pupillometry				
Baseline pupil diameter (mm)	5.3 (0.9)	5.2 (0.9)	5.3 (0.9)	0.137
Final pupil diameter (mm)	3.4 (0.6)	3.4 (0.6)	3.4 (0.6)	0.745
ACV (mm/s)	−3.9 0.7)	−3.9 (0.7)	−4.0 (0.7)	0.096
MCV (mm/s)	−5.2 (1.0)	−5.1 (1.0)	−5.4 (1.0)	0.021
Constriction amplitude (%)	35.1 (4.8)	34.5 (4.8)	35.6 (4.7)	0.017
ADV (mm/s)	1.1 (0.3)	1.2 (0.3)	1.1 (0.3)	0.750
T75 (s)	1.7 (0.7)	1.7 (0.7)	1.8 (0.7)	0.203

Table 3. Characteristics of the participants. Data reported as median (interquartile range) unless otherwise stated. BMI: body mass index; FVC: forced vital capacity; FEV_1: forced expiratory volume in the first second of FVC; FEF_{25-75}: forced expiratory flow in the middle portion of FVC; EBC: Exhaled breath condensate; ACV: Average constriction velocity; MCV: Maximum constriction velocity; ADV: Average dilation velocity; T75: the total time taken by the pupil to recover 75 of its initial resting diameter after it reached the peak of constriction.

construction sites, land without current use, and railways. PC1 was characterized as green urban areas, and PC2 as built areas. Afterwards, the PC1 and PC2 scores were ranked from 1 to 20, and the rank numbers were divided by 20.

Mixed-effect models with a random effect of school were used to measure the effect of schools on lung function, airway inflammation and autonomic nervous system in children. The intraclass correlation coefficient (ICC) and the proportion of explained variation were used to quantify the effect of schools and to quantify the effect of individual, neighbouring environment, and walkability on the school effect. The effect of schools' neighbourhoods on children health were analysed using a multilevel with individual-level and neighbourhood-level factors. All individual- and neighbourhood-level (as median values) factors were used in the multilevel analysis as independent variables. Five models were considered for the analysis: crude model (model 0 and 1), neighbourhood-level model (model 2), and an individual-neighbourhood level model (mixed effects model, models 3–5). Model 0, only included the PC1 and PC2 score or walkability; Model 1, the null model, baseline model without any exposure variable; Model 2 is adjusted for PC 1 and PC2 score or walkability; Model 3 is additionally adjusted for age, sex and asthma and atopy for exhaled NO; Model 4 is additionally adjusted for WHO z-score for BMI; and Model 5 is additionally adjusted for parental education level and family history of asthma or allergy for lung function parameters, exhaled NO and EBC pH. To minimize errors due to multiple comparisons, the Bonferroni correction was used to assess statistical significance. PCA, mixed-effect models and ICC were computed using the software RStudio, version 1.0.

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Author contributions

I.P., J.C.R., J.M., E.O.F., P.P., P.M., M.F.P., J.P.T. and A.M. were involved in investigation and I.P., J.C.R., D.S., C.M., F.M., T.R., A.R., J.M., L.D., E.O.F., P.P., P.M., M.S., M.F.P., J.P.T., H.B., L.R., T.H. and A.M. gave constructive criticism of the study manuscript. I.P., T.H. and A.M. were involved on study conceptualization and interpretation. I.P. and A.M. conducted the mapping of schools' land use neighborhood and MFP was involved in the determination of the walkability. I.P., M.S., L.R. and A.M. contributed to the statistical analysis. I.P. and A.M. wrote the manuscript with input from all authors. E.O.F. and A.M. obtained funding.

Competing interests

The authors declare no competing interests.

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