Early life environmental exposures and childhood respiratory health

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A la meva família

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We must always attempt to lift as we climb.

– Angela Y. Davis

Abstract

The high prevalence and burden of asthma and other chronic respiratory diseases worldwide has raised concerns about the potential role of environmental exposures. However, the evidence is still inadequate. This Thesis is based on the hypothesis that being exposed to a harmful environment during prenatal life can have a long-term impact on disease later in life (the Developmental Origins of Health and Disease paradigm). The main aim of this doctoral Thesis was to investigate how early life environmental stressors, particularly ubiquitous chemical pollutants and the urban environment, influence children's respiratory health.

In this Thesis we first reviewed the current evidence on the role of prenatal exposure to chemical pollutants, mainly organic pollutants, on children's lung function. Then, we explored the associations of prenatal exposure to chemical pollutants, namely organochlorine compounds and bisphenols, on children's respiratory health using 8 European population-based birth cohort studies. Last, we used a large longitudinal database, the Information System for Research in Primary Care, to establish a new birth cohort by linking electronic health records of parents and children.

Results of this Thesis show that 1) Evidence on prenatal exposure to organic pollutants on childhood lung function, especially organochlorine compounds and bisphenols, is limited. 2) Prenatal exposure to *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) is prevalent and may decrease lung function during childhood. 3)

Prenatal exposure to bisphenol A is prevalent and is associated with higher risk of wheeze and asthma among school-age girls. 4) The urban environment during pregnancy, mainly air pollution, road traffic noise, and availability of blue spaces can affect respiratory health in childhood. 5) We successfully linked the electronic health records of 719,858 children born from 2005 to 2018 to the electronic health records of at least one potential parent in the Information System for Research in Primary Care

In conclusion, prenatal exposure to organochlorine compounds, bisphenol A and to exposures of the urban environment is prevalent and influences children's respiratory health. This Thesis highlights that early identification of the environmental determinants of children's respiratory health is of utmost importance, given their long-term effect on disease throughout life, and provides a new large birth cohort from the linkage of parent's and children's electronic health records.

Resum

La gran prevalença d'asma i altres malalties respiratòries cròniques al món ha despertat preocupacions sobre el possible paper de les exposicions ambientals. Tot i així, l'evidència és inadequada. Aquesta Tesi es basa en la hipòtesi que postula que l'exposició a un ambient nociu durant l'etapa prenatal por tenir un impacte a llarg termini en la salut (el paradigma de l'origen durant el desenvolupament de la salut i la malaltia). L'objectiu principal d'aquesta Tesi doctoral és investigar com influeixen en la salut respiratòria infantil les exposicions ambientals, especialment els contaminants químics i el medi urbà, durant les primeres etapes de la vida.

En aquesta Tesi primer vam revisar l'evidència actual del paper de l'exposició prenatal a contaminants químics, principalment contaminants orgànics, sobre la funció pulmonar durant la infància. A continuació, vam explorar les associacions entre l'exposició prenatal a contaminants químics, principalment compostos organoclorats i bisfenols, i la salut respiratòria infantil mitjançant 8 estudis de cohort de naixement europeus. Per últim, vam utilitzar una gran base de dades longitudinal, el Sistema d'Informació per al Desenvolupament de la Investigació en Atenció Primària, per establir una nova cohort de naixement mitjançant la vinculació de la història clínica electrònica de pares i fills.

Els resultats d'aquesta Tesi mostren que 1) L'evidència sobre l'exposició prenatal a contaminants orgànics sobre la funció

pulmonar infantil, especialment compostos organoclorats i bisfenols, és limitada. 2) L'exposició prenatal a *p,p′*-diclorodifenildicloroetilè (*p, p′*-DDE) és freqüent i pot disminuir la funció pulmonar durant la infància. 3) L'exposició prenatal al bisfenol A és freqüent i s'associa amb un major risc de sibilàncies i asma entre les nenes en edat escolar. 4) L'entorn urbà durant l'embaràs, principalment la contaminació atmosfèrica, el soroll del trànsit i la disponibilitat d'espais blaus, poden afectar la salut respiratòria durant la infància. 5) Hem vinculat amb èxit la història clínica electrònica de 719.858 nens nascuts del 2005 al 2018 amb les d'almenys un dels potencials progenitors al Sistema d'Informació per al Desenvolupament de la Investigació en Atenció Primària.

En conclusió, l'exposició prenatal a compostos organoclorats, bisfenol A i a exposicions del medi urbà és freqüent i influeix en la salut respiratòria dels infants. Aquesta Tesi posa en relleu que la identificació precoç dels determinants ambientals de la salut respiratòria infantil és de màxima importància, atès el seu efecte a llarg termini sobre les malalties al llarg de la vida, i proporciona una nova gran cohort de naixement creada a partir de la vinculació de la història clínica electrònica de pares i fills.

Preface

This Thesis was developed through a strong collaboration between the Barcelona Institute for Global Health (ISGlobal) and the Institute for Primary Health Care Research Jordi Gol (IDIAPJGol). It complies with the procedures and regulations of the Biomedicine PhD program of the Department of Experimental and Health Sciences of the *Universitat Pompeu Fabra*, Barcelona, Spain. The Thesis was supervised by Dr Maribel Casas and Dr Talita Duarte-Salles, was tutorised by Dr Jordi Sunyer, and was developed between 2017 and 2021.

The Thesis contains one review and four original research papers (2 published, 1 under review, 2 in preparation). For all the scientific papers, the PhD candidate formulated the research objective, conceptualized the study design, performed the extensive data management and statistical analyses, interpreted the findings and wrote the scientific articles for publication. The Thesis contributed to the understanding of: 1) the current knowledge on the effects of prenatal exposure to organic pollutants on children's lung function, 2) the effects of prenatal exposure to organochlorine compounds on children's lung function, 3) the effects of prenatal exposure to bisphenols on children's respiratory health, 4) the effects of maternal exposure to the urban environment during pregnancy on children's respiratory health, and 5) the creation of a new parent-child cohort linking electronic health records from the Information System for Research in Primary Care database in Catalonia.

During the Thesis, the PhD candidate earned a Horizon2020-funded LifeCycle Project fellowship to conduct a 6-months research stay at the Erasmus Medical Centre, Rotterdam, The Netherlands. In addition, the PhD candidate attended several national and international conferences where she presented the results of the papers. Also, the PhD candidate was closely involved in the fieldwork and data management of the INMA Spanish birth cohort by developing three yearly follow-up newsletters for the INMA Sabadell participants, developing a protocol to describe the spirometry cleaning procedure, and by cleaning the spirometries performed at 18 years in the INMA Menorca cohort. She also contributed to the development of the standard operating procedures (SOP) for spirometry of the new visit of the HELIX subcohort children at the age of 12-18 years. Further, she wrote a children's health status report for the municipality of Sabadell. Also, the PhD candidate participated in the INMA Respiratory and Allergy working group and in the Chronic Airway Disease Early Stratification (CADSET) consortium.

Besides the Thesis projects, she co-authored seven original research papers, supervised a Master's Thesis (Master in Public Health, *Universitat Pompeu Fabra*), taught a seminar on endocrine disruptors (Superior Degree in Nutrition, *IES Guineueta*), and carried out outreach activities giving scientific talks and writing dissemination articles.

Abbreviations

ALSPAC	Avon Longitudinal Study of Parents And Children
ATS	American Thoracic Society
BiB	Born in Bradford
BKMR	Bayesian Kernel Machine Regression
BMI	Body mass index
BPA	Bisphenol A
BPF	Bisphenol F
BPS	Bisphenol S
COPD	Chronic obstructive pulmonary disease
DOHaD	Developmental Origins of Health and Disease
EDEN	*Étude des déterminants pré et postnatals du développement de la santé de l'enfant*
EHR	Electronic health records
ERS	European Respiratory Society
ESCAPE	European Study of Cohorts for Air Pollution Effects
EU	European Union
ExWAS	Exposome wide association study
$FEF_{25-75\%}$	Mid expiratory flow
FEV_1	Forced expiratory volume in 1 s
FVC	Forced vital capacity
GIS	Geographic information system
GLI	Global Lung Function Initiative
HCB	Hexachlorobenzene
HELIX	Human Early-Life Exposome

ICD-10	International Classification of Diseases 10th revision
ICS	*Institut Català de la Salut*
INMA	*INfancia y Medio Ambiente*
IPD	Individual participant data
IQ	Intelligence quotient
IQR	Interquartile range
ISAAC	International Study of Asthma and Allergies in Childhood
KANC	Kaunas cohort
Lden	Noise levels during day, evening, and night
Ln	Noise levels at night
LUR	Land use regression
MeDALL	Mechanisms of the Development of ALLergy
MoBa	Norwegian Mother and Child Cohort Study
NASS	Number of affiliation to the social security
NDVI	Normalised Difference Vegetation Index
NO_2	Nitrogen dioxide
NO_x	Nitrogen oxides
OC	Organochlorine compound
PBPK	Physiologically based pharmacokinetic
PCB	Polychlorinated biphenyl
PIAMA	Prevention and Incidence of Asthma and Mite Allergy
PIC	Personal identification code
PM	Particulate matter

$PM_{2.5\ abs}$	Particulate matter absorbance with diameter <2.5 μm
$PM_{2.5}$	Particulate matter with diameter <2.5 μm
PM_{10}	Particulate matter with diameter <10 μm
PM_{coarse}	Particulate matter with diameter 2.5-10 μm
p,p'-DDE	Dichlorodiphenyldichloroethylene
p-p'-DDT	Dichlorodiphenyl-trichloroethane
RHEA	Mother Child Cohort study in Crete
SES	Socioeconomic status
SIDIAP	Information System for Research in Primary Care
TTP	Third trusted party
WHO	World Health Organization

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1. INTRODUCTION

1.1. Burden of altered lung function and asthma

Lung function

Lung function is an objective marker of respiratory health and a predictor of morbidity and mortality (1). Lung function deficits are a common feature of respiratory morbidity like severe asthma and chronic obstructive pulmonary disease (COPD). However, deficits can also go unnoticed without respiratory symptoms. Even when lung function deficits do not present clinical manifestations, they still pose a substantial risk for future respiratory morbidity. Diminished lung function is also associated with cardiovascular and metabolic morbidity, early multimorbidity, and premature mortality (2,3).

Lung function follows a continuum of three phases over the lifespan. First, there is a period of growth from birth to early adulthood (20-25 years of age), when it reaches its maximal capacity, later it stabilizes for a few years and then declines due to physiological ageing of the lung (4). Many genetic and environmental factors can disrupt these phases. For example, lung function at birth and during childhood can be influenced by conditions during pregnancy such as maternal smoking (5) or intrauterine growth restriction (6) that might limit the correct foetal lung development.

The effects of both genetic and environmental factors lead to different patterns of lung function growth and decline over the lifespan. Many studies have identified several lung function

trajectories (4,7–10) and some of these trajectories have been associated with relevant implications for health. Lung function deficits in childhood resulting from impaired lung development in early life are of special importance because they are a risk factor for reduced lung function and respiratory morbidity in adulthood (11–13). Individuals who do not reach maximal lung growth are at increased risk of chronic respiratory diseases such as asthma (14,15), COPD and asthma-COPD overlap syndrome in adulthood (16).

Asthma

Chronic respiratory diseases, such as asthma and COPD, remain among the leading causes of mortality and morbidity worldwide and contribute substantially to the global burden of non-communicable diseases (17). They pose a considerable burden on society with regard to premature mortality, disability, healthcare costs, and loss of production costs. It is estimated that €380 billion is attributable to the cost of chronic respiratory diseases in the 28 European Union (EU) member states annually. In EU, asthma alone poses €72.2 billion including direct and indirect costs and 697,000 disability-adjusted life years lost per year (18).

Asthma is the most common chronic respiratory disease, affecting more than 300 million people worldwide (19) and is the most common chronic disease in children (20). The prevalence of asthma symptoms in children presents a great variability across countries. In EU countries, according to the International Study of Asthma and Allergies in Childhood (ISAAC) surveillance, prevalence of asthma

symptoms varied from 5% to over 20%. For example, in Spain, the prevalence was close to 10% among children aged 6-7 and 13-14 years of age (21).

Asthma is a complex and heterogeneous disease usually characterised by chronic airway inflammation and airway hyperresponsiveness. According to the Global Initiative for Asthma, asthma is defined as a *heterogeneous disease, usually characterised 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* (22). In population-based studies, asthma is commonly assessed following the ISAAC (23) and Mechanisms of the Development of ALLergy (MeDALL) (24) validated questionnaires and recommendations, that include "ever been diagnosed by a doctor of asthma", "wheezing episodes in the 12 months prior to the assessment", and "asthma medication use in the 12 months prior to the assessment". ISAAC is an epidemiological study that aimed to assess asthma and allergic diseases worldwide and to establish a standardised methodology to facilitate international collaboration in the study of such diseases (23). MeDALL aimed to increase the amount of harmonised questions beyond those proposed within ISAAC and to describe existing asthma and allergic phenotypes (24).

There is growing consensus in the research community to not consider asthma as a disease itself, but as an umbrella term of several

diseases presenting similar manifestations, usually referred to as asthma phenotypes, that may originate from different underlying pathophysiological mechanisms (25,26). Asthma phenotypes are clusters of demographic, clinical, and pathophysiological characteristics (22). The most common phenotypes are: allergic asthma, non-allergic asthma, adult or late-onset, asthma with persistent airflow limitation, and asthma with obesity (22). Frequent manifestations across phenotypes, although not necessarily always present, include airflow limitation, eosinophilic airway inflammation, cough, and wheeze.

Wheezing is the major clinical expression of asthma. Severe and persistent wheeze in childhood is a risk factor for asthma and abnormal lung function later in life (27). Wheezing patterns across childhood (28,29) and until adulthood (30) have been identified. Four wheezing patterns and its prevalence were described in children from the Tucson Children's Respiratory Study: never wheezing (51.5%), early wheezing (≤3 years only; 19.9%), late wheezing (3 to 6 years only; 15.0%), and persistent wheezing (≤3 years and 3 to 6 years; 13.7%) (28). A latter study identified in addition an intermediate-onset wheezing pattern (≥2 years) using latent class analysis in the Avon Longitudinal Study of Parents And Children (ALSPAC) and the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) studies (29). Some wheezing patterns were associated with reduced lung function, asthma, and atopy (28,29), also in adults (30).

Asthma can develop at any age, but most cases of chronic asthma develop in the first years of life (31), as well as most symptoms such as wheeze and reduced lung function. The aetiology of asthma is complex and partially unknown. Its heterogeneity among the population has complicated the understanding of its natural course. Along with genetic predisposition, many lifestyle and environmental factors are also involved in its natural history such as tobacco smoking, psychological distress, unhealthy diet, and air pollution (32–35). Identifying preventable risk factors for asthma is key because childhood asthma is associated with reduced quality of life (36), increased healthcare utilization (18), school absenteeism (37), and is an important risk factor for COPD (38,39).

Overall, asthma and lung function during childhood are an important predictor of future morbidity. Lung function growth and asthma development, in turn, are influenced by genetics and environmental factors during early life. Therefore, early identification of preventable environmental factors is relevant given their long-term effect on health and disease.

1.2. Early life origins of respiratory health and disease

1.2.1. The Developmental Origins of Health and Disease

The Developmental Origins of Health and Disease (DOHaD) paradigm postulates that early life environmental factors play a role on health and disease later in life (40). This concept emerged from studies by Barker and colleagues in the mid 1980s where they

observed that poor nutrition in early life, related with impaired infant growth and infant mortality, was associated with cardiovascular disease in adulthood (41–43).

These first studies opened the door to the exploration of many environmental exposures during early development on diverse health outcomes. An increasing number of studies were developed exploring these associations and led to the emergence of a new field of research that considers that the health status of the population can be shaped even before birth. The aim of this field is to clarify whether and how environmental influences during critical windows of development can induce permanent structural and physiological changes that may increase the risk for morbidity later in life. Prenatal and early postnatal years are particularly critical periods of cell differentiation and proliferation and tissue development, including the lung, in which repair mechanisms and detoxifying pathways have not fully developed. This makes these periods especially vulnerable to the effects of environmental exposures.

1.2.2. Overview of the lung and immune system development

The development of the lung starts at the foetal stage and continues after birth until 20 years of age. It consists of five consecutive stages: embryonic, pseudoglandular, canalicular, saccular, and alveolar. Primary lung buds start to develop at 4 weeks of gestation to form the trachea, bronchi, and pulmonary vein and artery. During the second trimester of pregnancy, during the pseudoglandular and canalicular stages, the bronchi continue their segmentation, primitive alveoli are

formed, and the synthesis of surfactant starts. This is considered the most critical stage for the respiratory system since it is when all conducting airways are formed. The saccular and alveolar are the last prenatal stages. They develop from the third trimester of gestation until the second year after birth. During these stages the gas exchange region expands. The expansion triggers the production of extracellular matrix, collagen, and elastin. During these phases, there is further growth of the vascular system associated with the respiratory system. After birth and until the second year of age, alveoli continue developing. The gas exchange regions keep expanding through a branching process in concordance to its associated vascular and nervous systems. From 2 years until adulthood, the process of lung growth and expansion continues by a sustained cellular proliferation (44,45).

The immune system starts developing during the first weeks of gestation and continues developing and maturing into adult life (46). The immune system encompasses the innate and the adaptative immune systems. The innate immune system is an early first line of defence to pathogens and involves neutrophils, monocytes, macrophages and dendritic cells. The adaptative immune system provides humoral responses to eliminate pathogens and induce immunological memory for long-term protection. It involves antibodies and cytokines produced by B cells and T cells, respectively. The developmental processes during gestation are highly influenced by maternal molecular and cellular components that are transferred to the foetus (e.g., maternal antibodies,

inflammatory mediators, micronutrients, chemicals) (47). These complex and multiple signals are responsible for the programming of the immune system and can modulate its homeostatic regulation (48). Each type of immune cell is differentiated and matured at different times during gestation. For example, neutrophils are present at the end of the first trimester and greatly proliferate shortly before birth, even reaching adult levels, but they are not fully functional (49); monocytes proliferate continuously during gestation, but their cytokine production is limited until years after birth (50); B cells appear between 8 to 13 weeks of gestation (48); and T cells appear by 15 weeks of gestation (51). At birth, the immune system is relatively immature and evolves during the postnatal period with the exposure to multiple antigens. During the first months of life, the immune response is mainly driven by the immature innate immune system. However, mechanisms of antigen presentation, phagocytosis, or cytotoxicity are not fully developed until years after birth (49,50). The innate and adaptative systems need to be well balanced and coordinated to ensure an adequate protection against external pathogens, and to avoid inappropriate reactions that lead to autoimmune or allergic diseases (48).

1.3. Early life environmental exposures and lung function and asthma

Environmental exposures that occur during pregnancy and the first years of life can influence the development of the respiratory and immune systems and consequently affect health and disease throughout the life course. In this Thesis we have focused on two

common environmental exposures: 1) chemical pollutants, namely organochlorine compounds (OCs) and bisphenols, and 2) the urban environment. We have focused on OCs and bisphenols because exposure to these compounds occur worldwide and because *in vitro* and *in vivo* studies have demonstrated their capacity to alter the immune system and lung development; however, evidence from epidemiological studies is still limited (52). We have focused on the urban environment because half of the populations live in cities and it is expected to increase in the coming decades, and its related exposures are suspected to influence the immune and lung development. The urban environment is composed of different features including air pollution, noise, traffic, natural spaces, and built environment. Although the respiratory health effects of air pollution have been extensively investigated, there are still discrepancies across studies, especially in the prenatal period, and other components of the urban environment have not been studied in relation to respiratory health (53).

1.3.1. Chemical pollutants

Millions of tons of synthetic chemicals are produced and consumed every year (54). They can be classified into inorganic and organic pollutants. Inorganic pollutants include a variety of heavy metals such as mercury, lead, arsenic, or cadmium; they will not be studied in the present Thesis. Organic pollutants are usually divided into persistent and non-persistent according to their half-lives in the human body. Persistent organic pollutants are characterised by their long biological half-lives (e.g., from months to years) and non-persistent, by their short biological half-lives that range from hours

to days. Organochlorine compounds and bisphenols are the two organic pollutants, persistent and non-persistent, respectively, that are studied in the present Thesis.

Organochlorine compounds (OCs) and bisphenols are among chemical pollutants that have been produced or are currently produced in large quantities worldwide (millions of tons annually) and consequently, human populations are continuously exposed to them. Both groups are classified as endocrine disrupting chemicals, this means that they have the capacity to interfere with the endocrine system and consequently alter many essential body functions such as growth, behaviour, and reproduction (55). Indeed, the term "endocrine disrupting chemical" includes a large number of substances (in some lists more than 1,500) (56) whose primary effect is on the endocrine system through interaction with cellular hormone receptors, hormone synthesis or clearance (55). Of importance, these chemicals can have effects at very low exposure levels, as endogenous hormones do. Low doses can have more potent effects than higher doses, and exposure to multiple chemicals can result in synergistic, antagonistic, or cumulative effects (57). Further, because they can interfere with sex hormones, their potential effects may be sex-dependent (58–60). Foetuses and infants are especially sensitive to chemicals that mimic hormones because the protective mechanisms (i.e., detoxification) existing in adulthood are not completely functional in early life. In the EU, the medical cost associated with exposure to endocrine disrupting chemicals has been estimated at €163 billion a year (61). Nowadays, the reduction of

exposure to endocrine disrupting chemicals represents a priority for action in the EU Commission owing to their high annual production and potential toxicity (62).

Organochlorine compounds

Organochlorine compounds (OCs) are synthetic persistent organic pollutants mainly used as pesticides or in industrial products such as electrical insulators and flame retardants. Their use and production of some of these compounds has been banned for decades (63,64). For example, the former widely used pesticide dichlorodiphenyl-trichloroethane (*p,p'*-DDT) was banned in the EU in the early 80s (64). Due to the strong evidence of the adverse health effects of other OCs, these compounds were internationally banned at the Stockholm Convention in 2001 (65). However, they can bioaccumulate and persist in the environment for long periods. They have long biological half-lives that can range from several years to decades in some compounds (66). Currently, the main source of exposure to OCs is diet (i.e., fatty fish, meat, dairy). Biomonitoring studies show that current populations of pregnant women and children are exposed to these substances, finding detectable levels in over 80% of maternal and child blood samples (67). OCs have been associated with adverse health outcomes such as low birth weight, cognitive impairment, and childhood obesity (52).

OCs may interfere in the lung morphogenesis as well as play a role in inflammation processes both pre- and postnatally. These compounds have estrogenic and anti-androgenic properties (68),

therefore, they may interact with oestrogenic and androgenic receptors and alter the related signalling pathways, including the activation of the aryl hydrocarbon pathway, which has been shown to delay lung development in animal studies (69). Furthermore, exposure to OCs has also been related to inflammation, observing increased interleukins and immunoglobulins in children exposed to them (70–72).

Before this Thesis, only two studies assessed the effects of prenatal exposure to OCs and lung function (73,74). In a Danish birth cohort established in the 80s, prenatal exposure to very high levels of dichlorodiphenyldichloroethylene (*p,p'*-DDE), hexachlorobenzene (HCB), and polychlorinated biphenyls (PCBs) was associated with increased risk of airway obstruction at 20 years of age (73). More recently, in a study assessing the effects of the exposome – the totality of environmental exposures from conception onwards (75) – on lung function, *p,p'*-DDT and *p,p'*-DDE tended to be associated with reduced forced expiratory volume in 1s (FEV_1) in school age children, but results did not reach statistical significance (74).

Several studies have explored the association between prenatal exposure to OCs and respiratory outcomes in the offspring (71,76–82). These studies found that prenatal exposure to OCs, particularly *p,p'*-DDE, HCB, and PCBs, can increase the risk, even at low exposure levels, of lower respiratory tract infections, wheeze, and asthma in children (71,76–82).

In summary, and as reviewed by Vrijheid et al. (52), the evidence of the effects of OCs on respiratory symptoms including wheeze and asthma was moderate; whereas the OCs effects on lung function was insufficient. Contributing to the understanding of this knowledge gap will be the main objective of paper II of this Thesis.

Bisphenols

Bisphenols are non-persistent organic pollutants widely present in daily life products such as plastic packaging, children's toys, thermal paper, and canned food. General population is continuously exposed to them through dermal contact, inhalation or ingestion. Bisphenol A (BPA) is the most produced bisphenol and has been found in >90% of urine samples in the general population (83,84). BPA has been related to adverse health effects (e.g., metabolic and reproductive disorders, behavioural problems) and in 2017 it was considered a "substance of very high concern" by the European Chemical Agency (85). Its production and use has been prohibited in some products and in some countries, giving rise to the production of substitute products of similar structure such as bisphenol S (BPS) and bisphenol F (BPF) (86). After exposure, bisphenols are rapidly excreted from the body (half-life of less than 6 hours).

Bisphenols might interfere in the developing lung and immune system thanks to their capacity to cross the placental barrier and the ubiquitous daily exposure after birth. A study in mice observed that exposure to BPA during gestation severely retarded foetal lung maturation (87). This immaturity was characterized by diminished

alveolar airspace and thickened septa and by diminished number of type I pneumocytes (87). Bisphenols can also induce oxidative stress, endocrine disruption and mitochondrial dysfunction (88–90). These chemicals can alter inflammatory responses through different signalling pathways. They can activate the reactive oxygen species pathway and the mitogen-activated protein kinase signalling pathways that lead to DNA damage and cellular death. Bisphenols might also alter immune responses by the stimulation of pro-inflammatory cytokines and the inhibition of anti-inflammatory cytokines production (91).

Evidence on prenatal BPA exposure and lung function is scarce. A study observed that increasing maternal urinary BPA levels were associated with decreased lung function at 4 years of age but did not see such association at 5 years (92). In the *Étude des déterminants pré et postnatals du développement de la santé de l'enfant* (EDEN) birth cohort study, no association was observed between prenatal BPA levels and predicted FEV_1 at 5 years (93). No association was observed in the Human Early-Life Exposome (HELIX) cohorts between prenatal BPA levels and lung function in school-age children in the first exposome study (74). A recent study assessed the effects of prenatal exposure to 20 endocrine disruptors simultaneously, including BPA, on lung function at 7 years. By using the Bayesian Kernel Machine Regression (BKMR), a statistical tool that allows the identification of the most relevant group and pollutant within the group associated with a specific outcome, authors did not observe any association between BPA and lung function (94).

Prenatal BPA exposure may increase the risk of respiratory symptoms (52), but evidence is limited. Previous to the present Thesis, nine prospective birth cohort studies assessed the association between prenatal exposure to BPA and asthma-related symptoms. Six of them found that prenatal exposure to BPA was associated with increased risk of lower respiratory tract infections, wheeze, and asthma during childhood (92,93,95–98). However, such associations were not found in two other cohort studies that included immune markers (99,100), and one study found prenatal BPA exposure to be associated with a decreased risk of wheeze (101). To the best of our knowledge, there is no evidence of prenatal BPA substitutes on asthma-related outcomes and lung function.

There is insufficient evidence of the effects of prenatal exposure to BPA on respiratory health, both asthma-related symptoms and lung function (52). Also, no study thus far has explored whether BPA substitutes, increasingly present in daily-life products, can affect respiratory health. This gap of knowledge is addressed in paper III of this Thesis.

1.3.2. Urban environment

Half of the global population currently lives in urban settings, and this proportion is expected to increase up to 70% by 2050 with the emergence of new cities and the expansion of existing ones (102). Despite urban living offers many opportunities to improve health, and well-being such as better access to services and goods, employment and innovation, and social interaction, it can also imply to increasing exposure to harmful environments (i.e., air pollution,

noise, lack of natural spaces) and potentiate sedentary lifestyles if appropriate environmental and urban planning policies are not in place (103,104). Urban living factors have been associated with premature mortality and morbidity (105). In children, aspects of the urban environment have been linked to adverse birth and respiratory outcomes (53). However, up to now, the majority of studies have been focused on air pollution exposures only and evidence about the potential health effects of other features of the urban environment (e.g., noise, blue spaces, green spaces, built environment) and the concurrence of several exposures is scarce (53). To date, only one study has assessed maternal exposure to the full urban environment during pregnancy and observed no association with children's lung function but did not study wheeze or asthma as outcomes (74). Environmental exposures related to the urban environment differ between locations and urban settings and this might lead to differences in its consequent health effects between regions. Thus, exploring the urban environment as a whole in relation to respiratory health, especially in vulnerable developing periods of life, merits further investigation. This gap in knowledge is the main objective of paper IV of this Thesis. In the following sections current knowledge about the main exposures related to the urban environment included in this Thesis is described.

Air pollution

Air pollution is a well-recognised global public health issue. Air pollution has been associated with increased risk of premature mortality, cancer, and respiratory and cardiovascular disease in adults

(106–109). It was estimated that air pollution was responsible for 9 million premature deaths in 2015 (110). Air pollution is a mixture of several pollutants including gases and particles.

Particulate matter (PM) and nitrogen oxides (NO_x) are two main air pollutants (111). PM contains a mixture of organic and inorganic particles in solid and liquid form suspended in the air. According to their diameter, particulate matter is classified into PM_{10} (<10 μm), PM_{coarse} (2.5-10 μm), and $PM_{2.5}$ (<2.5 μm). PMs of lesser diameter are especially of concern because they can penetrate deeper in the respiratory system (111). Composition of PM from different sources is greatly variable in time, location, composition, and sources. Anthropogenic sources of PM include industry, fossil fuel combustion, and traffic emissions. NOx are usually generated from combustion reactions. In urban settings, the main source of NO_x is combustion for motorized vehicles and nitrogen dioxide (NO_2) is one of the most prevalent nitrogen oxides formed. NO_2 is a toxic gas that can cause significant inflammation of the airways at short-term high exposure (112). The main anthropogenic sources of NO_2 include road vehicle and ship emissions, heating, and power generation (111). Current EU legislation establishes an air pollution annual mean limit of 40 $\mu g/m^3$ for PM_{10}, 25 $\mu g/m^3$ for $PM_{2.5}$, and 40$\mu g/m^3$ for NO_2 (113), while the World Health Organization (WHO) recommendations establish a lower annual mean threshold of 20 $\mu g/m^3$ for PM_{10}, 10 $\mu g/m^3$ for $PM_{2.5}$, and 40$\mu g/m^3$ for NO_2 (114). However, most of the world's population do not meet these limits. It is estimated that 87% live in areas exceeding the WHO guidelines for

$PM_{2.5}$ (115). In EU, 60% of the cities currently exceed the WHO guidelines despite the improvements in air quality in the last decade (116).

Air pollutants inhaled by the mother during pregnancy can cross the placenta and thus have a direct effect on the foetus development (117). Both PM and gases can induce inflammation and oxidative stress at various levels (i.e., trachea, bronchi, bronchiole, alveoli) depending on its deposition site according to their size (111). Oxidative stress, inflammation processes, and also epigenetic changes are suspected to play an important role on the respiratory effects of air pollution. Indeed, oxidative stress and inflammation are mechanisms involved in asthma exacerbation, severity, asthma development (118), and foetal growth and development (119,120). However, mechanisms could be specific per pollutant (121). Air pollution could directly affect lung development by disturbing organogenesis due to the aforementioned mechanisms, and indirectly affect it through adverse birth outcomes such as intrauterine growth restriction (122,123). Animal studies have shown that perinatal air pollution exposure caused alterations in the development of distal airways, pulmonary parenchyma, elastic properties of the lung, and persistent airway hyperresponsiveness (124–126).

Current evidence suggests that air pollution exposure after birth is negatively associated with lung function during childhood and adolescence (127,128), but there are relatively few studies that assessed prenatal exposures and reported mixed findings (121,129–

136). A recent study from the ALSPAC birth cohort found that prenatal exposure to road traffic PM_{10} was linked to small reductions in lung function at 8 years of age, but not at 15 years (130). Regarding $PM_{2.5}$, two studies found an association of prenatal exposure with impaired lung function at age 5 (131) and 7 years (121). An adverse association of prenatal NO_2 with lung function was found at 4 years (132) and in asthmatic children aged 6-11 years (133). A recent study reported reduced mid expiratory flow ($FEF_{25-75\%}$) in relation to prenatal NO_x but no association with the other lung function parameters at 8-9 years (134). No associations were observed between the birth address air pollution levels and lung function at age 6-8 years in five cohorts belonging to the European Study of Cohorts for Air Pollution Effects (ESCAPE) (135), and between air pollution at birth and lung function at age 15 years in two German birth cohorts (136).

Although postnatal exposure to air pollution has been shown to increase the risk of developing wheeze and asthma (35) and exacerbate both pre-existing conditions (137), there is less clear evidence regarding the prenatal effects on wheeze and asthma during childhood. A recent systematic review showed an association between prenatal exposure to NO_2 and early childhood wheeze and between prenatal PM_{10} and NO_2 and asthma in young children (138). Two later studies found that prenatal exposure to NO_2 (139) and $PM_{2.5}$ (139,140) were associated with increased odds of asthma in early childhood. Contrarily, such associations were not observed in a recent meta-analysis of five birth cohorts covering seven areas in Europe and until 8 years of age (141). However, most studies that

reported negative associations were conducted until preschool ages (5-6 years) (138–140) and there is less evidence whether such observed effects persist later on (141). Some studies have examined these associations at later ages but have assessed air pollution exposure from birth or the first year of life and not during pregnancy (142–147). Some (142,144–147), but not all (143) studies found associations between air pollution, particularly NO_2, at birth and higher asthma incidence between 10 and 20 years of age.

Differences in results between studies may partly be due to spatial and source differences that lead to diverse pollutant composition and hence, of toxicity of the different pollutants.

Noise

Road traffic noise constitutes the most prevalent source of urban noise and is considered one of the major environmental health hazards in Europe (148). Chronic traffic noise exposure has been associated with cardiovascular morbidity and mortality, and all-cause mortality in the adult general population (149). In children, chronic noise has been associated with increased blood pressure and stress hormone levels such as cortisol, however evidence is mainly based on cross-sectional studies (150,151). Noise exposure has also been linked with sleep disturbances and cognitive impairment in children (152,153). Current European regulations establish a limit of annual noise levels during day, evening and night (Lden) <55 dB and annual noise levels at night (Ln) <50dB (154), while WHO recommendations are lower: Lden <53dB and Ln <45dB (155). It is

estimated that 20% and 15% of the European population is exposed to Lden and Ln levels respectively over the EU regulations (154).

Noise acts as a physiological and psychological stressor. It is suggested to promote stress responses through the hypothalamic-pituitary-adrenal axis leading to the release of stress hormones such as cortisol (156). This was supported in studies that reported children exposed to higher levels of noise at night (157) and those with higher annoyance to noise (158) showed increased saliva cortisol levels. Stress-related responses and biomarkers have been associated with respiratory disease in children and adults (159–163). Stress mechanisms may also disrupt sleep and night-time recovery of the immune system, leading to inflammatory processes also in the respiratory system (156). Further, chronic stress-reactions and sleep disturbances may lead to alterations in the endocrine system that could impair critical developmental processes of the lung and immune system (156,164,165).

There is emerging evidence on the effects of environmental noise exposure and respiratory morbidity in adults (163), but little is known on the effects in children. In adults, short-term noise exposure has been related with respiratory hospital admissions and respiratory mortality (166–169). Night road traffic noise has also been associated with asthma prevalence in adults (170). There are only two studies that have explored noise exposure during pregnancy and reported null associations (74,171). A study that assessed the totality of urban exposures did not find any association of prenatal day and night noise

levels with lung function at school-age (74). A study from over 4,000 participants from the BAMSE (Children, Allergy, Milieu, Stockholm, Epidemiology) birth cohort in Sweden explored the role of prenatal and postnatal exposure to residential noise, with objective noise measurements, and wheeze and asthma until adolescence and did not find consistent associations in relation to asthma and wheeze until 16 years of age. However, they observed a tendency of increased risk of ever suffering from asthma until adolescence (171).

Natural spaces

Natural spaces include green and blue spaces. Green spaces are those partly or completely covered by vegetation (i.e., park, garden, forest) and blue spaces are those covered by water bodies (i.e., river, sea, lake). Green spaces have been associated with beneficial health outcomes such as improved mental health and reduced risk of mortality (172). Higher greenness surrounding the home of the mother during pregnancy has also been related to beneficial birth outcomes (e.g., lower risk of low birth weight) (173–175). Blue spaces and health research is an emerging field of research (176). Blue spaces have been associated with physical and mental well-being (177,178) but evidence with other health outcomes is limited and inconsistent (176).

There are several potential mechanisms by which natural spaces may affect lung and asthma development (179). First, green and blue spaces could act as mitigators of harmful exposures such as air pollution (180). Second, by promoting healthier lifestyles and

reducing stress (e.g., increasing physical activity, promoting social cohesion) (181). Third, by influencing individual's microbiome as postulated by the biodiversity hypothesis (182,183). This hypothesis holds that urban environments are usually associated with a lack of natural exposures and biodiversity that affect the human microbiome and its immunoregulatory capacity necessary for a healthy development (184). Last, green space could imply the presence of allergens such as pollen that have been linked to reduced lung function and asthma (185,186).

Current evidence regarding the effects of prenatal exposure to green spaces on lung function in children is scarce and inconsistent (187). In a study encompassing 6 birth cohorts in Europe, no associations were found for prenatal exposure to green spaces with lung function from 6-11 years of age (74). Contrarily, a study that assessed green spaces exposure at birth found that greenness in a 100m buffer from the home and the presence of urban green spaces in a 300m buffer was associated with increased lung function up to 24 years of age (188). Regarding wheeze and asthma, a study in Canada found decreased risk of asthma until 5 years of age (189) but not at later ages (190) in relation to green space exposure during pregnancy. Two studies in New York found opposite results in relation to green spaces; one showed decreased risk of asthma at 5 years (191) and the other increased risk at 7 years (192). Regarding blue spaces, evidence is even more limited. Only one study previously assessed prenatal exposure to blue spaces in relation to lung function and reported null associations (74). To date, no study has examined the association of

prenatal exposure to blue spaces with childhood respiratory outcomes.

Built environment

The built environment refers to how the urban environment is shaped. It considers the physical parts of the urban setting including road and street design, infrastructures, buildings, and facilities, among others. The most commonly assessed built environment features include population density, building density, connectivity density, access to public transport, facilities density and richness, land use Shannon's Evenness Index, and walkability. Each indicator will be explained in chapter 4. Built environment features, particularly walkability, have been related to reduced risk of obesity, type 2 diabetes and hypertension in adults (193).

The features of the built environment have the potential to influence the health of its inhabitants through the influence on the magnitude of environmental stressors and on health-related behaviours (194). The complexity of the urban design makes every urban setting unique in terms of its health-related effects. In example, a pedestrian focused design in detriment of traffic would enhance the choice of active transport of the population, leading to less traffic-related air pollution and increase in physical activity, that would lead to beneficial health effects. Higher levels of fitness have been associated with reduced asthma symptoms, airway inflammation and bronchial hyperreactivity in both adults (195) and children (196) with asthma. On the contrary, lower levels of physical activity during pregnancy

(197) and in childhood (198) have been associated with increased risk of asthma symptoms in children and adolescents.

To date, only one study has assessed the influence of the built environment during pregnancy with lung function during childhood and reported null findings (74). To the best of our knowledge, there is no study assessing the built environment during pregnancy in relation to childhood wheeze and asthma.

2. RATIONALE

Altered lung function and asthma during childhood are known risks factors of chronic respiratory diseases later in life. Chronic respiratory diseases such as asthma and COPD remain among the leading causes of morbidity and mortality worldwide. Apart from genetic predisposition, environmental risk factors contribute to the substantial prevalence of this diseases. Early life is a critical period of development that is especially vulnerable to environmental exposures. Therefore, some of the burden due to altered lung function and asthma could be preventable. Identifying environmental risk factors is key to informing policy decisions on early prevention strategies.

OCs, bisphenols, and exposures related to the urban environment are some of the most common environmental exposures. OCs were extensively produced until their international ban in 2004. However, they are highly persistent and are able to bioaccumulate. In consequence, current populations are still exposed to them. Of concern, *in vitro* and *in vivo* studies have demonstrated their capacity to alter the lung and immune development. However, evidence from epidemiological studies is still limited. Bisphenols are currently produced in large quantities worldwide. They are present in many daily life products (e.g., plastic packaging, children's toys, thermal paper, canned food). Consequently, exposure to these chemical pollutants is continuous and widespread. Bisphenols have the potential to alter the lung and immune development. Evidence is scarce and focused only on BPA and respiratory symptoms, although

there are other analogues such as BPF and BPS that are increasingly present in the market. Currently, half of the global population live in cities, and it is expected to increase in the near future. Exposures related to the urban environment are suspected to influence the lung and immune development. The urban environment is composed of several features (e.g., air pollution, noise, traffic, natural spaces, built environment), but most of the evidence is based on air pollution and shows inconsistencies across studies. Evidence on other exposures of the urban environment and the co-occurrence of all the urban exposures is limited.

Consequently, there is a strong need for further research to elucidate the role of prenatal exposure to OCs, bisphenols, and urban environment exposures on respiratory health during childhood.

3. OBJECTIVES

The general objective of this Thesis is to investigate how early life environmental stressors, particularly ubiquitous chemical pollutants and the urban environment, influence children's respiratory health. Also, given the relevance of research on early life determinants of children's health we aimed to establish a new large parent-child cohort in a real-world data setting.

This is addressed through the following specific objectives:

1. To review current knowledge about the role of prenatal exposure to organic pollutants on children's lung function (Paper I)

2. To assess the association between prenatal exposure to organochlorine compounds and lung function during childhood in the Spanish INMA birth cohort (Paper II)

3. To assess the associations of prenatal exposure to bisphenols with lung function, wheeze, and asthma during childhood, using data from eight European birth cohorts (Paper III)

4. To assess the associations of maternal exposure to the urban environment with lung function, wheeze, and asthma during childhood in the Dutch Generation R birth cohort (Paper IV).

5. To establish a new cohort of 719,858 parent-child pairs by linking electronic health records in a large longitudinal database in Catalonia, Spain: the Information System for Research in Primary Care (SIDIAP) (Paper V).

4. METHODS

This section provides a general overview of the study design, study population, and exposure and outcome assessment used in this Thesis. Specific methodological details are given in the methods section of each paper included in chapter 5.

4.1. Study design and population

The present Thesis has used data from population-based birth cohorts in Europe and real-world data from electronic health records (EHR) from the public health system in Catalonia (Figure 1).

4.1.1. Birth cohorts

Birth cohort studies provide a valuable source of information to study early life environmental exposures and childhood development and health because of their prospective nature. Population based birth cohorts give information of a somewhat representative portion of the population since they are recruited without necessarily presenting any health outcome of interest. Extensive information on parental and child characteristics can be collected as well as a variety of health determinants for study purposes. In the present Thesis we included the following birth cohort studies: INMA (*INfancia y Medio Ambiente* – Environment and Childhood) Gipuzkoa, INMA Sabadell, INMA Valencia, Generation R, and the HELIX (The Human Early-Life Exposome) cohorts BiB (Born in Bradford), EDEN, MoBa (Norwegian Mother and Child Cohort Study), and RHEA (Mother Child Cohort study in Crete).

INMA (*Infancia y Medio Ambiente* – Environment and Childhood)

The INMA Project is an ongoing population-based birth cohort study that encompasses more than 3,000 mother-child pairs from seven ongoing cohorts located in seven geographical regions of Spain: Asturias, Gipuzkoa, Granada, Menorca, Ribera d'Ebre, Sabadell, and Valencia. The cohorts from Granada, Menorca, and Ribera d'Ebre recruited its population between 1997 and 2000. The cohorts from Asturias, Gipuzkoa, Sabadell, and Valencia, performed the recruitment between 2004 and 2006 (199). The objectives of the INMA Project are to study environmental prenatal and postnatal exposures, their impact on fetal and infant growth, health, and development, and the interaction between exposures and genetic characteristics on development and health (199). For this Thesis we used data from 3 cohorts: Gipuzkoa (n=638), Valencia (n=855), Sabadell (n=657), because they had relevant exposure and outcome information for papers II and III. In these three cohorts, pregnant women were recruited at the first prenatal visit, around 12 weeks of gestation, in the main health center or public hospital of their region. They followed common follow-up protocols during the prenatal and childhood assessments at 1.5, 4, 7, 9, and 11 years.

Generation R

The Generation R Study is an ongoing population-based prospective birth cohort located in Rotterdam, The Netherlands. It includes 9,778 mother-child pairs followed from fetal life until young adulthood. The aims of the study are to study growth, physical, cognitive and

behavioral development, diseases in childhood, health and healthcare for pregnant women and children, and to identify early environmental and genetic causes of development and health (200). Pregnant women were recruited during the whole pregnancy at their health care centers between 2002 and 2006. Periodic follow-ups were conducted during pregnancy, at birth, at 6 and 10 years of age (200–202).

HELIX (The Human Early-Life Exposome)

The HELIX project is a network of six established and ongoing population-based birth cohorts from six countries in Europe: BiB, the United Kingdom; EDEN, France; INMA, Spain; Kaunas cohort (KANC), Lithuania; MoBa, Norway; and RHEA, Greece. The aims of the HELIX project are to describe multiple prenatal and postnatal environmental exposures and to prospectively associate them with child health outcomes and molecular omics signatures (203,204). In a subcohort of 1,301 mother-child pairs, extensive information on prenatal and postnatal environmental exposures was collected and child health outcomes were measured in a common follow-up between 6 and 11 years of age following common standardized protocols. These HELIX cohorts recruited pregnant women between 1999 and 2010 at the first or second trimester of pregnancy and performed the common follow-up between 2013 and 2016 (204). The HELIX cohorts BiB (n=205), EDEN (n=198), MoBa (n=272), INMA Sabadell (n=223), and RHEA (n=199) were included in the Thesis for paper III because they had relevant information on both the exposures and outcomes of interest (the KANC cohort did not collect

urine samples during pregnancy and hence BPA could not be determined).

4.1.2. Real-world data

Real-world data includes information related to the population's health or healthcare utilisation that is routinely collected from different sources (e.g., electronic health records (EHRs), claims from insurance companies, administrative registries). Real-world data is gaining interest in research because they normally represent real-world populations covering a large number of individuals, which increases the generalisability and external validity of studies related to that data. EHRs are one of the most common real-world data sources. In paper V of this Thesis we used real-world data from the Information System for the Development of Research in Primary Care (SIDIAP) in Catalonia, Spain.

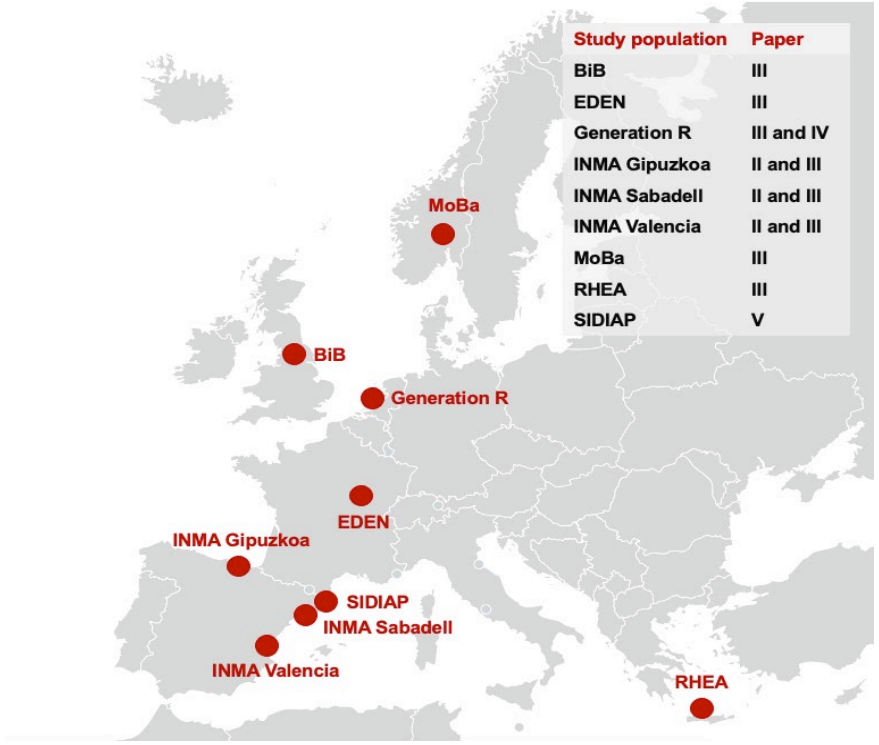
SIDIAP (The Information System for the Development of Research in Primary Care)

The SIDIAP database contains pseudo-anonymised individual level data collected routinely by healthcare professionals in primary care. It contains information on approximately 80% of the population residing in Catalonia (205), and it is shown to be representative of the overall population in terms of geographical, age, and sex distributions (206).

In Catalonia, universal public health care is provided. The major healthcare provider is the ICS (*Institut Català de la Salut* – Catalan Health Institute) and includes 328 primary care centres. Primary care

professionals from centres belonging to the ICS use the software *e-CAP* to record the patient's information in a structured format during their visits. Since 2006, all health records from *e-CAP* are stored in the SIDIAP database, which is updated bi-annually. The SIDIAP database stores information from individuals that are born in or move into an area covered by the ICS. Individuals are followed-up until they move out of the ICS area or die. SIDIAP contains information on disease diagnoses, clinical measurements, laboratory tests, drug prescriptions and/or dispensations in the pharmacies, lifestyle information, sociodemographic, and socioeconomic characteristics.

Figure 1. Study populations included in each paper of this Thesis.



4.2. Exposure assessment

4.2.1. Organochlorine compounds

Complete details on the assessment of OCs are found in the Methods section of paper II. Briefly, concentrations of *p,p′*-DDT, *p,p′*-DDE, HCB, and PCB congeners (PCB-28, -52, -101, -118, -138, -153, and -180) were determined in maternal or cord serum in the INMA Gipuzkoa, Sabadell, and Valencia cohorts. Because *p,p′*-DDT and PCB congeners 28, 52, 101, and 118 presented detectable levels in <25% of samples, they were excluded from the analyses. Since cord serum is considered the best proxy of OCs prenatal exposure (207), we estimated the equivalent concentrations in cord serum from the concentrations measured in maternal serum by applying cohort specific conversion factors.

4.2.2. Bisphenols

Concentrations of BPA, BPF and BPS were determined in maternal urine samples in the INMA (Gipuzkoa, Sabadell, Valencia), Generation R, BiB, EDEN, MoBa, and RHEA cohorts. BPA was measured in all included cohorts; BPF and BPS were measured in the three INMA cohorts and in Generation R. Concentrations were determined in a single or a two spot urine sample collected throughout pregnancy. Bisphenol concentrations were adjusted for creatinine to correct for urine dilution. Complete details are found in Supplementary Methods section of paper III.

4.2.3. Urban environment

In paper IV, we assessed exposures related to the urban environment at the home address of the mothers during pregnancy using geographic information system (GIS) modelling. Complete details on the exposures data pre-processing are found in the Methods section and Supplementary Table S1 of paper IV.

Air pollution

Air pollution was estimated using land use regression (LUR) models developed within the ESCAPE framework (208,209). Exposures were estimated for the Rotterdam region at every study year and were temporally adjusted following the ESCAPE guidelines (210). In brief, the LUR spatial estimates in each geocode were combined with a temporal adjusting factor from the routine monitoring data. The following air pollutants were estimated: NO_X, NO_2, $PM_{2.5}$, PM_{10}, $PM_{2.5abs}$, PM_{coarse}, and elemental components of $PM_{2.5}$: Copper (Cu), Iron (Fe), Potassium (K), Nickel (Ni), Sulfur (S), Si (Silicon), Vanadium (V), Zinc (Zn).

Noise

Noise was assessed with Lden and Ln indicators. Lden is the annual average of day, evening and night noise levels (dB). It is defined by the European Noise Directive to assess long-term noise annoyance. Ln is the annual average of night noise levels (dB) and is defined to assess sleep disturbances. Lden and Ln were assessed from the European road traffic noise maps created in the framework of the European Noise Directive 2002/49/EC (211).

Traffic

Traffic was included as an additional proxy of traffic related air pollution. It was estimated using the Dutch National Database of Road Traffic Data (Nationale Databank Wegverkeersgegevens). Traffic was assessed as the inverse distance to the nearest road (m^{-1}).

Natural spaces

Green spaces

Exposure to green spaces was based on indicators of availability and accessibility.

Availability of green spaces was estimated with the following indicators:

- Normalised Difference Vegetation Index (NDVI): quantifies the average greenness by measuring the difference between near-infrared light, that is strongly reflected by vegetation, and red light, that is absorbed by vegetation. The index values range from -1 to 1. Negative values represent water bodies and positive increasing values represent greener spaces and were set to 0. NDVI was calculated using LANDSAT 4-5, 7 Thematic Mappers, and Landsat 8 Operational Land Imager/Thermal Infrared Sensor with resolution of 30 m x 30 m (212).
- Size of the nearest major green space was calculated using Urban Atlas maps (213). This indicator estimates the size of the nearest green space >5000 m^2 from the home address.

Accessibility to green spaces, considered as the residential proximity to major green spaces (>5000 m^2), was estimated using Urban Atlas maps (213) with the following indicators:

- Distance to the nearest major green space in a straight line.
- Availability of a major green space within 300 m of the home address, which is considered approximately a 15-minute walk.

Blue spaces

Blue spaces availability and accessibility were estimated using Urban Atlas maps (213). Similar to green spaces, availability was estimated as the size of the nearest major blue space (>5000 m^2). Accessibility to blue spaces was considered as the straight-line distance to the nearest major blue space and the availability of a major blue space in a 300 m buffer of the home address.

Built environment

Several indicators of the built environment were estimated using different data sources. The following indicators were used:

- Population density: number of inhabitants per km^2. Calculated from the Global Human Settlement layer (214).
- Building density: area occupied by buildings (m2) per km^2. Calculated from the European Settlement Map (215).
- Street connectivity: number of intersections per km^2. Calculated from NAVTEQ (216).
- Accessibility to public transport: assessed by the number of public transport lines and public transport stops in buffers of

100 m, 300 m, and 500 m from the home address. Calculated from Open Street Maps (217).

- Facilities: considering all points of daily life activities (e.g., schools, medical centres, shops, restaurants, libraries). Facilities density (number of facilities per km^2) and facility richness index (number of different facility types present divided by the maximum potential number of facility types specified) in a buffer of 300 m from the home address were calculated from NAVTEQ (216).
- Land use mix (Shannon's Evenness Index): index ranging from 0 to 1 that indicates the diversity of land uses (e.g., residential, agricultural, commercial). A higher value represents a more even distribution of different types of land uses. This was calculated within a buffer of 300 m from the home address from Urban Atlas (213).
- Walkability index: quantifies how walkable is an area based on land use mix, facility richness, population density, and street connectivity following methods described elsewhere (218). This was calculated in a buffer of 300 m from the home address.
- Unhealthy food environment: assessed by the number of unhealthy food facilities (number of facilities per km^2) in a buffer of 300 m of the home address. Calculated from NAVTEQ (216).

4.3. Outcome assessment

4.3.1. Lung function

Lung function was examined in papers II, III, and IV. In all birth cohort studies, lung function was assessed from spirometry tests following the American Thoracic Society and the European Respiratory Society guidelines (ATS/ERS) (219). Forced vital capacity (FVC), FEV_1, FEV_1/FVC were the main lung function parameters used. In papers III and IV, we additionally examined $FEF_{25-75\%}$. Criteria of acceptability and reproducibility of the spirometry tests following the ATS/ERS criteria were taken into account (219). Lung function parameters were additionally standardized into sex-, age-, height- and ethnicity-adjusted z-scores based on the Global Lung Function Initiative (GLI) reference values (220) for a better comparability between the cohorts included.

Spirometry

During the spirometry test, the participant was asked to inhale fully and perform a forced complete expiration (i.e., as quickly and forcefully as possible) while seated. This procedure could be repeated up to a maximum of 8 times to ideally obtain at least 3 reproducible manoeuvres, although this was not always achieved. Measures of volume, time, and flow were recorded. The following parameters were selected:

- FVC: is the maximal volume or air exhaled. This parameter is dependent on the total lung volume, muscular strength, and elasticity of the thorax and can reflect airway restriction.

- FEV_1: is the maximal volume of air exhaled in the first second of the forced expiration. It is dependent on the diameter and elasticity of the airways and can reflect airflow obstruction.
- FEV_1/FVC: shows the relation of the previous two parameters. It can reflect airways obstruction.
- $FEF_{25-75\%}$: is the flow measured within 25% and 75% of the FVC. This parameter reflects airflow limitations in the mid-to-small airways.

4.3.2. Wheeze

Wheeze was assessed in paper III and IV from parental-administered questionnaires adapted from ISAAC (23). Information on wheeze was obtained throughout childhood. We considered two wheeze variables: wheeze in the last year and wheezing patterns. Wheeze in the last year was assessed between 6-11 years in paper III and at 10 years in paper IV. In paper III, wheezing patterns were constructed as: 1) never wheezing; 2) early wheezing, ≤4 years; 3) late wheezing, >4 years; and 4) persistent wheezing, ≤4 and >4 years. In paper IV, the same wheezing patterns were constructed but setting the cut-off at 3 years instead of 4 years. Previously defined wheezing patterns (221) established a cut-point at 3 years. However, in paper III, due to the availability of the data among cohorts and for a better harmonisation we established the cut-point at 4 years.

4.3.3. Asthma

Information on asthma was used in papers II, III, and IV, and asthma medication information in papers III and IV. Information on asthma and asthma medication was assessed from parental-administered

questionnaires adapted from ISAAC (23). In paper II asthma was considered simply as “ever been diagnosed by a doctor of having asthma” for a sensitivity analysis. In paper III, current asthma at school-age was defined following the MeDALL definition (24) because it is a suitable definition for harmonisation of asthma assessments across different cohorts. It was defined as having a positive answer to two of the following: 1) ever asthma diagnosis; 2) wheezing in the last year; 3) asthma medication in the last year. In paper IV, the definition was slightly stricter than the MeDALL definition. Current asthma was defined as ever physician-diagnosed asthma with either wheezing or asthma medication use in the last year (33).

Table 1. Summary of study populations, exposure assessments, and outcome assessments in papers II to IV.

Paper	Study population	Exposure	Exposure period	Exposure assessment	Outcome assessment	Outcome assessment	Age at outcome assessment
Paper II	INMA Gipuzkoa Sabadell Valencia	OCs: p,p'-DDE, HCB, PCB-138, PCB-153, PCB-180	Pregnancy	Maternal serum and/or cord blood	Lung function	Spirometry	4 years 7 years
Paper III	INMA Gipuzkoa Sabadell Valencia Generation R BiB EDEN MoBa RHEA	Bisphenols: BPA, BPF, BPS	Pregnancy	Maternal urine	Lung function	Spirometry	7-11 years
					Wheezing patterns	Questionnaire	0-11 years
					Current asthma Wheeze in the last year	Questionnaire	7-11 years
Paper IV	Generation R	Urban environment: Air pollution, noise, traffic, natural spaces, built environment	Pregnancy	GIS estimations at the home address	Lung function	Spirometry	10 years
					Wheezing patterns	Questionnaire	0-11 years
					Current asthma	Questionnaire	10 years

5. RESULTS

Paper I: *In utero* exposure to organic pollutants and lung function in the offspring.

Paper II: Prenatal exposure to organochlorine compounds and lung function during childhood.

Paper III: *In utero* exposure to bisphenols and asthma, wheeze, and lung function in school-age children: A prospective meta-analysis of 8 European birth cohorts.

Paper IV: Urban environment during pregnancy and lung function, wheeze, and asthma in school-age children. The Generation R Study.

Paper V: Linkage of 719,858 parent and child electronic health records in a large longitudinal database in Spain: the Information System for Research in Primary Care (SIDIAP).

5.1. Paper I

Abellan A, Casas M.

***In Utero* Exposure to Organic Pollutants and Lung Function in the Offspring**

BRN Rev. 2021; 7(1):62-79

BRN Reviews REVIEW

In Utero Exposure to Organic Pollutants and Lung Function in the Offspring

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ABSTRACT

Humans are exposed daily to thousands of chemicals present in many consumer products that can interfere with hormonal signalling systems. The prenatal period is critical because the developing lung is intrinsically subject to hormonal regulation. Alterations at this time may predispose to reduced lung function in later life. In this review, we summarise current knowledge about the role of prenatal exposure to organic pollutants on lung function in the offspring. We divide pollutants into persistent: organochlorine and perfluoroalkyl compounds, and non-persistent: bisphenols, parabens, triclosan, benzophenones, phthalates, and currently used pesticides. Eleven prospective cohort studies, mainly from Europe and the US, have been identified. Overall, the literature is scarce and inconsistent. The observed associations have identified small changes in lung function parameters. Main challenges for future studies include assessment of exposure to non-persistent pollutants and the study of multipollutant effects. In parallel, public health strategies should be implemented to reduce exposure to organic pollutants, particularly in pregnant women.

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INTRODUCTION

Impaired lung development in early life may predispose individuals to reduced lung function in adulthood[1,2]. This can lead to persistent respiratory morbidity and chronic respiratory diseases later in life[3,4]. Chronic respiratory diseases are among the leading causes of mortality and morbidity worldwide and asthma and chronic obstructive pulmonary disease (COPD) are the most common – 358 million people are affected by asthma and 175 million by COPD[5]. There is no curative therapy for either of them. The natural history of both diseases is extensive yet incomplete. It is postulated that they have at least part of their origins in early life when the lung is still undergoing rapid development[6,7]. This fits well with the concept of the Developmental Origins of Health and Disease, which describes how early-life exposures may have a long-term impact on disease in adulthood[8]. Identification of early determinants of lung development is of utmost importance, given their long-term effect on disease throughout life.

The developing lung is extremely susceptible to the effects of environmental exposures[9]. Exposure to an adverse environment during critical periods of pre- and early postnatal life might lead to developmental adaptations resulting in impaired lung growth with smaller airways and lower lung volume, altered immunological responses, and related inflammation. The dramatic increase in childhood asthma prevalence over the last decades[5] has raised concerns about the potential role of environmental pollutants. Exposure to a number of common environmental pollutants, including environmental tobacco smoke[10] and air pollution[11,12] has been associated with childhood respiratory tract illnesses. More recently, concern is growing over the impact of environmental chemicals on childhood lung function.

Environmental chemicals can be classified within organic and inorganic pollutants. Organic pollutants include polychlorinated biphenyls (PCBs), pesticides, perfluoroalkyl substances (PFASs), phenols, and polyaromatic hydrocarbons (PAHs), among others; inorganic pollutants include a variety of heavy metals such as mercury, lead, arsenic, or cadmium. Both organic and inorganic pollutants present in the environment may have the capacity to interfere with the endocrine system and consequently alter many essential body functions such as growth, behaviour, and reproduction[13]. The term "endocrine disrupting chemical" includes a large number of substances (in some lists more than 1,500[14]) whose primary effect is on the endocrine system through interaction with cellular hormone receptors, hormone synthesis or clearance[13]. Endocrine disrupting chemicals can have effects at very low exposure levels, as endogenous hormones do; low doses can have more potent effects than higher doses; and exposure to multiple chemicals can result in synergistic, antagonistic, or cumulative effects[15].

Endocrine disrupting chemicals are produced in large quantities worldwide (millions of tons annually) and consequently, human populations are continuously exposed to them through food, food packaging, cosmetics, dust inhalation, and consumer products. Human biomonitoring studies have shown low but very widespread human exposure[16–18]. Exposure to these chemicals is of special concern

Table 1. Concentrations of organic pollutants in 1,301 pregnant women and children in 6 European population-based cohort studies participating in the HELIX project *(extracted from Haug et al.[18] with permission from the author)*

Pollutant	Maternal samples			Child samples		
	% quantifiable samples	Median (P25-P75)	Max	% quantifiable samples	Median (P25-P75)	Max
Organochlorine compounds – blood (ng/g lipid)						
DDE	99.9	52.3 (25.9, 111)	1903	100	21.8 (11.6, 45.6)	2158
PCB-153	99.6	17.6 (10.4, 30.5)	214	100	11.6 (7.28, 18.6)	217
HCB	99.1	8.16 (5.59, 13.0)	164	99.9	8.19 (6.27, 11.4)	88.1
Perfluoroalkyl substances – blood (μg/L)						
PFOS	100	6.41 (4.12, 9.63)	48.0	99.8	2.03 (1.26, 3.22)	33.8
PFOA	99.7	2.30 (1.38, 3.34)	31.6	100	1.55 (1.19, 1.97)	6.66
Bisphenols – urine (μg/g creatinine)						
Bisphenol A	99.4	2.82 (1.55, 6.60)	107	98.3	4.06 (2.42, 7.17)	362
Parabens, Triclosan, and Benzophenones – urine (μg/g creatinine)						
Methyl-paraben	99.8	167 (39.5, 389)	39,241	99.7	6.50 (3.28, 26.4)	23,963
Ethyl-paraben	97.4	6.26 (1.14, 26.72)	817	99.3	0.67 (0.43, 1.22)	2033
Triclosan	98.5	6.28 (1.50, 79.9)	1653	100	0.61 (0.32, 1.5)	702
Benzophenone-3	99.3	4.90 (1.46, 27.5)	12,837	99.9	2.16 (0.86, 6.96)	7985
Phthalates – urine (μg/g creatinine)						
DEHP metabolite MEHP	99.5	8.73 (4.42, 15.3)	417	96.8	2.88 (1.70, 5.10)	282
MiBP	99.9	38.7 (23.3, 60.7)	705	100	41.8 (25.9, 73.3)	861
Currently used pesticides - urine (μg/g creatinine)						
DAP metabolite DMP	90.8	8.37 (4.13, 16.4)	321	49.3	0.78 (0.29, 4.70)	83.3
DAP metabolite DEP	97.8	3.33 (1.86, 6.44)	198	80.9	1.83 (0.47, 4.52)	665

% quantifiable samples: % of the biomarker measurements with concentrations reported.
DAP: dialkyl phosphate; DDE: dichlorodiphenyldichloroethylene; DEHP: di-(2-ethylhexyl) phthalate; DEP: diethyl phosphate
DMP: dimethyl phosphate; HCB: hexachlorobenzene; MEHP: mono-2-ethylhexyl phthalate; MiBP: mono-isobutyl phthalate; P25: 25th percentile; P75: 75th percentile; PCB-153: polychlorinated biphenyl-153; PFOA: perfluorooctanoate; PFOS: perfluorooctane sulfonate.

in pregnant women and children, with many compounds being detected in more than 90% of samples (Table 1). Foetus and infants are especially sensitive to chemicals that mimic hormones because the protective mechanisms (i.e., detoxification) existing in adulthood are not completely functional in early life. In the EU, the medical cost associated with exposure to endocrine disrupting chemicals has been estimated at €163 billion a year[19]. Nowadays, the reduction of exposure to endocrine disrupting chemicals, and particularly the organic ones, represent a priority for action in the European Commission owing to their high annual production and potential toxicity[20].

In this narrative review, we summarize current knowledge about the role of exposure

to organic pollutants during pregnancy on lung function in the offspring. We have focused the review on the prenatal exposure because is when the lung is more susceptible to the effects of harmful environmental exposures[9]. This summary is mainly based on epidemiological literature published between 2014 and 2020. We have primarily identified relevant literature using the PubMed search engine (National Library of Medicine). Search strategies include keywords for the various combinations of organic pollutants (organochlorine compounds (OCs), PCBs, dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), PFASs, bisphenols, parabens, triclosan, benzophenone-3, phthalates, and organophosphate pesticides) and respiratory function (lung function, spirometry, forced expiratory volume in one second [FEV_1], forced vital capacity [FVC], FEV_1/FVC ratio, and forced expiratory flow at 25-75%). We have divided organic pollutants into persistent, characterized as having long biological half-lives in the body (e.g., from months to years), and non-persistent, with short biological half-lives (e.g., from hours to days).

OVERVIEW OF LUNG DEVELOPMENT

The development of the lung starts at the foetal stage and continues after birth until 20 years of age. It consists of five consecutive stages: embryonic, pseudoglandular, canalicular, saccular, and alveolar (Fig. 1). Primary lung buds start to develop at the 4th week of gestation to form the trachea, bronchi, and pulmonary vein and artery. During the second trimester of pregnancy, during the pseudoglandular and canalicular stages, the bronchi continue their segmentation, primitive alveoli are formed, and the synthesis of surfactant starts. This is considered to be the most critical stage for the respiratory system since it is when all conducting airways are formed. The saccular and alveolar are the last prenatal stages. They develop from the third trimester of gestation until the second year after birth. During these stages, the gas exchange region expands. The expansion triggers the production of extracellular matrix, collagen, and elastin. During these phases, there is further growth of the vascular system associated with the respiratory system. After birth and until the second year of age, alveoli continue developing. The gas exchange regions keep expanding through a branching process in concordance to its associated vascular and nervous systems. From two years until adulthood, the process of lung growth and expansion continues by a sustained cellular proliferation[9,21]. Across the lifespan, lung function grows from birth to late adolescence, reaching its maximal levels in early adulthood. This is followed by a plateau period when lung function remains stable for several years before it starts to gradually decline[22].

The developing lung is intrinsically subject to endogenous hormonal regulation[9,23,24]: glucocorticoids, oestrogens, and thyroid hormones promote the structural development of the lung and the production of pulmonary surfactant, while androgens retard surfactant production. Other receptors also play a prominent role on lung development: aryl hydrocarbon receptor (AhR) activation decrease thyroid hormone levels and peroxisome proliferator–activated receptor (PPAR) alter airway cell differentiation and surfactant

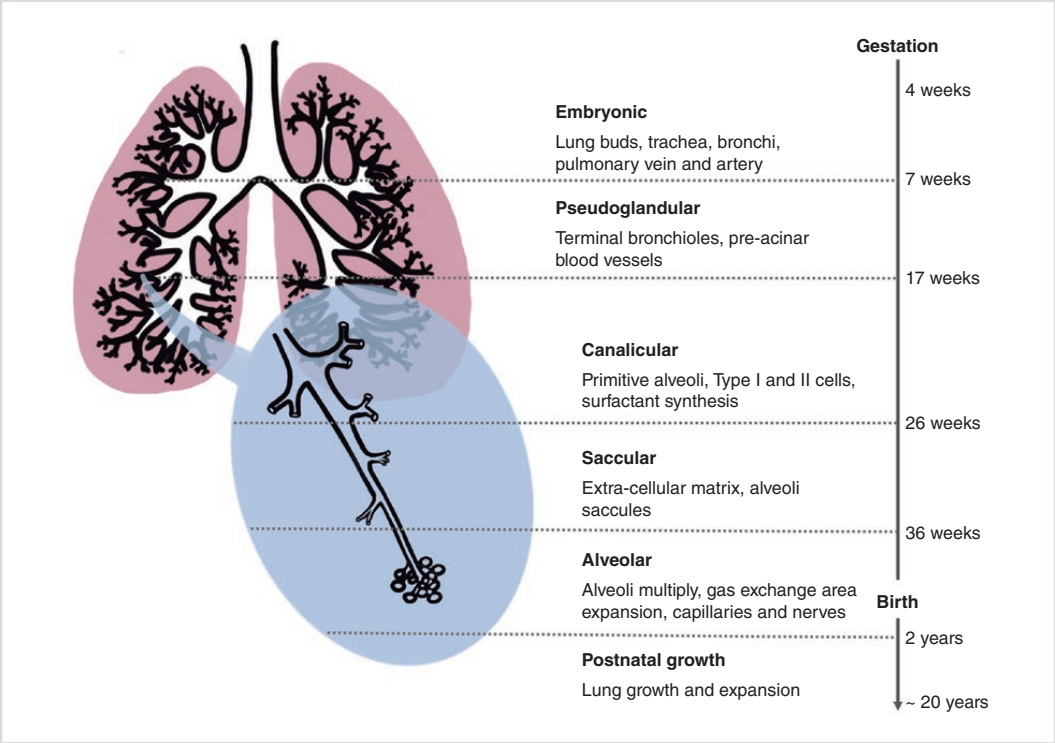

Figure 1. Developmental stages of the lung.

production, among other mechanisms. All these receptors are expressed in the human lung[9]. Of concern, endocrine disrupting chemicals have affinity with glucocorticoids (e.g. pesticides), oestrogens (e.g. bisphenols, pesticides), androgens (e.g. phthalates), and/or thyroid hormones receptors (e.g. bisphenols, metals), to AhR (e.g. pesticides), and/or PPAR (e.g. perfluoroalkyl compounds)[25–28]. Hence, disruption of fundamental biologic processes and associated signalling events due to exposure to endocrine disrupting chemicals may result in significant alterations in lung development.

PERSISTENT ORGANIC POLLUTANTS AND LUNG FUNCTION

Organochlorine compounds

OCs are synthetic persistent organic pollutants mainly used as pesticides or in industrial products such as electrical insulators and flame retardants (Table 2). Their use and production of some of these compounds has been banned for decades[29,30]. For example, the former widely used pesticide dichlorodiphenyl-trichloroethane (DDT) was banned

Table 2. Main sources of exposure to organic pollutants

	Pollutants	Main sources of exposure
Persistent organic pollutants	Organochlorine compounds	Pesticides Electrical insulators Industrial products
	Perfluoroalkyl substances	Surfactants for industrial products coating (i.e., paper packaging, textile, leather) Fire-fighting foams Lubricants Photography industry Non-stick cookware
Non-persistent organic pollutants	Bisphenols	Polycarbonate plastic Canned food Sport bottles Thermal receipt paper Children's toys
	Parabens, Triclosan, Benzophenone-3	Cosmetics Toothpaste Sunscreen Perfumes Pharmaceutical products Detergents Soaps Plastic packaging Textiles
	Phthalates	Polyvinyl chloride (PVC) plastics Vinyl building products Adhesives Fragrances Medical equipment Detergents
	Currently used pesticides	Agriculture Residential settings

Source[101].

in the European Union in the early 80s[30]. Due to the strong evidence of the adverse health effects of other OCs, these compounds were internationally banned at the Stockholm Convention in 2001[31]. However, they can bioaccumulate and persist in the environment for long periods. They have long biological half-lives that can range from several years to decades in some compounds[32]. Currently, the main source of exposure to OCs is diet. Biomonitoring studies show that current populations are exposed to these substances finding detectable levels in blood samples[18] (Table 1).

OCs may interfere in the lung morphogenesis as well as play a role in inflammation processes both pre- and postnatally. These compounds are endocrine disruptors with estrogenic and anti-androgenic properties[33]. Therefore, they may interact with oestrogenic and androgenic receptors and alter the related signalling pathways, including the activation of the AhR pathway, which has been shown to delay lung development in animal studies[34]. Furthermore, exposure to OCs has also been related to inflammation, observing increased interleukins and immunoglobulins in children exposed to them[35–37].

Table 3. Studies on persistent organic pollutants and lung function by year of publication

Pollutant	Author (year)	Country, study design (cohort name)	N	Year of recruitment	Outcome age	Exposure assessment	Statistically significant main findings
Organochlorine compounds	Hansen et al. (2016)[38]	Denmark, birth cohort (Danish Fetal Origins Birth Cohort)	414	1988-1989	20y	Blood (pregnancy)	OCs - Airway obstruction: PCBs OR = 2.96 (1.14 to 7.70)[a] HCB OR = 2.63 (1.07 to 6.46)[a] DDE OR = 2.87 (1.09 to 7.57)[a]
	Abellan et al. (2019)[39]	Spain, birth cohort (INMA)	1308	2004-2008	4y and 7y	Blood (pregnancy/ birth)	DDE - FEV_1 4y: β = –53.61 (–89.87 to –17.35)[b]; FEV_1 7y: β = –36.07 (–65.21 to –6.92)[c]; FVC 7y β = –39.45 mL (–71.23 to –7.66)[c] HCB - FVC 7y: β = –56.68 (–89.87 to –23.49)[c]
	Agier et al. (2019)[41]	6 EU countries, birth cohort (HELIX)	1033	2003-2009	6-12y	Blood (pregnancy)	None
Perfluoroalkyl substances	Impinen et al. (2018)[49]	Norway, birth cohort (ECA)	641	1992-1993	Birth	Blood (birth)	None
	Agier et al. (2019)[41]	6 EU countries, birth cohort (HELIX)	1033	2003-2009	6-12y	Blood (pregnancy)	PFOA - FEV_1: β = –1.4 (–2.7 to –0.1)[d] PFNA - FEV_1: β = –1.4 (–2.7 to –0.1)[d]
	Manzano-Salgado et al. (2019)[50]	Spain, birth cohort (INMA)	992	2003-2008	4y and 7y	Plasma (pregnancy)	PFOA - FVC 4y: β = –0.17 (–0.34 to –0.01)[e]

[a]Coefficient estimates are Odds Ratios of airway obstruction (FEV_1/FVC < 75%) given on the third versus the first (lowest) quartile of exposure. [b]Coefficient estimates are given for a change in FEV_1 (ml) on the third versus the first (lowest) quartile of exposure. [c]Coefficient estimates are given for a change in FEV_1 or FVC (ml) on the second versus the first (lowest) quartile of exposure. [d]Coefficient estimates are given for a change in mean FEV_1% for an interquartile range change in PFASs concentration. [e]Coefficient estimates are given for a change in FVC z-score for each doubling of PFASs concentration.
DDE: dichlorodiphenyldichloroethylene; ECA: The Environment and Childhood Asthma; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; HCB: hexachlorobenzene; HELIX: Human Early-Life Exposome; INMA: *INfancia y Medio Ambiente* (Environment and Childhood); OCs: organochlorine compounds; PCBs: polychlorinated biphenyl; PFASs: perfluoroalkyl substances; PFNA: perfluorononanoate; PFOA: perfluorooctanoate; y: years.

Current evidence suggests that prenatal exposure to OCs is associated with reduced lung function in the offspring (Table 3). Prenatal exposure to very high levels of DDE, HCB, and PCBs was associated with increased risk of airway obstruction at 20 years of age in a Danish birth cohort established in the 80s[38]. In a recent study from a Spanish birth cohort, prenatal exposure to DDE was associated with lower lung function at 4 and 7 years of age, even at low exposure levels. In this study they also observed reduced forced vital capacity (FVC) at certain exposure levels of HCB and found inconsistent results with PCBs[39]. Recently, in a study assessing the effects of the "exposome" - the totality of environmental exposures from conception onwards[40]- on lung function, DDT and DDE tended to be associated with reduced FEV_1 in school-age children, but results did not reach statistical significance[41].

Perfluoroalkyl substances

PFASs are synthetic fluorinated organic compounds produced in large quantities since the

1950s. PFASs have been widely used as surfactants in industrial and commercial products including paper, textile, and leather coatings, fire-fighting foams, lubricants, and photography industry[28] (Table 2). Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are the most common PFASs and are widely detected in the environment (i.e. water)[42] and in human blood[17] (Table 1). Although they are not stored in the adipose tissue, they are classified as persistent organic pollutants because they form chemical adducts with liver and serum proteins (e.g. albumin) and have long biological half-lives in humans (3-5 years)[43]. PFASs can also cross the placental barrier[44].

Studies assessing the levels of PFASs in different organs in mice and humans, observed the highest levels in lungs[45,46], suggesting the lung to be a target of PFASs toxicity. Indeed, new-born rats prenatally exposed to PFASs had retarded lung maturation, namely diminished alveolar airspace and increased alveolar thickness[47]. PFASs are synthetic surfactant molecules and therefore, have the potential to interfere with the integrity of the surface-active interfacial films formed by natural surfactant in the alveolar space. A recent in vitro study showed that PFASs can inhibit lung surfactant function and also induce a pro-inflammatory response in human bronchial epithelial cells[48].

Up to now, three prospective studies in humans have evaluated whether prenatal exposure to PFASs impairs lung function in the offspring (Table 3). Impinen et al. (2018)[49] did not find any association between exposure to PFASs during pregnancy and lung function at birth. In the first exposome study, *in utero* exposure to PFOA and perfluorononanoate (PFNA) was associated with reduced FEV_1 in children aged 6–12 years[41]. In a recent study in the Environment and Childhood - *INfancia y Medio Ambiente* (INMA) cohort, although authors observed a reduction in FVC at 4 years associated with prenatal exposure to PFOA, this association disappeared after applying a more stringent reproducibility criterion for the spirometry test[50].

NON-PERSISTENT ORGANIC POLLUTANTS AND LUNG FUNCTION

Bisphenols

Bisphenols are widely present in daily life products such as plastic packaging, children's toys, thermal paper, and canned food (Table 2). The general population is continuously exposed to them through dermal contact, inhalation or ingestion. Bisphenol A (BPA) is the most produced bisphenol and has been found in > 90% of urine samples in general population[51,52] (Table 1). It has been related to adverse health effects (e.g., metabolic and reproductive disorders, behavioural problems) and in 2017 it was considered a "substance of very high concern" by the European Chemical Agency[53]. Its production and use have been prohibited in some products and in some countries, giving rise to the production of substitute products of similar structure such as bisphenol S (BPS) and bisphenol F (BPF)[54]. After exposure, bisphenols are rapidly excreted from the body (half-life of less than 6 hours).

Bisphenols might interfere in the developing lung thanks to their capacity to cross the placental barrier and the ubiquitous daily

exposure after birth. A study in mice observed that exposure to BPA during gestation severely retarded foetal lung maturation[55]. This immaturity was characterized by diminished alveolar airspace and thickened septa and by a diminished number of type I pneumocytes[55]. Bisphenols can also induce oxidative stress, endocrine disruption and mitochondrial dysfunction[56–58]. These chemicals can alter inflammatory responses through different signalling pathways. They can activate the reactive oxygen species (ROS) pathway and the mitogen-activated protein kinase (MAPK) signalling pathways that lead to DNA damage and cellular death. Bisphenols might also alter immune responses by the stimulation of pro-inflammatory cytokines and the inhibition of anti-inflammatory cytokines production[59].

Current evidence suggests that BPA exposure increases the risk of respiratory symptoms (reviewed by Vrijheid et al, 2016[60]) but there is limited evidence on the effects of BPA and their substitutes on lung function (Table 4). A study observed that increasing maternal urinary BPA levels were associated with 14.2% (95% CI: -24.5, -3.9) decrease in the % predicted FEV_1 at 4 years of age but did not see such association at 5 years[61]. In the Étude des déterminants pré et postnatals du développement de la santé de l'enfant (EDEN) birth cohort study, no association was observed between prenatal BPA levels and predicted FEV_1 at 5 years[62]. No association was observed in the Human Early-Life Exposome (HELIX) cohorts between prenatal BPA levels and lung function in school-age children in the first exposome study[41]. Recently, Berger et al. (2020)[63] assessed the effects of prenatal exposure to 20 endocrine disruptors at the same time, including BPA, on lung function at 7 years. By using the Bayesian Kernel Machine Regression (BKMR), a statistical tool that allows the identification of the most relevant group and pollutant within the group associated with a specific outcome, the authors did not observe any association between BPA and lung function[63]. These findings were previously observed in the same cohort in a study assessing BPA and eight phthalates[73]. We should consider that all previous studies have used one or two spot urines, which, due to the high temporal variability of bisphenols and their short half-lives, might have led to exposure misclassification that biases the associations towards the null.

Parabens, Triclosan, and Benzophenones

Parabens, triclosan, and benzophenones belong to the chemical group of phenols. Parabens have bactericide and fungicide properties, and they are used in the cosmetic and pharmaceutical industry (Table 2). Triclosan is an antibacterial agent and preservative in detergents and personal care products. Benzophenones act as filters for ultraviolet radiation and are present in sunscreens, perfumes, soaps, and plastic packaging. Human exposure to these chemicals is widespread[18] (Table 1). The most common parabens used are methyl-, ethyl-, propyl-, and butyl-paraben whereas benzophenone-3 (BP-3) is the most used benzophenone.

Experimental studies suggest that parabens, triclosan, and BP-3 lead to reduced lung function through their capacity to link to estrogenic receptor and PPAR[64]. For

Table 4. Studies on non-persistent organic pollutants and lung function by year of publication

Pollutant	Author, Year	Country, study design (cohort name)	N	Year of recruitment	Outcome age	Exposure assessment	Statistically significant main findings
Bisphenols	Spanier et al. (2014)[61]	US, birth cohort (HOME)	208	2003-2006	4-5y	2 urines (pregnancy)	BPA - FEV_1 4y: $\beta = -14.2$ (−24.5 to −3.90)[a]
	Vernet et al. (2017)[62]	France, birth cohort (EDEN)	228	2002-2006	5y	1 urine (pregnancy)	None
	Berger et al. (2019)[69,b]	US, birth cohort (CHAMACOS)	260	1999-2000	7y	2 urines (pregnancy)	None
	Agier et al. (2019)[41]	6 EU countries, birth cohort (HELIX)	1,033	2003-2009	6-12y	1 urine (pregnancy and childhood)	None
	Berger et al. (2020)[63,b]	US, birth cohort (CHAMACOS)	282	1999-2000	7y	2 urines (pregnancy)	None
Parabens, Triclosan, Benzophenones	Vernet et al. (2017)[62]	France, birth cohort (EDEN)	228	2002-2006	5y	1 urine (pregnancy)	None
	Berger et al. (2018)[69,b]	US, birth cohort (CHAMACOS)	296	1999-2000	7y	2 urine (pregnancy)	None
	Agier et al. (2019)[41]	6 EU countries, birth cohort (HELIX)	1,033	2003-2009	6-12y	1 urine (pregnancy and childhood)	None
	Berger et al. (2020)[63,b]	US, birth cohort (CHAMACOS)	282	1999-2000	7y	2 urines (pregnancy)	None
Phthalates	Vernet et al. (2017)[62]	France, birth cohort (EDEN)	228	2002-2006	5y	1 urine (pregnancy)	None
	Berger et al. (2018)[69,b]	US, birth cohort (CHAMACOS)	296	1999-2000	7y	2 urines (pregnancy)	MEP - FEF_{25-75}: $\beta = -3.22$ (−6.02 to −0.34)[c]
	Berger et al. (2019)[74,b]	US, birth cohort (CHAMACOS)	260	1999-2000	7y	2 urines (pregnancy)	MCOP - FEV_1: $\beta = -0.09$ (−0.15 to −0.03)[c]; FEF_{25-75}: $\beta = -7.06$ (−11.04 to −2.90)[c]
	Agier et al. (2019)[41]	6 EU countries, birth cohort (HELIX)	1,033	2003-2009	6-12y	1 urine (pregnancy and childhood)	None
	Berger et al. (2020)[63,b]	US, birth cohort (CHAMACOS)	282	1999-2000	7y	2 urines (pregnancy)	MCOP - FEV_1: PIP = −0.07 (0.05)[d]
Currently used pesticides	Raanan et al. (2017)[81]	US, birth cohort (CHAMACOS)	279	1999-2000	7y	2 urines (pregnancy), 5 urines (childhood)	None
	Agier et al. (2019)[41]	6 EU countries, birth cohort (HELIX)	1,033	2003-2009	6-12y	1 urine (pregnancy and childhood)	None

[a]Coefficient estimates are given for a change in FEV_1% for every 10-fold increase in the mean BPA concentration. [b]In Berger et al.[69] three phthalates were assessed together with three parabens and four phenols; in Berger et al.[74] eight phthalates were assessed together with BPA; in Berger et al.[63] eleven phthalates were assessed together with four parabens and five phenols. [c]Coefficient estimates are given for a change in L/s of FEF_{25-75} percent difference or L of FEV_1 for each doubling of phthalates concentration. [d]Predicted probability and standard deviation obtained in the Bayesian Kernel Regression Model for an interquartile range change in phthalates concentration.
BPA: bisphenol A; DAPs: dialkyl phosphate metabolites; DEHP: di-(2-ethylhexyl) phthalate; Développement et de la Santé de l'Enfant; EDEN: Étude des déterminants pré et postnatals du développement de la santé de l'enfant; FEF_{25-75}: forced expiratory flow at 25–75% of FVC; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; HELIX: Human Early-Life Exposome; HOME: Health Outcomes and Measures of the Environment; MCOP: monocarboxyisooctyl phthalate; MEP: monoethyl phthalate; PIP: posterior inclusion probability; y: years.

example, studies in rats show that prenatal exposure to BP-3 can impair the expression of estrogenic receptor -α and -β[65,66]. Estrogenic receptor-β is abundantly expressed and biologically active in the lungs and regulates alveolar formation and surfactant homeostasis[9]. BP-3 may also disrupt the levels of PPARγ receptor, down-regulating surfactant protein expression in alveolar type II cells[9], essential for lung maturation. Triclosan was shown to decrease the viability, growth and morphology of lung epithelial cells[67]. Parabens, triclosan, and BP-3 can also increase biomarkers of oxidative stress and inflammation[68].

Four studies in humans have evaluated whether prenatal exposure to parabens, triclosan, and BP-3 can affect lung function in the offspring (Table 4). The French cohort EDEN evaluated exposure to parabens, triclosan, and BP-3 in 228 pregnant women and their male's offspring and observed that exposure to ethyl-paraben was associated with reduced FEV_1 at 5 years; however, this association did not reach statistical significance. No association was observed for the other phenols[62]. In 392 pregnant women and their children of the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort, an agricultural community in California, US, Berger et al. (2018)[69] assessed the association between prenatal exposure to parabens, triclosan, and BP-3 and lung function but did not observe any association. These null results have recently been confirmed in another study in the same cohort evaluating the combined effect of 20 endocrine disruptors on lung function at 7 years[63]. Parabens and BP-3 showed a low contribution in the overall model including eleven phthalates, five phenols, and four parabens; for triclosan, they observed a positive association but in the BKMR model, its importance was low. Finally, in the HELIX cohort, Agier et al. (2019)[41] did not observe any association between prenatal exposure to ethyl-paraben and lung function at 6-12 years. Prenatal exposure to the other phenols was also not associated with lung function at school age.

Phthalates

Phthalates are phthalic acid diester compounds commonly used as plasticizers to increase flexibility and transparency of hard polyvinyl chloride (PVC) plastics. Phthalates are divided into long- and short-chain phthalates. The long-chain are the ones used in PVC plastics whereas the short-chain are used in non-PVC products including adhesives and personal care products[28] (Table 2). Both groups of phthalates are produced in large quantities worldwide and humans are continuously exposed to them through food, cosmetics products, and indoor air[28]. After exposure, they are metabolized and excreted from the body in hours or days. In order to avoid external contamination (e.g., phthalates present in lab plastic material), the metabolites and not their parent compounds are detected in biological samples, preferably in urine.

In vivo studies have shown that exposure to phthalates during gestation can lead to delayed lung maturation in newborn rats. Offspring of rats exposed to the long-chain di-(2-ethylhexyl) phthalate (DEHP) during gestation had increased lung interstitial tissue proportion and diminished number of

airspace units leading to a reduction of the gas-exchange surface[70–72]. A significant increase in the number and dimension of type-II pneumocytes, responsible for secretion of pulmonary surfactant and implicated in the epithelial repair, was observed[71]. Moreover, an *in vitro* study showed that exposure to DEHP altered the structure and migration of A549 cells, cell lines used as prototypes of type-II pneumocytes, and the production of pulmonary surfactant[73].

A total of five studies, three of them from the same cohort, have evaluated this association (Table 4). In the French cohort EDEN, the high molecular weight phthalate monocarboxyisooctyl phthalate (MCOP) and the low molecular weight phthalate mono-isobutyl phthalate (MiBP) tended to be associated with reduced FEV_1% at 5 years, but associations did not reach statistical significance[62]. A total of three studies have been conducted within the CHAMACOS cohort in the US[63,69,74]. These three studies differ in the number of phthalate metabolites included and the statistical tools used to assess the combined effect. The most consistent finding of these studies was the reduction of FEV_1 at 7 years associated with exposure to MCOP, even after accounting for other pollutants. Indeed, the most recent study included three pollutant groups (i.e. phthalates, parabens, and other phenols), and the BKMR model identified the phthalate group, and particularly MCOP, as the most important associated with impaired lung function[63]. Finally, in the HELIX study including a large number of pollutants and other environmental factors, exposure to DEHP metabolites during pregnancy was not associated with lung function at 6-12 years of age[41].

Currently used pesticides

Restriction on the use of persistent pesticides such as DDT has led to the use of non-persistent alternatives such as organophosphate pesticides, carbamates, and pyrethroids. They are widely produced and used worldwide for controlling pests in both agricultural and residential settings (Table 2). In 2018, around 400,000 tonnes of pesticides were sold in Europe with the majority used in agriculture[75]. Organophosphate pesticides (e.g., chlorpyrifos, malathion) are the most widely used active substances, followed by pyrethroids and carbamates. Exposure to pesticides is ubiquitous in humans (Table 1), primarily through their diet. However, the detection frequencies in biospecimens are usually low due to the intermittent exposure and their rapid elimination from the body[76–78].

Organophosphate pesticides can affect lung function by inhibition of the acetylcholinesterase (AChE) enzyme, preventing the metabolism of acetylcholine and consequently increasing bronchoconstriction and mucus secretion. It is also postulated that organophosphate pesticides can produce bronchoconstriction at levels below those needed to inhibit AChE via direct effect on muscarinic receptors; receptors responsible of controlling muscle tone, mucus secretion, vasodilatation, and inflammation[79,80].

We have identified two studies that evaluated the lung function effects of prenatal exposure to currently used pesticides (Table 4); the two were focused on organophosphate pesticides[41,81]. The first study was conducted in the CHAMACOS cohort. Organophosphate pesticides, particularly six dialkyl phosphate

Table 5. Summary of the effects of in utero exposure to organic pollutants on lung function

Pollutants	Number of studies	Effects observed	Evidence[a]
Persistent organic pollutants			
Organochlorine compounds	3	3 ↓ lung function	Insufficient
Perfluoroalkyl substances	3	2 ↓ lung function / 1 no effects	Insufficient
Non-persistent organic pollutants			
Bisphenols	5	1 ↓ lung function / 4 no effects	Insufficient
Parabens, Triclosan, Benzophenones	4	4 no effects	Insufficient
Phthalates	5	3 ↓ lung function / 2 no effects	Insufficient
Currently used pesticides	2	2 no effects	Insufficient

[a]Good evidence: for an association based on consistent results from multiple studies and meta-analyses; Moderate evidence: of an association based on multiple studies, but with some inconsistencies; Insufficient evidence: evidence for an association based on only a few studies, or with substantial inconsistencies; 0: no or very few studies[102].

metabolites (DAPs), were measured twice during pregnancy[81]. Prenatal exposure to DAPs was not associated with FEV_1 or FVC at 7 years. In the HELIX cohorts, DAPs were also measured during pregnancy but no association was found with lung function at school-age[41].

DISCUSSION

In this review, we provide a broad summary of the current evidence of the effects of prenatal exposure to organic pollutants on lung function in the offspring. Overall, evidence is insufficient for all organic pollutants, with few studies and inconsistent results across them (Table 5). Few studies have found associations and the effects observed (i.e. changes in lung function parameters) are small (e.g., 50 mL reduction in FEV_1 for DDE exposure[39]). Inconsistencies across studies may reflect differences in sociodemographic characteristics of the populations, different use of consumer products between countries, measurement error associated with the assessment of the outcome and the exposure, and the statistical approaches used to assess the lung function effects of organic pollutants. Hereby we discuss different possibilities that may explain the inconsistent results across studies.

Outcome measurement: The evidence on the effects of organic pollutants on respiratory health is larger and more consistent in relation to respiratory symptoms (i.e. respiratory tract infections, wheezing, asthma) (reviewed by Vrijheid et al., 2016[60]). More studies have been conducted in this regard because information on respiratory symptoms can be easily obtained from questionnaires administered to the parents. On the contrary, spirometry is not easily conducted in young untrained children participating in population-based studies and needs to be performed by a pulmonologist or trained nurse; however, spirometry offers an objective measurement of lung function less subject to bias than parental administered questionnaires. All the studies included in this review performed spirometry from 4 to 20 years of age except Impinen et al. (2018)[49] where children performed tidal breathing shortly after birth. Spirometry is a good

method to measure pulmonary function and the most commonly performed[82]. However, there are other techniques that can be performed to complement or improve the outcome measurement. The reliability of spirometry performance in very young children has been argued. Techniques such as body plethysmography and interrupter resistance technique (R_{int}) can be a good complement or alternative to spirometry testing in younger children, being the latter the most suitable for young children due to its minimal difficulty to be performed[83-85]. In very young children (less than 4 years of age), pulmonary function can be measured with non-invasive techniques that do not require sedation such as multiple-breath washout test, tidal breathing (as did Impinen et al., 2018[49]), and forced oscillation technique[3,86,87]. These techniques are simple to perform, require little cooperation, and provide information on lung development, including its volume and ventilation inhomogeneity (multiple-breath washout), airway size (tidal breathing and forced oscillation), mechanical properties (forced oscillation), respiratory control (tidal breathing), and small airway function (multiple-breath washout and forced oscillation). With these techniques, researchers have been able to detect changes in lung function in relation to prenatal exposure to air pollution[86]. In older children and adults, the reversibility test can be performed to measure airflow limitation. This technique measures lung function performing spirometry before and after the administration of a bronchodilator such as salbutamol. A positive result is given when there is a significant improvement in lung function after the administration of the bronchodilator (change in $FEV_1 \geq 12\%$ or ≥ 200ml). It is considered consistent with the diagnosis of asthma and COPD[88,89], although in population studies a positive result cannot discern between asthma and COPD[90]. The reversibility test is easy to conduct and does not require medical assistance.

Exposure assessment: We have restricted ourselves to articles assessing the levels of organic pollutants in human biospecimens. Studies where exposure to organic pollutants (e.g. pesticides) was estimated by means of residential proximity to a contaminated area (e.g. crops) have not been included (e.g. Raanan et al., 2017[91]). Biomonitoring is the most extensive strategy for the assessment of environmental influences on health because samples are easy to collect, multiple exposures can be measured in the same biospecimen, and levels provide information of exposure for those pollutants found in many sources, as the case of organic pollutants[92]. However, biomarker levels could be subject to physiological distortion (e.g., renal clearance in the case of PFASs), could not reflect the internal dose (more directly related with the health outcome), and in the case of non-persistent pollutants, a single spot urine sample only reflects exposure for a short period of time (leading to exposure misclassification and attenuation of the results). We should consider all these limitations when interpreting the results. Alternatively, physiological conditions can be considered such as the glomerular filtration rate[93], internal dose can be estimated by using physiologically based pharmacokinetic modelling (PBPK)[94], and multiple samples per subject can be collected to obtain information on long-term exposure[76,95].

Statistical approach: The majority of studies included in this review assessed one pollutant

at each time. However, humans are exposed to multiple organic pollutants at the same time. In the last decade, the exposome approach has been proposed as a new to paradigm to encompass the totality of human environmental (meaning all non-genetic) exposures from conception onwards, complementing the genome[96]. Some of the studies included in this review have already developed multipollutant models with the aim to identify the most relevant pollutant associated with lung function[41,63,69,74]. Novel statistical tools are being developed to study chemical mixtures[15]. For the study of the health effects of endocrine disrupting chemicals, these methods should consider non-linear exposure-outcome relationships (lower doses can have more harmful effects than higher doses[39]) and potential interactions and confounding between pollutants, as the BKMR method used by Berger et al. (2020)[63] offers.

External validity: Particularly for non-persistent organic pollutants, we should consider that these pollutants have been studied among the same few population-based cohort studies (i.e., in the US: CHAMACOS; and in Europe: HELIX, EDEN, INMA), thus, limiting the generalization of the results in other population settings with different use of chemicals (and in consequence different exposure levels) and sociodemographic characteristics.

Public health implications: Although the changes in lung function associated with exposure to organic pollutants may not be clinically relevant (e.g. 50 mL reduction in FEV_1 for DDE exposure[39], as mentioned before), they can be important from an etiological perspective and at a population level. A good example to illustrate the societal impact of small effects associated with exposure to environmental pollutants is the case of lead exposure in children and the reduction of intelligence quotient (IQ) of 6 points[97,98]. It is estimated that an average drop of 6 points in IQ across the population would nearly double the number of people with an IQ below 70[99]. Therefore, when there is absence of consistent evidence about the health effects of a given exposure, such as the association of organic pollutants with lung function, the precautionary principle should prevail and apply policy measures to reduce its exposure.

CONCLUSION

In conclusion, although in the last decade the number of studies assessing the lung function effects of prenatal exposure to organic pollutants have increased, the evidence is still limited and inconsistent. Many studies did not find any association; this can reflect a real null effect or methodological limitations such as exposure misclassification, an important challenge of the study of the health effects of non-persistent pollutants, or measurement error of lung function parameters performed at young age. Further studies, with larger sample size to study susceptible groups, conducted in different population settings, with a thoughtful sampling design, and considering multiple pollutants, are needed. In parallel, public health strategies, such as the one that the Federation of Gynecology and Obstetrics, the University of California, and the Health and Environment Alliance has recently launched[100], are needed to reduce exposure to organic pollutants in the community and particularly in pregnant women.

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DISCLOSURES

Dr. Abellan and Dr. Casas have nothing to disclose.

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5.2. Paper II

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Prenatal exposure to organochlorine compounds and lung function during childhood

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ABSTRACT

Introduction: Prenatal exposure to organochlorine compounds (OCs) can increase the risk of reported respiratory symptoms in children. It remains unclear whether these compounds can also impact on lung function. We assessed the association between prenatal exposure to OCs and lung function during childhood.

Methods: We included 1308 mother-child pairs enrolled in a prospective cohort study. Prenatal concentrations of *p,p′*-dichlorodiphenyltrichloroethane [*p,p′*-DDT], *p,p′*-dichlorodiphenyldichloroethylene [*p,p′*-DDE], hexachlorobenzene [HCB], and seven polychlorinated biphenyls [PCBs] were measured in cord blood. Spirometry was performed in the offspring at ages 4 (n = 636) and 7 years (n = 1192).

Results: More than 80% of samples presented quantifiable levels of *p,p′*-DDE, HCB, PCB-138, PCB-153, and PCB-180; *p,p′*-DDE was the compound with the highest median concentrations. At 4 years, prenatal *p,p′*-DDE exposure was associated with a decrease in forced expiratory volume in 1 s (FEV_1) in all quartiles of exposure (e.g., third quartile [0.23–0.34 ng/mL]: β for FEV_1 −53.61 mL, 95% CI −89.87, −17.35, vs. the lowest). Prenatal *p,p′*-DDE levels also decreased forced vital capacity (FVC) and FEV_1/FVC, but associations did not reach statistical significance in most exposure quartiles. At 7 years, *p,p′*-DDE was associated with a decrease in FVC and FEV_1 in only the second quartile of exposure (e.g. β for FEV_1 −36.96 mL, 95% CI −66.22, −7.70, vs. the lowest). Prenatal exposure to HCB was associated with decreased FVC and FEV_1, but in only the second quartile and at 7 years (e.g. [0.07–0.14 ng/mL]: β for FEV_1 −25.79 mL, 95% CI −55.98, 4.39, vs. the lowest). PCBs were not consistently associated with lung function.

Conclusion: Prenatal exposure to *p,p′*-DDE may decrease lung function during childhood, especially FEV_1 and at medium levels of exposure. Further and deeper knowledge on the impact of environmental chemicals during pregnancy on lung development is needed.

1. Introduction

Respiratory diseases are among the leading causes of paediatric morbidity worldwide and the aetiology of some of them, such as asthma, remains unclear (European Respiratory Society, 2017). Adverse environmental exposures such as air pollution and smoking during prenatal life have been associated with respiratory diseases and alterations in the lung development in childhood and adulthood (Gehring

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et al., 2015; Kajekar, 2007; Miller and Marty, 2010). Organochlorine compounds (OCs), synthetic persistent pollutants extensively used as pesticides, electrical insulators, and other industrial products until their ban in 1970s, have also been suspected to influence the development of the lung (Hansen et al., 2016). Exposure to environmental chemicals such as OCs during prenatal life may induce developmental adaptations of the lung and airways, leading to relatively small airways. Such adaptations could lead to a reduction in expiratory flows - reflected by lower lung function values - and consequently increasing the risk of adverse respiratory outcomes (Miller and Marty, 2010). Due to their persistence, OCs are still detectable in current populations' blood (Haug et al., 2018). General population is exposed mainly through diet, whereas foetuses and new-borns can be exposed to OCs through placental transfer and breastfeeding (Sunyer et al., 2005).

Several studies have assessed the effects of prenatal exposure to OCs on adverse respiratory outcomes in the offspring, mainly using parental-reported symptoms (Dallaire et al., 2006; Gascon et al., 2014a, 2014b, 2012; Hansen et al., 2016, 2014; Sunyer et al., 2010, 2006, 2005). These studies showed that prenatal exposure to OCs, particularly *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) but also hexachlorobenzene (HCB) and polychlorinated biphenyls (PCBs), can increase the risk of lower respiratory tract infections, wheeze, and asthma in children (Gascon et al., 2014a, 2014b, 2012; Sunyer et al., 2006, 2005) and asthma medication use in adults (Hansen et al., 2014), even at low exposure levels (Sunyer et al., 2005). Only one study assessed the impact of OCs exposure during prenatal life on lung function (Hansen et al., 2016). Authors observed that prenatal exposure to *p,p′*-DDE, HCB, and PCBs was associated with offspring reduced forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) ratio (increased risk of airway obstruction) at 20 years of age. In this cohort however, the sample size was quite small (n = 414) and the OCs concentrations were determined in the 80's and might not reflect current exposure (Hansen et al., 2016).

Investigating the effects of prenatal exposure to OCs on lung function is relevant because reduced lung function in infancy not only poses a burden of childhood morbidity but is also a risk factor for the development of chronic diseases during adulthood such as bronchial asthma (Antó et al., 2010; Martinez, 2009) and chronic obstructive pulmonary disease (Bui et al., 2017; Martinez, 2016). The present study therefore aims to estimate the association between exposure to OCs during pregnancy and lung function in childhood.

2. Material and methods

2.1. Study population

This study is based in three geographic areas belonging to the Spanish INMA (INfancia y Medio Ambiente – Environment and Childhood) population-based birth cohort: Gipuzkoa, Sabadell, and Valencia. Pregnant women attending for prenatal care visits during the first trimester of their pregnancy who fulfilled the eligibility criteria were invited to participate in the cohort between 2004 and 2008 (Guxens et al., 2012). Eligible women had to be 16 years or older, residing in the study area, willing to deliver in the reference hospital, not have followed assisted reproduction, not to be twin pregnant, and not have any communication problems.

For the present study, population was restricted to children with prenatal OCs concentrations measured in maternal serum drawn during pregnancy or in cord serum that additionally had available and acceptable lung function data at 4 or at 7 years of age. A total of 1308 children had available data on exposure and outcome at 4 or at 7 years (Fig. 1). The study was approved by the ethical committees of the centres involved in the study. Written informed consent was obtained from the parents of all children.

2.2. Prenatal OCs exposure assessment

Quantification of *p,p′*-dichlorodiphenyltrichloroethane (*p,p′*-DDT), *p,p′*-DDE, HCB, and several PCB congeners (PCB-28, -52, -101, -118, -138, -153, and -180) was determined in maternal or cord serum. Maternal serum samples were collected between the 7th and 26th week of pregnancy (median = 13 weeks; interquartile range (IQR) = 1.6) and cord serum samples were extracted at birth. Samples were analysed by gas chromatography methods described elsewhere (Goñi et al., 2007). Limits of detection (LOD) in Gipuzkoa and Sabadell were 0.073 ng/mL for all compounds and in Valencia they ranged between 0.01 and 0.071 ng/mL. *p,p′*-DDT and PCB congeners 28, 52, 101, and 118 presented detectable levels in < 25% of analysed samples and were excluded from the analyses. We therefore calculated the sum of PCBs (ΣPCB) as the sum of PCB-138, PCB-153, and PCB-180. Samples with *p,p′*-DDE, HCB, PCB-138, -153, and -180 concentrations below the LOD were imputed on a defined range between 0 and each corresponding LOD (data not shown). Since cord serum is considered the best proxy of OCs exposure during foetal life (Korrick et al., 2000), we estimated the equivalent concentrations in cord serum from the concentrations measured in maternal serum by applying cohort specific conversion factors using paired cord and maternal measurements available in Gipuzkoa and Valencia (Supplementary Methods 1).

2.3. Lung function assessment

Lung function was assessed from spirometry tests performed at 4 years (mean = 4.4 years, standard deviation (SD) = 0.2) in Gipuzkoa and Sabadell, and at 7 years in Gipuzkoa, Sabadell, and Valencia (mean = 7.4 years, SD = 0.5) following the American Thoracic Society (ATS) and the European Respiratory Society (ERS) guidelines (Miller et al., 2005). Children with at least one acceptable manoeuvre were included. Lung function parameters selected for the analyses were FVC (mL), FEV_1 (mL), and FEV_1/FVC (%).

2.4. Covariates

Information on maternal ethnicity, country of birth, education level, socio-economic status (coded according to the International Standard Classification of Occupations-88 system), smoking during pregnancy, alcohol consumption, history of allergy, rhinitis or eczema, age at delivery, and parity was obtained by questionnaires administered to the mothers at 12th and 32th weeks of pregnancy. Information on maternal diet was obtained from a 100-item semi-quantitative food frequency questionnaire administered to the mothers at 12th and 32th weeks of pregnancy. Measured maternal height and reported weight by the mother at the first trimester visit were used to calculate pre-pregnancy body mass index (BMI) (kg/m^2). Gestational age, child's sex, and birth weight and height were obtained from clinical records. Preterm birth and low birth weight were defined as < 37 weeks of gestational age and < 2500 g at birth, respectively. Postnatal questionnaires provided information on number of breastfeeding, day care attendance, and doctor diagnosed asthma. Child's height and weight at 4 and 7 years were measured and age was recorded at the time of the spirometry test.

2.5. Statistical analysis

The analyses were performed separately for children who had outcome data at 4 years (n = 636) and at 7 years (n = 1192) (Fig. 1). The shape of the relationship between OCs and lung function was tested by General Additive Models (GAMs). None of the associations showed linearity (Supplementary Fig. S1) and therefore OCs concentrations were included in the models as exposure categories using age-specific quartiles as cut-offs. To reduce the likelihood of bias due to follow-up losses and missing data, we performed multiple imputations by chained equations of missing values for the covariates where 10 complete

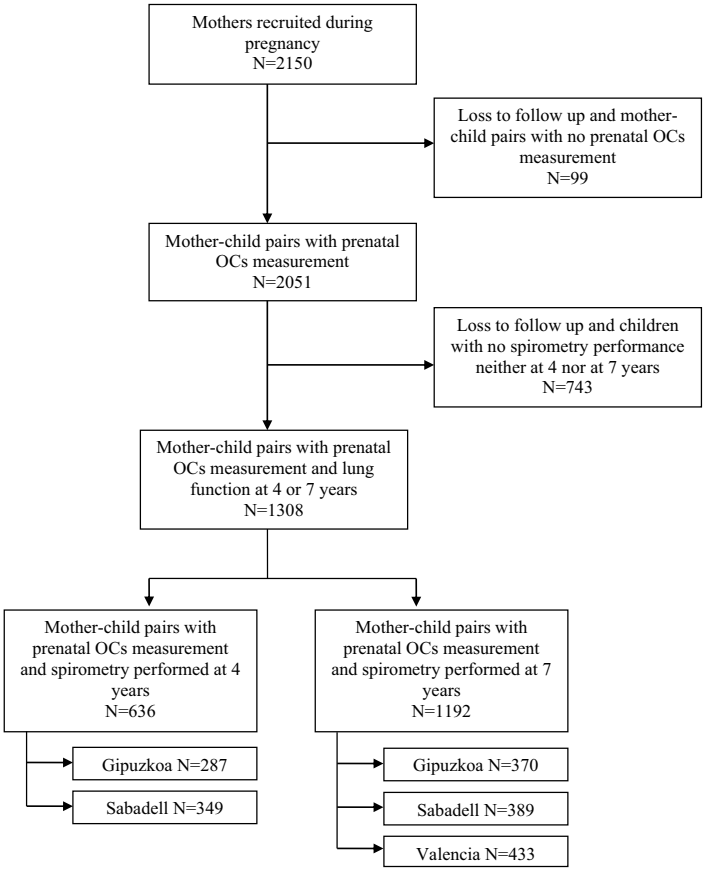

Fig. 1. Flowchart of the included study population.

datasets were generated (Supplementary Methods 2). Distributions among variables from the imputed and the observed datasets did not differ (Supplementary Table S1). Potential confounders, effect modifiers, and mediators of the association of interest were assessed by the construction of a directed acyclic graph (DAG) using DAGitty software (Supplementary Fig. S2) (Textor et al., 2011). Multivariable linear regression models were used to estimate the association between quartiles of OCs concentrations and lung function separately at 4 (n = 636) and 7 (n = 1192) years of age. Multivariable linear mixed models were used in the population with two lung function measurements available (n = 520) with random intercepts for subjects and cohorts. Covariates included in the models were those selected from the DAG: maternal age at delivery, maternal pre-pregnancy BMI, maternal educational level, maternal social class, maternal country of birth, parity, maternal lifestyle (smoking and alcohol consumption during pregnancy), and maternal diet (specifically fish, meat, vegetables and fruits). Additionally, models were adjusted for region of residence and child's sex, age, and height at the time of the lung function assessment because these child characteristics are the most important predictors of pulmonary function (Quanjer et al., 2012). Effect modification by preterm birth, low birth weight, maternal history of asthma, rhinitis or eczema, and child's sex was assessed through inclusion of the interaction terms in the models (p-value < 0.10) and stratified analyses, when possible. OCs can influence height of the child and hence potentially mediate the association of OCs and lung function (Balte et al., 2017). We therefore, tested whether height met the conditions to act as mediator: i) it was associated with OCs; ii) it was associated with lung function and iii) after including it in the models as confounder the association was no longer significant. In case all these conditions were fulfilled we then performed mediation analysis. To assess the robustness of our results, various sensitivity analyses were performed. We performed all models again by using the complete case dataset, the lipid-adjusted OCs concentrations, the maternal serum concentrations, and the lung function parameters expressed as sex, height, age, and ethnicity -adjusted z-scores according to the Global Lung Function Initiative reference values (Quanjer et al., 2012). Models were also repeated by excluding children who were unable to perform reproducible spirometry tests and children with asthma. Finally, to differentiate the role of each OC, we performed a multipollutant model in which all main exposure variables (*p,p′*-DDE, HCB, and ΣPCB) were included.

3. Results

3.1. Study population

Children from the study population were representative of the total sample in terms of sex, gestational age, and birth weight, among other characteristics. Mothers included in the analyses were slightly older, had higher education levels but lower social class, and were more likely to be European in comparison to the total sample (Supplementary Table S2). Complete details of the characteristics of the study populations at 4 and 7 years are shown in Table 1. *p,p′*-DDE was the compound with the

Table 1
Maternal and child characteristics of the study populations at 4 and 7 years of age[a].

	4 years (n = 636)	7 years (n = 1192)
Maternal characteristics		
Age at delivery (years), mean (SD)	32.3 (3.8)	32.1 (3.9)
Pre-pregnancy BMI (kg/m^2), mean (SD)	23.3 (4.3)	23.5 (4.3)
Educational level, %		
Less than primary or primary	18.3	21.2
Secondary	37.4	40.4
University	44.2	38.3
Social class, %		
Low	27.5	25.3
Medium	32.4	29.2
High	40.1	45.5
Country of birth, %		
European	96.5	96.4
Non-European	3.5	3.6
Parity, %		
Nulliparous	57.6	56.6
Multiparous	42.4	43.4
Smoking during pregnancy, %		
Never smoker	49.8	46.2
Smoker before pregnancy	36.4	37
Smoker during pregnancy	13.9	16.8
History of asthma, rhinitis, or eczema, % yes	28.8	28
Diet (servings/day), mean (SD)		
Vegetables	4.9 (1.8)	4.9 (1.9)
Fish	0.7 (0.3)	0.7 (0.3)
Meat	1.1 (0.4)	1.1 (0.4)
Alcohol consumption (g/day), mean (SD)	0.3 (0.9)	0.3 (1.0)
Child characteristics		
Sex, % female	48.3	49.3
Gestational age (weeks), mean (SD)	39.9 (1.8)	39.9 (1.8)
Preterm, yes (< 37 weeks), mean (SD)	2.7	3.9
Birth weight (g), mean (SD)	3266 (423)	3257 (456)
Low birth weight, % < 2500 g	4.4	5.1
Breastfeeding, % yes	93	89.6
Day care attendance, % yes	87.8	83.1
Height at follow-up (cm), mean (SD)	106.3 (4.3)	125.3 (6)
Weight at follow-up (kg), mean (SD)	18.4 (2.7)	27.4 (5.6)
Ever asthma, % yes	2.9	6.2
FVC (L), mean (SD)	1.0 (0.2)	1.8 (0.3)
FEV_1 (L), mean (SD)	0.9 (0.2)	1.5 (0.2)
FEV_1/FVC (%), mean (SD)	92.9 (6.7)	86.7 (6.7)
≥ 2 acceptable and reproducible manoeuvres, %	61.2	63.6

BMI: body mass index; SD: standard deviation; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s.
[a] Values come from the observed dataset.

Table 2
Concentrations (ng/mL) of *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), hexachlorobenzene (HCB), polychlorinated biphenyl congeners (PCB-138, PCB-153, PCB-180), and the sum of polychlorinated biphenyls (ΣPCB) in the study population[a].

	% < LOD	Minimum	p25	Median	p75	Maximum
p,p′-DDE	1.6	0.00	0.18	0.28	0.48	43.39
HCB	13.6	0.00	0.07	0.13	0.23	4.64
PCB-138	20.3	0.00	0.05	0.08	0.11	1.61
PCB-153	12.8	0.00	0.07	0.11	0.16	1.44
PCB-180	20.6	0.00	0.05	0.08	0.12	1.33
ΣPCB	–	0.01	0.18	0.28	0.39	4.02

LOD: limit of detection; p: percentile.
[a] Cord serum concentrations (ng/mL) from mother-child pairs with prenatal OCs measurement and lung function assessment at 4 or 7 years of age (n = 1308). LODs in Gipuzkoa and Sabadell were 0.071 ng/mL and in Valencia ranged between 0.01 and 0.071 ng/mL.

highest median concentrations in cord blood (0.28 ng/mL; IQR = 0.3 ng/mL) (Table 2). HCB and the ΣPCB presented median concentrations of 0.13 ng/mL and 0.28 ng/mL, respectively. Among the three study regions, the highest median levels of OCs were observed in Valencia (Supplementary Table S3).

3.2. *p,p′*-DDE and lung function

At 4 years, prenatal exposure to *p,p′*-DDE seemed to be associated with a decrease in FVC in all quartiles of exposure compared to the lowest quartile, but associations did not reach statistical significance (e.g. fourth quartile [> 0.35 ng/mL] β −37.56 mL, 95% CI −80.58, 5.46) (Fig. 2a and Supplementary Table S4). Prenatal exposure to *p,p′*-DDE was associated with a decrease in FEV_1 in all quartiles of exposure compared to the lowest quartile (e.g. fourth quartile β −42.82 mL, 95% CI −80.65, −4.99) (Fig. 2a and Supplementary Table S4). Being exposed to the second and third quartiles of *p,p′*-DDE concentrations were also associated with decreased FEV_1/FVC, compared to the lowest quartile; however only the third quartile showed statistical significance (e.g. third quartile [0.23–0.34 ng/mL] β −2%, 95% CI −3.5, −0.5) (Fig. 2a and Supplementary Table S4). At 7 years, children exposed to the second quartile of *p,p′*-DDE concentrations (0.17–0.28 ng/mL), had a decreased FVC and FEV_1 (β for FVC −39.45 mL, 95% CI −71.23, −7.66; and FEV_1 −36.07 mL, 95% CI −65.21, −6.92) compared to the lowest quartile of exposure; such associations were not observed in children exposed to the third and fourth quartiles (Fig. 2a and Supplementary Table S4). No association was observed for FEV_1/FVC at 7 years. In children with two available lung function measurements (n = 520), we observed an overall association between prenatal exposure to the second and third quartiles of *p,p′*-DDE and reduced FEV_1 (e.g. third quartile [23–34 ng/mL] β −37.17 mL, 95% CI −71.92, −2.42), although results on the second quartile were borderline statistically significant ([0.14–0.23 ng/mL] β −33.82 mL, 95% CI −68.22, 0.59) (Table 3). We did not observe any associations with FVC nor FEV_1/FVC (Table 3).

3.3. HCB and lung function

At 4 years, cord blood HCB seemed to be related to reduced FVC and FEV_1, particularly in the third and fourth quartiles, when compared to the lowest exposed group, although associations did not reach statistical significance (Fig. 2b and Supplementary Table S4). HCB was not associated with FEV_1/FVC at 4 years. At 7 years, only children exposed to the second quartile of HCB concentrations (0.07–0.14 ng/mL) had reduced FVC and FEV_1 (e.g. β for FVC −56.68 mL, 95% CI −89.87, −23.49) and increased FEV_1/FVC (β 1.24%, 95% CI 0.14, 2.34) (Fig. 2b and Supplementary Table S4) but no associations were observed among the third and fourth quartiles of exposure. In the multivariate linear mixed models we did not observe any association between HCB and lung function (Table 3).

3.4. PCBs and lung function

Regarding PCBs, no consistent associations were observed between any of the analysed PCBs, including the ΣPCB, and lung function parameters neither at 4 nor at 7 years (Fig. 2c and d, and Supplementary Fig. S3 and Table S4). We only observed an increase in FEV_1/FVC ratio at 7 years (β 1.2%, 95% CI 0.1, 2.3) associated with being exposed to the second quartile of PCB-153 concentrations (0.08–0.12 ng/mL) compared to the lowest quartile (Fig. 2c and Supplementary Table S4). No associations were observed in the analysis considering those children with two available lung function measurements (Table 3).

3.5. Additional analyses

Interaction and stratification analyses could only be performed for

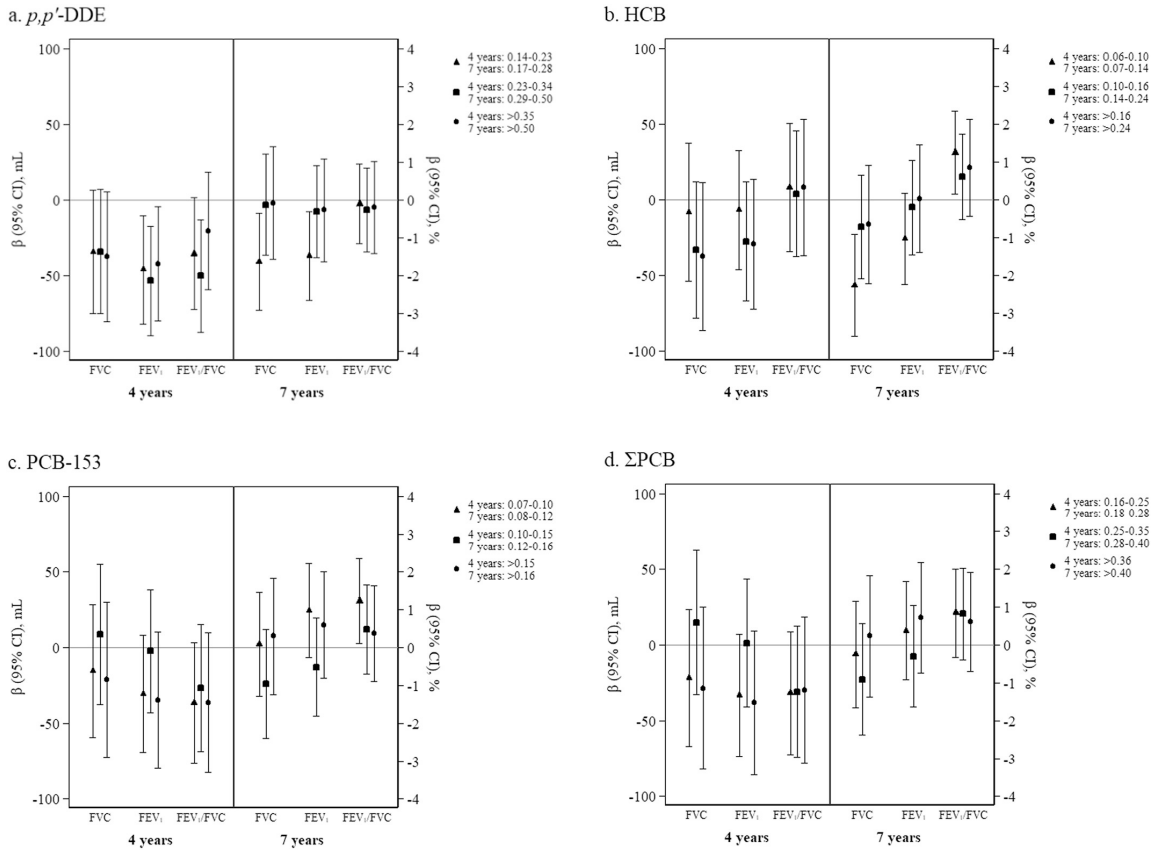

Fig. 2. Associations (β (95% CI)) between cord serum *p,p′*-DDE (a), HCB (b), PCB-153 (c), and ΣPCB (d), in quartile ranges (ng/mL), and lung function at 4 (n = 636) and 7 (n = 1192) years of age[a].

[a]Models adjusted for children's region of residence, sex, age and height at the time of the lung function assessment, maternal age at delivery, maternal pre-pregnancy BMI, maternal educational level, maternal social class, maternal country of birth, parity, maternal smoking during pregnancy, maternal diet, and maternal alcohol consumption. Values represent mL for FVC and FEV_1 and % for FEV_1/FVC.

child's sex due to the low number of preterm, low birth weight babies, and mothers with history of asthma, rhinitis or eczema in each quartile of exposure. Child's sex did not show any interaction in our associations of interest (data not shown). Height was not associated with any quartile of OCs and hence we did not perform subsequent mediation analysis.

Overall, effect estimates did not change in complete case analyses (Supplementary Table S5), using the lipid-adjusted OCs concentrations (data not shown), the maternal serum concentrations (Supplementary Table S5), and the lung function standardised z-scores (Supplementary Fig. S4). Results were similar after excluding those children who were not able to perform reproducible spirometry tests (Supplementary Table S6) and children with asthma (data not shown). Finally, results remained robust when models were adjusted for all main OCs together (data not shown).

4. Discussion

In this prospective study, we observed that prenatal exposure to *p,p′*-DDE was associated with reduced FEV_1 and also with reduced FVC and FEV_1/FVC at 4 years, although most of the associations with FVC and FEV_1/FVC did not reach statistical significance. Prenatal *p,p′*-DDE levels also reduced FVC and FEV_1 at 7 years but only at medium levels of exposure. Additionally, HCB seemed to be associated with reduced FVC and FEV_1 at certain exposure levels at both ages, but most of the associations were borderline statistically significant. Inconsistent results were observed among analysed PCBs.

To our knowledge, only one study has assessed the effects of prenatal exposure to OCs and lung function in the offspring (Hansen et al., 2016). In a Danish birth cohort of 414 participants established in the 80s, they observed an increased risk of airway obstruction (FEV_1/FVC < 75%) at 20 years of age associated with exposure to very high concentrations of *p,p′*-DDE, HCB, and PCBs (e.g. *p,p′*-DDE = 3.2–38.8 ng/mL). They did not find any association with reduced FEV_1 (% predicted value < 90%). Two other studies have assessed postnatal exposure to *p,p′*-DDE in relation to lung function and found decreases in FVC and FEV_1 with increasing blood concentrations of *p,p′*-DDE in adults (n = 1696; median = 152 ng/g lipid; IQR = 213.1) (Ye et al., 2015) and in school-aged children (n = 328; median = 0.30 ng/mL, IQR = 0.2) (Balte et al., 2017). These two studies did not assess neither HCB nor PCBs (Balte et al., 2017; Ye et al., 2015). In our study we observed a reduction in lung function, especially in FEV_1, at pre-school and school ages in relation to prenatal *p,p′*-DDE concentrations around 0.30 ng/mL, at similar concentrations and even lower than these previous studies. These associations were not seen at higher *p,p′*-DDE exposure levels in the case of 7-year-old children. We

Table 3
Associations (β (95% CI)) between cord serum *p,p′*-DDE (a), HCB (b), PCB-153 (c), and ΣPCB (d), in quartile range (ng/mL), and lung function during childhood in children with repeated measurements (n = 520)[a].

	FVC (mL)	FEV_1 (mL)	FEV_1/FVC (%)
ng/mL	β (95% CI)	β (95% CI)	β (95% CI)
p,p′-DDE			
< 0.14	Ref	Ref	Ref
0.14–0.23	−16.71 (−55.43, 22.01)	−33.82 (−68.22, 0.59)	−1.21 (−2.54, 0.12)
0.23–0.34	−25.03 (−64.15, 14.09)	−37.17 (−71.92, −2.42)	−1.12 (−2.46, 0.22)
> 0.35	−7.83 (−48.61, 32.95)	−19.84 (−56.07, 16.39)	−0.70 (−2.09, 0.70)
HCB			
< 0.06	Ref	Ref	Ref
0.06–0.10	−14.78 (−57.91, 28.36)	−11.68 (−48.60, 25.25)	0.35 (−1.19, 1.88)
0.10–0.16	−29.65 (−72.58, 13.27)	−12.52 (−50.23, 25.18)	0.76 (−0.74, 2.27)
> 0.16	−29.01 (−76.37, 18.35)	−22.43 (−64.00, 19.15)	0.47 (−1.18, 2.12)
PCB-138			
< 0.04	Ref	Ref	Ref
0.04–0.07	−6.94 (−50.47, 36.59)	−9.22 (−47.78, 29.34)	−0.28 (−1.76, 1.19)
0.07–0.10	−16.58 (−60.84, 27.68)	−30.07 (−69.41, 9.27)	−1.02 (−2.55, 0.51)
> 0.10	−6.69 (−54.19, 40.81)	4.95 (−37.28, 47.18)	0.73 (−0.86, 2.32)
PCB-153			
< 0.07	Ref	Ref	Ref
0.07–0.10	−16.28 (−57.33, 24.77)	−16.23 (−53.06, 20.59)	−0.38 (−1.82, 1.06)
0.10–0.15	6.07 (−36.98, 49.13)	2.13 (−36.35, 40.61)	−0.31 (−1.80, 1.18)
> 0.15	−4.76 (−52.50, 42.99)	−5.05 (−47.71, 37.61)	−0.27 (−1.90, 1.37)
PCB-180			
< 0.05	Ref	Ref	Ref
0.05–0.07	−5.11 (−51.95, 41.73)	−12.07 (−54.06, 29.91)	−0.71 (−2.28, 0.86)
0.07–0.11	13.79 (−34.88, 62.46)	18.49 (−24.91, 61.89)	0.14 (−1.51, 1.78)
> 0.11	2.21 (−52.70, 57.11)	7.67 (−41.37, 56.71)	0.08 (−1.76, 1.91)
ΣPCBs			
< 0.16	Ref	Ref	Ref
0.16–0.25	−31.43 (−74.09, 11.24)	−28.20 (−67.30, 10.91)	−0.29 (−1.77, 1.20)
0.25–0.35	0.46 (−44.56, 45.48)	−7.72 (−48.48, 33.05)	−0.61 (−2.14, 0.93)
> 0.36	−13.91 (−64.24, 36.42)	−7.73 (−53.19, 37.72)	0.17 (−1.54, 1.88)

[a] Models adjusted for children's region of residence, sex, age and height at the time of the lung function assessment, maternal age at delivery, maternal pre-pregnancy BMI, maternal educational level, maternal social class, maternal country of birth, parity, maternal smoking during pregnancy, maternal diet, and maternal alcohol consumption.

observed a similar exposure-response relationship in relation to prenatal HCB exposure and lung function at school-age, where associations were not seen at higher HCB exposure levels.

Similarly to previous studies on OCs and respiratory health, for *p,p′*-DDE and HCB we only observed associations at certain exposure concentrations but not at higher levels (Dallaire et al., 2004; Dewailly et al., 2000; Glynn et al., 2008; Karmaus et al., 2003). It is well established that endocrine disrupting chemicals, including OCs, can have a non-monotonic exposure-response having effects at low doses that are not predicted by effects at higher doses (Vandenberg et al., 2012). This means that increasing concentrations not necessarily have greater effects on the complete exposure range. This exposure-response pattern is very common in natural hormones and endocrine disruptors since they act at extremely low concentrations. Biological mechanisms involved in this pattern are related to cytotoxicity and cellular or tissue specific receptors including negative feedback, down-regulation and desensitization, receptor selectivity, and receptor competition (Vandenberg et al., 2012).

Reduced lung function in early life can predispose to asthma-related symptoms in childhood (Martinez, 2009). In recent years, many studies have reported associations between prenatal exposure to OCs, mainly *p,p′*-DDE, and low respiratory tract infections, wheezing, asthma, and asthma medication use during childhood and early adulthood assessed through parental-reported symptoms (Dallaire et al., 2006; Gascon et al., 2014a, 2014b, 2012; Hansen et al., 2016, 2014; Sunyer et al., 2010, 2006, 2005). These findings are in accordance with our results since we observed a decrease in FEV_1, FVC and/or FEV_1/FVC, which reflect decreased airway patency in obstructive lung diseases such as asthma, associated with exposure to *p,p′*-DDE during foetal life. These associations did not change after excluding children with asthma diagnosis, probably because these children often perform spirometry tests within the normal range (Bacharier et al., 2004).

We suspect OCs may interact with cellular receptors and alter signalling pathways which are associated with lung morphogenesis and inflammation. The development of the lung starts at around 4 weeks of gestation and continues postnatally with alveolarisation and lung growth and expansion (Kajekar, 2007). Lung development is the result of a complex interaction between growth factors, hormones, genetic factors, and environmental factors, among others (Frey and Gerritsen, 2006). Estrogenic and androgenic receptors are expressed in the lung and their modulation plays an important role on the development and functioning of the lungs during foetal development (Carey et al., 2007). OCs are endocrine disruptors able to interfere with hormonal activities due to their estrogenic and anti-androgenic properties. They can interact with the estrogenic receptor (ER) signalling pathway directly and indirectly through the aryl hydrocarbon receptor (AhR) (Shanle and Xu, 2011), and consequently alter the development and the correct functioning of the airways. *p,p′*-DDE, HCB, and PCB-153 have been shown to induce AhR expression in vitro (Gaspar-Ramírez et al., 2015). Activation of AhR has been associated to delayed lung development in rats and to adverse immunological effects through different mechanisms such as inflammation (Kransler et al., 2009). In children, OCs have been associated with increased inflammatory markers such as interleukins and immunoglobulins (Brooks et al., 2007; Gascon et al., 2014b; Karmaus et al., 2005). Induction of AhR expression and activity modulation by exposure to OCs might be crucial in the developing lung and

immune system.

The importance for the study of OCs relies on the widespread human exposure to these compounds and their adverse health effects. Even though OCs have been banned since the 1970s, detectable levels of *p,p′*-DDE, HCB, and some PCBs have been found in > 80% of pregnant women included in our study, even the recruitment started after 2004. Indeed, OCs concentrations have been detected in almost all children aged 6–11 years participating in the HELIX cohort between 2013 and 2016 including INMA (Haug et al., 2018); showing that current populations, and consequently the future generations, are still exposed to these compounds.

The main strengths of the present study are its population-based prospective design, the relatively large sample size, and the objective assessment of lung function. The present study, however, has some limitations. First, differences were observed between mothers of included children and those from the total sample: included mothers tended to be older, more educated, and European. This might have biased the obtained estimates, but we would not expect the estimates to change since such variables did not affect the associations (data not shown). Although our results may not be generalisable to the general population, it is not likely to affect internal validity, i.e. the association estimates between exposure to OCs and lung function in the offspring. Second, the acceptability criteria of the lung function tests were similar in all INMA regions of residence but were not identical. This could have led to a differential lung function parameter selection between the studied regions. We took this into account by adjusting the models for region of residence. Third, the reliability of spirometry results in children, particularly FVC and in 4 year-olds, might be arguable. Thus, results obtained need to be interpreted carefully. Nonetheless, to ensure the optimal reliability achievable, tests were performed by pulmonologists or trained nurses and each obtained manoeuvre was thoroughly examined to be considered acceptable and reproducible for the analyses following the ATS/ERS guidelines. Furthermore, the estimates did not substantially change when analyses were restricted to children who performed reproducible manoeuvres. Forth, in our study population we did not measure postnatal exposure to OCs, which may be relevant since the lung mainly develops until 2 years of age (Kajekar, 2007). Further studies assessing the childhood exposure to OCs in relation to lung function, and preferable using physiologically based pharmacokinetic models to estimate lactational exposure to OCs are needed to elucidate which is the most susceptible period of exposure to OCs for lung development. Fifth, we could not perform stratified analysis for most of the effect modifiers due to the small sample size. Studies pooling information from different birth cohorts would allow an appropriate analysis of effect modification to detect susceptible groups of the population to these exposures. Sixth, we only considered OCs exposure although the foetus is exposed to a wide range of environmental chemicals including perfluoroalkyl compounds (PFASs), phenols, phthalates, and organophosphate pesticides that can also potentially affect lung development (Gascon et al., 2015; Qin et al., 2017; Raanan et al., 2016). Nonetheless, recent exposome studies reveal that correlations between exposures in different exposure groups (e.g. between OCs and PFASs) are much lower than among exposures in the same group (e.g. within OCs) (Tamayo-Uria et al., 2019). Therefore, we do not expect our estimates to be largely affected by other exposure groups not considered in the present study. Finally, we did not correct for multiple comparisons which may have led to false-positive findings (type I error). However, statistical correction for multiple comparisons increases the chances of false negative findings (type II error), which in the context of public health research might have worse consequences. Also, it assumes that the tested hypotheses are independent, which may not be the case in this study since the exposures tested, as well as the outcomes, are related.

5. Conclusion

In conclusion, our results suggest that prenatal exposure to *p,p′*-DDE may decrease lung function during childhood, especially FEV_1 and at medium levels of exposure. Since lung function in infancy predicts pulmonary function throughout life, further and deeper knowledge on the impact of environmental chemicals during pregnancy on lung development is needed for the development of public health policies targeted at the prevention of chronic respiratory diseases.

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Valencia

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Declaration of Competing Interest

All authors declare they do not have any conflict of interest in the presented work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105049.

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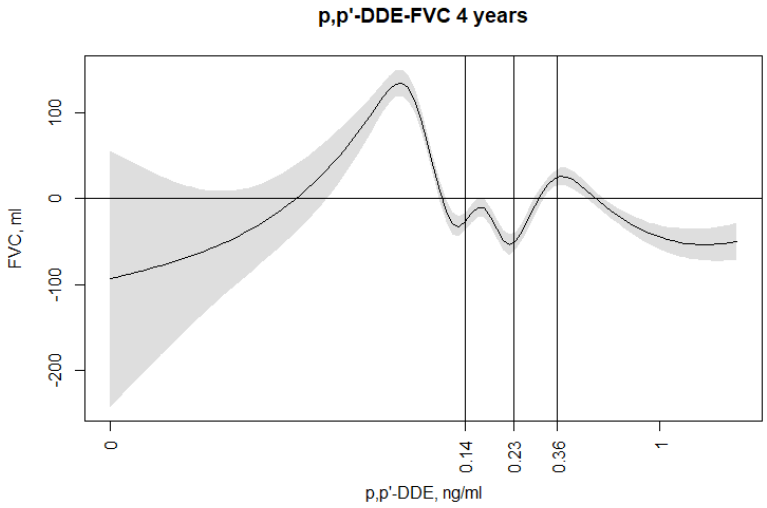
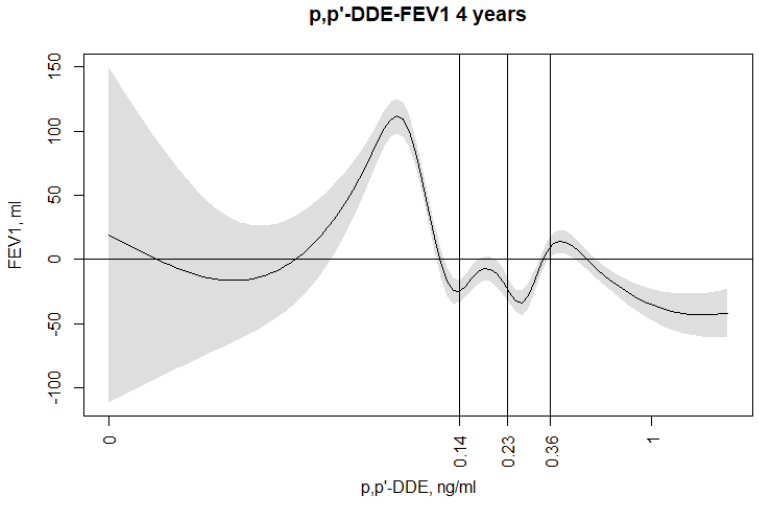
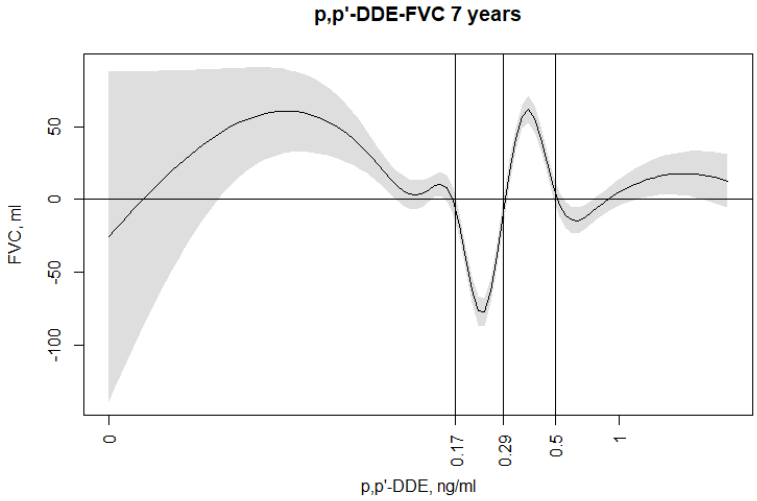
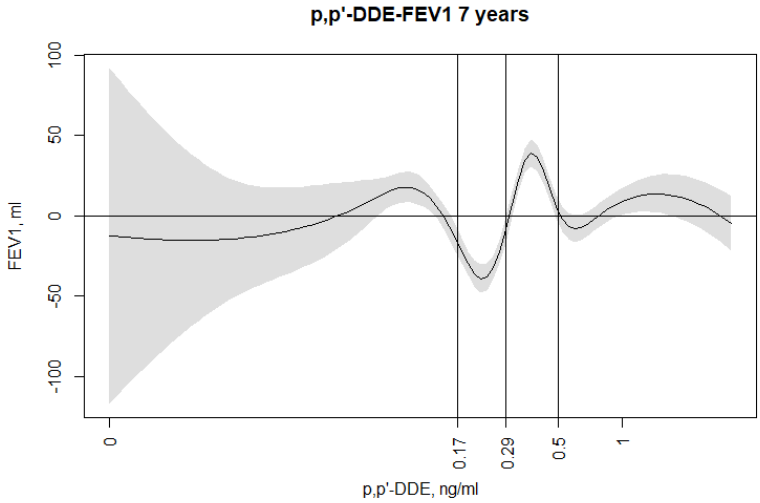
SUPPLEMENTARY MATERIAL

Figure S1. Generalised Additive Models to examine the shape of relationships between exposure to organochlorine compounds and cord serum *p,p'*-DDE and lung function at 4 (n=636) and 7 (n=1192) years.









[a] Models adjusted for children's region of residence, sex, age, weight, and height at the time of the lung function assessment, maternal age at delivery, maternal pre-pregnancy BMI, maternal educational level, maternal social class, maternal country of birth, parity, maternal smoking during pregnancy, maternal diet, and maternal alcohol consumption.

Figure S2. Directed acyclic graph of the association between prenatal exposure to OCs and lung function

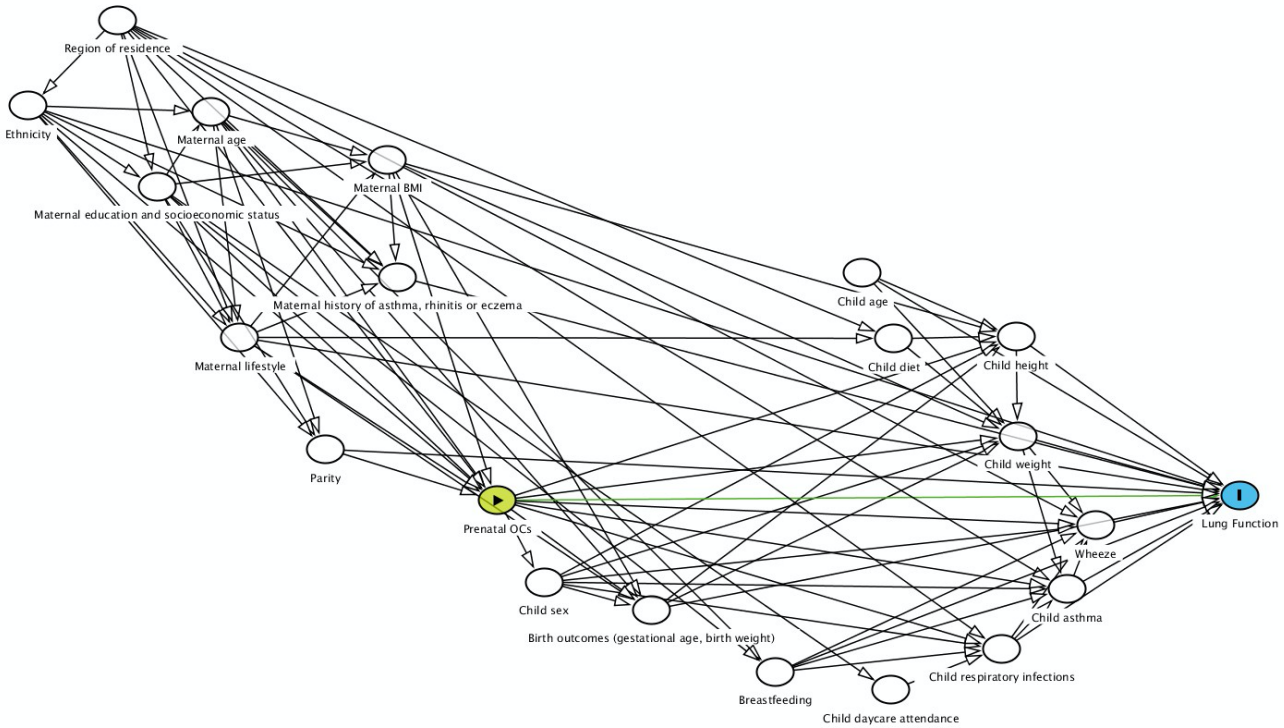


Table S1. Differences in maternal and child characteristics between observed and imputed datasets in the Gipuzkoa study region[a] as example

	% missing	Observed dataset	Imputed dataset
Maternal characteristics			
Age at delivery (years), mean (SD)	0.0	32.6 (3.3)	32.6 (3.3)
Pre-pregnancy BMI (kg/m^2), mean (SD)	0.0	22.8 (3.4)	22.8 (3.4)
Educational level, %	0.5		
Less than primary or primary		10.4	10.5
Secondary		35.9	36.2
University		53.2	53.3
Social class, %	0.0		
Low		32.9	32.9
Medium		29.6	29.6
High		37.6	37.6
Country of birth, %	0.0		
European		98.4	98.4
Non-European		1.6	1.6
Parity, %	0.0		
Nulliparous		55.3	55.3
Multiparous		44.7	44.7
Smoking during pregnancy, %	3.1		
Never smoker		50.7	50.8
Smoker before pregnancy		37.3	37.0
Smoker during pregnancy		11.9	12.2
History of asthma, rhinitis or eczema, % yes	0.0	24.6	24.6
Diet (servings/day), mean (SD)			
Vegetables	4	5.0 (1.7)	5.0 (1.7)
Fish	4	0.8 (0.3)	0.8 (0.3)
Meat	4	0.9 (0.3)	0.9 (0.3)

Alcohol consumption (g/day), mean (SD)	4	0.2 (0.5)	0.2 (0.5)
Child characteristics			
Sex, % female	0	51.5	51.5
Gestational age (weeks), mean (SD)	0	39.9 (1.8)	39.9 (1.8)
Preterm, % <37 weeks	0	3.3	3.3
Birth weight (g), mean (SD)	0.7	3295 (442)	3295 (442)
Low birth weight, %<2500g	0.7	4.3	4.3
Breastfeeding, % yes	5	91.8	91.8
Day care attendance, % yes	6.4	82.1	82.0

BMI: body mass index; SD: standard deviation.

[a] Corresponding to the number of mother-child pairs with prenatal OCs measurement and lung function assessment at 4 or 7 years of age (n=423).

Table S2. Differences in maternal and child characteristics between the study population and the total sample

	Total sample (n=2,150)	**Study population (n=1,308)**	**p-value**
Maternal characteristics			
Age at delivery (years), mean (SD)	31.8 (4.2)	32.1 (3.9)	0.034
Pre-pregnancy BMI (kg/m^2), mean (SD)	23.5 (4.3)	23.4 (4.2)	0.676
Educational level, %			
Less than primary or primary	26.6	21.2	0.000
Secondary	40.7	39.9	
University	32.7	38.9	
Social class, %			
Low	21.1	25.2	0.000
Medium	26.8	29.7	
High	52.2	45.1	
Country of birth, %			
European	92.9	96.3	0.000
Non-European	7.1	3.7	
Parity, %			
Nulliparous	55.2	56.4	0.196
Multiparous	44.8	43.7	
Smoking during pregnancy, %			
Never smoker	43.9	46.6	0.118
Smoker before pregnancy	37.7	37.6	
Smoker during pregnancy	18.4	15.8	
History of asthma, rhinitis or eczema, % yes	27	28.2	0.430
Diet (servings/day), mean (SD)			
Vegetables	4.9 (1.9)	4.9 (1.9)	0.853
Fish	0.7 (0.3)	0.7 (0.3)	0.391
Meat	1.1 (0.4)	1.1 (0.4)	0.062
Alcohol consumption (g/day), mean (SD)	0.3 (1.0)	0.3 (1.0)	0.714
Child characteristics			
Sex, % female	48.6	49.9	0.487
Gestational age (weeks), mean (SD)	39.6 (1.7)	39.7 (1.5)	0.501

Preterm, % <37 weeks	4.1	3.7	0.579
Birth weight (g), mean (SD)	3252 (483)	3257 (449)	0.728
Low birth weight, %<2500g	4.7	4.7	0.996
Breastfeeding, % yes	88.4	90	0.166
Day care attendance, % yes	81.7	83.5	0.206

BMI: body mass index; SD: standard deviation.
[a]p-values (analytical vs total sample) were estimated using one-sample mean tests or chi-square tests.

Table S3. Prenatal OCs concentrations[a] (ng/mL) for each study region[b]

	p25			Median			p75		
	Gipuzkoa	**Sabadell**	**Valencia**	**Gipuzkoa**	**Sabadell**	**Valencia**	**Gipuzkoa**	**Sabadell**	**Valencia**
***p,p'*-DDE**	0.144	0.143	0.284	0.224	0.235	0.432	0.346	0.368	0.728
HCB	0.085	0.070	0.142	0.125	0.112	0.237	0.176	0.179	0.367
PCB138	0.081	0.046	0.075	0.102	0.063	0.102	0.138	0.088	0.140
PCB153	0.104	0.062	0.094	0.134	0.087	0.133	0.176	0.126	0.178
PCB180	0.083	0.047	0.071	0.107	0.066	0.097	0.145	0.089	0.131
ΣPCBs	0.179	0.127	0.238	0.301	0.195	0.326	0.432	0.287	0.446

[a] Values come from the imputed dataset.

[b] Cord serum concentrations (ng/ml) from mother-child pairs with prenatal OCs measurement and lung function assessment at 4 or 7 years of age (n=1308). Limits of detection (LOD) in Gipuzkoa and Sabadell were 0.071 ng/mL and in Valencia they ranged between 0.01 and 0.073 ng/mL (supplementary table S1).

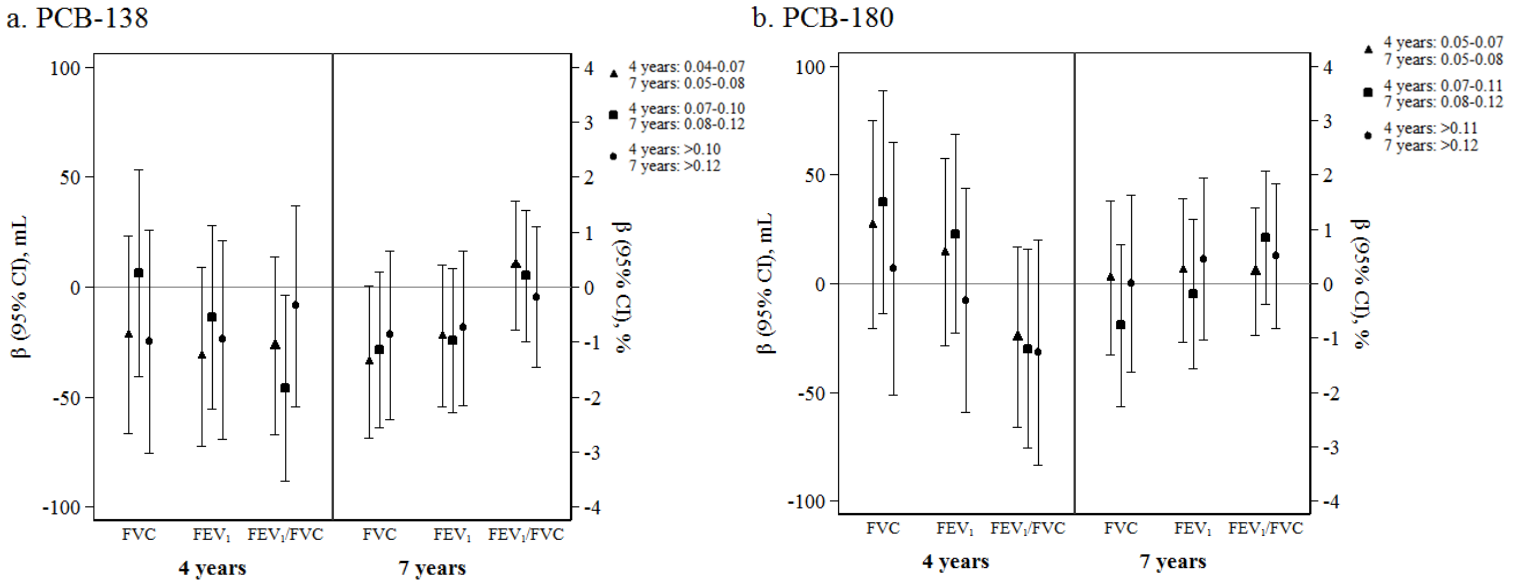
Table S4. Associations (β (95% CI)) between cord serum OCs in quartile ranges (ng/mL), and lung function at 4 (n=636) and 7 (n=1192) years of age[a]

4 years (n=636)					**7 years (n=1192)**				
		FVC (mL)	**FEV_1 (mL)**	**FEV_1/FVC (%)**			**FVC (mL)**	**FEV_1 (mL)**	**FEV_1/FVC (%)**
ng/mL	N	β (95% CI)	β (95% CI)	β (95% CI)	ng/mL	N	β (95% CI)	β (95% CI)	β (95% CI)
***p,p'*-DDE**									
<0.14	160	Ref	Ref	Ref	<0.17	299	Ref	Ref	Ref
0.14 – 0.23	159	-34.52 (-75.37, 6.33)	-46.12 (-82.05, -10.19)	-1.43 (-2.90, 0.05)	0.17 – 0.28	298	-41.04 (-73.18, -8.91)	-36.96 (-66.22, -7.70)	-0.11 (-1.17, 0.95)
0.23 – 0.34	159	-34.13 (-75.35, 7.10)	-53.61 (-89.87, -17.35)	-2.01 (-3.51, -0.52)	0.29 – 0.50	298	-3.22 (-36.61, 30.17)	-7.90 (-38.33, 22.53)	-0.27 (-1.37, 0.83)
>0.35	158	-37.51 (-80.40, 5.38)	-42.37 (-80.09, -4.65)	-0.82 (-2.38, 0.73)	>0.50	297	-2.07 (-39.11, 34.96)	-6.87 (-40.61, 26.88)	-0.20 (-1.42, 1.02)
HCB									
<0.06	164	Ref	Ref	Ref	<0.07	303	Ref	Ref	Ref
0.06 – 0.10	155	-8.36 (-53.87, 37.16)	-7.00 (-46.24, 32.23)	0.32 (-1.38, 2.02)	0.07 – 0.14	294	-56.77 (-90.34, -23.20)	-25.84 (-56.15, 4.47)	1.24 (0.14, 2.34)
0.10 – 0.16	158	-33.14 (-78.32, 12.03)	-27.66 (-67.21, 11.89)	0.15 (-1.51, 1.82)	0.14 – 0.24	297	-18.23 (-52.39, 15.93)	-5.16 (-36.27, 25.94)	0.60 (-0.53, 1.72)
>0.16	159	-37.75 (-86.68, 11.19)	-29.37 (-72.23, 13.49)	0.32 (-1.48, 2.12)	>0.24	298	-16.52 (-55.61, 22.57)	0.59 (-34.99, 36.16)	0.84 (-0.44, 2.13)
PCB-138									
<0.04	158	Ref	Ref	Ref	<0.05	292	Ref	Ref	Ref
0.04 – 0.07	159	-21.76 (-66.56, 23.03)	-31.33 (-71.96, 9.31)	-1.06 (-2.66, 0.55)	0.05 – 0.08	305	-34.03 (-68.83, 0.76)	-22.28 (-54.53, 9.96)	0.40 (-0.77, 1.58)
0.07 – 0.10	160	6.17 (-40.75, 53.08)	-13.54 (-55.38, 28.29)	-1.82 (-3.51, -0.13)	0.08 – 0.12	297	-27.31 (-63.31, 8.68)	-23.62 (-56.55, 9.32)	0.19 (-1.00, 1.38)

>0.10	159	-24.75 (-75.29, 25.79)	-23.45 (-68.49, 21.59)	-0.31 (-2.13, 1.51)	>0.12	298	-19.56 (-58.22, 19.09)	-17.47 (-52.90, 17.96)	-0.21 (-1.49, 1.07)	
PCB-153										
<0.07	161	Ref	Ref	Ref	<0.08	299	Ref	Ref	Ref	
0.07-0.10	158	-15.59 (-59.21, 28.03)	-30.68 (-69.10, 7.75)	-1.46 (-3.05, 0.12)	0.08 – 0.12	298	2.15 (-32.03, 36.34)	24.20 (-6.87, 55.26)	1.22 (0.10, 2.34)	
0.10 – 0.15	158	8.46 (-37.58, 54.49)	-2.55 (-43.17, 38.06)	-1.06 (-2.74, 0.61)	0.12 – 0.16	297	-23.99 (-59.82, 11.84)	-12.91 (-45.46, 19.63)	0.47 (-0.70, 1.64)	
>0.15	159	-21.16 (-72.13, 29.81)	-34.82 (-79.77, 10.13)	-1.46 (-3.32, 0.39)	>0.16	298	7.22 (-31.20, 45.64)	14.80 (-20.13, 49.72)	0.36 (-0.90, 1.62)	
PCB-180										
<0.05	153	Ref	Ref	Ref	<0.05	304	Ref	Ref	Ref	
0.05 – 0.07	166	27.07 (-20.68, 74.82)	14.37 (-28.65, 57.39)	-0.98 (-2.63, 0.68)	0.05 – 0.08	293	5.26 (-30.41, 40.93)	7.60 (-25.28, 40.48)	0.19 (-0.98, 1.36)	
0.07 – 0.11	158	37.50 (-13.58, 88.58)	23.08 (-22.43, 68.59)	-1.17 (-3.00, 0.65)	0.08 – 0.12	297	-16.27 (-53.81, 21.27)	-3.01 (-37.26, 31.25)	0.81 (-0.42, 2.04)	
>0.11	159	6.71 (-51.16, 64.58)	-7.46 (-59.06, 44.14)	-1.24 (-3.30, 0.83)	>0.12	298	4.67 (-36.15, 45.49)	13.82 (-23.39, 51.03)	0.44 (-0.90, 1.78)	
ΣPCBs										
<0.16	157	Ref	Ref	Ref	<0.18	294	Ref	Ref	Ref	
0.16 – 0.25	161	-21.78 (-66.89, 23.34)	-33.27 (-73.60, 7.06)	-1.27 (-2.89, 0.35)	0.18 – 0.28	304	-6.38 (-41.43, 28.67)	9.22 (-23.09, 41.52)	0.84 (-0.32, 2.00)	
0.25 – 0.35	159	14.69 (-32.86, 62.25)	1.07 (-41.10, 43.24)	-1.24 (-2.96, 0.48)	0.28 – 0.40	296	-22.75 (-59.25, 13.76)	-7.49 (-40.76, 25.78)	0.81 (-0.39, 2.02)	
>0.36	159	-28.68 (-82.27, 24.90)	-38.42 (-85.95, 9.11)	-1.20 (-3.14, 0.74)	>0.40	298	5.62 (-34.14, 45.38)	17.96 (-18.35, 54.27)	0.60 (-0.71, 1.91)	

[a] Models adjusted for children's region of residence, sex, age, and height at the time of the lung function assessment, maternal age at delivery, maternal pre-pregnancy BMI, maternal educational level, maternal social class, maternal country of birth, parity, maternal smoking during pregnancy, maternal diet, and maternal alcohol consumption.

Figure S3. Associations (β (95% CI)) between cord serum PCB-138 (a) and PCB-180 (b) in quartile ranges (ng/mL), and lung function at 4 and 7 years of age[a]



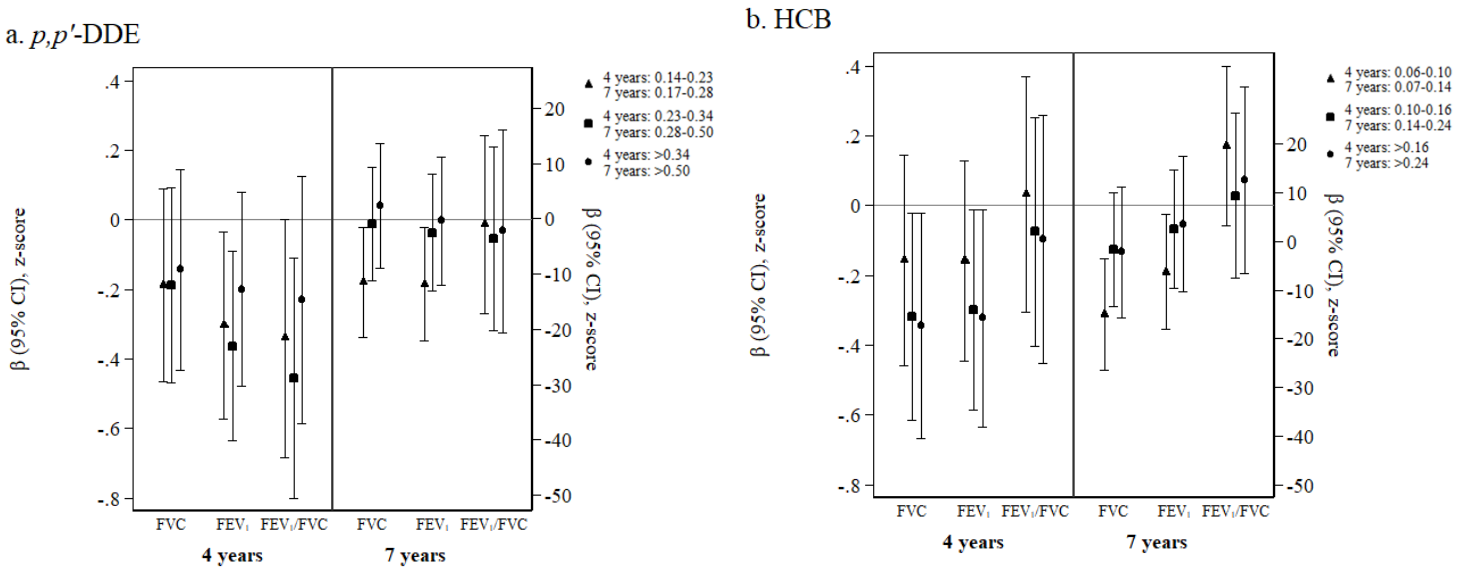
[a] Models adjusted for children's region of residence, sex, age, and height at the time of the lung function assessment, maternal age at delivery, maternal pre-pregnancy BMI, maternal educational level, maternal social class, maternal country of birth, parity, maternal smoking during pregnancy, maternal diet, and maternal alcohol consumption. Values represent mL for FVC and FEV_1, and % for FEV_1/FVC.

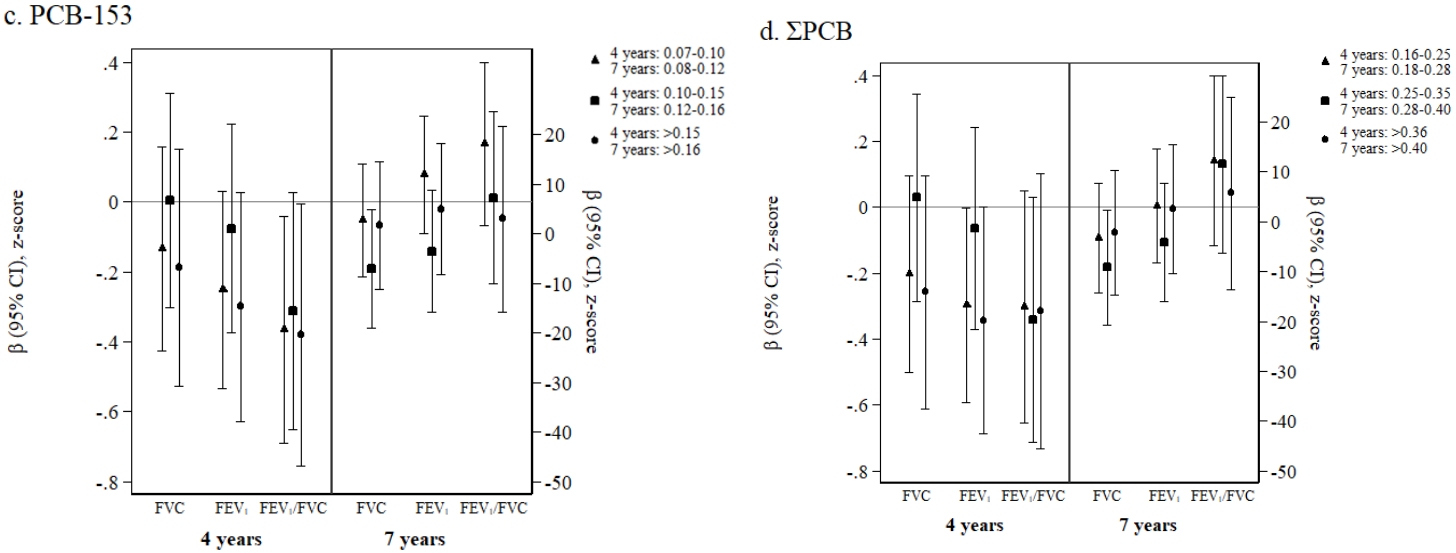
Table S5. Sensitivity analysis: associations (β (95% CI)) between cord serum *p,p'*-DDE in quartile ranges (ng/mL) and lung function at 7 years of age in the whole population (Main), in complete case, and using maternal serum *p,p'*-DDE concentrations[a]

Lung function	*p,p'*-DDE	Main (n=1192)		Complete Case (n=1131)		Maternal serum (n=1172) [b]		
at 7 years	ng/ml	N	β (95% CI)	N	β (95% CI)	ng/ml	N	β (95% CI)
FVC (mL)	<0.17	299	Ref	287	Ref	*<0.47*	293	Ref
	0.17 – 0.28	296	-41.04 (-73.18, -8.91)	282	-39.41 (-72.33, -6.49)	0.47 – 0.76	293	-20.30 (-30.49, -10.11)
	0.28 – 0.50	299	-3.22 (-36.61, 30.17)	302	-4.60 (-38.73, 29.53)	0.76 – 1.28	293	-4.43 (-14.85, 5.99)
	>0.50	298	-2.07 (-39.11, 34.96)	305	-3.59 (-41.18, 33.99)	>1.28	293	3.55 (-7.91, 15.01)
FEV_1 (mL)	<0.17	299	Ref	287	Ref	<0.47	293	Ref
	0.17 – 0.28	296	-36.96 (-66.22, -7.70)	282	-37.75 (-67.57, -7.93)	0.47 – 0.76	293	-31.22 (-40.51, -21.94)
	0.28 – 0.50	299	-7.90 (-38.33, 22.53)	302	-9.67 (-40.58, 21.24)	0.76 – 1.28	293	-11.90 (-21.39, -2.40)
	>0.50	298	-6.87 (-40.61, 26.88)	305	-10.40 (-44.45, 23.64)	>1.28	293	-11.32 (-21.77, -0.87)
FEV_1/FVC (%)	<0.17	299	Ref	287	Ref	<0.47	293	Ref
	0.17 – 0.28	296	-0.11 (-1.17, 0.95)	282	-0.24 (-1.33, 0.84)	0.47 – 0.76	293	-0.77 (-1.10, -0.43)
	0.28 – 0.50	299	-0.27 (-1.37, 0.83)	302	-0.31 (-1.44, 0.81)	0.76 – 1.28	293	-0.42 (-0.76, -0.08)
	>0.50	298	-0.20 (-1.42, 1.02)	305	-0.33 (-1.57, 0.91)	>1.28	293	-0.76 (-1.13, -0.38)

[a] Models adjusted for children's region of residence, sex, age, and height at the time of the lung function assessment, maternal age at delivery, maternal pre-pregnancy BMI, maternal educational level, maternal social class, maternal country of birth, parity, maternal smoking during pregnancy, maternal diet, and maternal alcohol consumption. Values represent mL for FVC and FEV_1, and % for FEV_1/FVC.

Figure S4. Sensitivity analysis: associations (β (95% CI)) between cord serum *p,p'*-DDE (a), HCB (b), PCB-153 (c), and ΣPCB (d) in quartile ranges (ng/mL), and lung function parameters converted into sex, height, age, and ethnicity -adjusted z-scores at 4 (n=636) and 7 (n=1192) years of age[a]





[a] Models adjusted for children's region of residence, sex, age, and height at the time of the lung function assessment, maternal age at delivery, maternal pre-pregnancy BMI, maternal educational level, maternal social class, maternal country of birth, parity, maternal smoking during pregnancy, maternal diet, and maternal alcohol consumption.

[b] Maternal serum concentrations are equivalent to cord serum concentrations applying cohort-specific conversion factors (supplementary methods 1).

Table S6. Sensitivity analysis: associations (β (95% CI)) between cord serum *p,p'*-DDE in quartile ranges (ng/mL) and lung function at 4 and 7 years of age in the whole population (Main) and restricting to those children with at least 2 acceptable and reproducible manoeuvres[a]

	4 years					7 years				
	p,p'-DDE	**Main (n=636)**		**≥ 2 acceptable and reproducible (n=389)**			**Main (n=1192)**		**≥ 2 acceptable and reproducible (n=758)**	
	ng/ml	N	β (95% CI)	N	β (95% CI)	ng/ml	N	β (95% CI)	N	β (95% CI)
FVC (mL)	<0.14	160	Ref	140	Ref	<0.17	299	Ref	174	Ref
	0.14 – 0.23	159	-34.52 (-75.37, 6.33)	107	-64.19 (-111.72, -16.67)	0.17 – 0.28	296	-41.04 (-73.18, -8.91)	179	-19.89 (-59.65, 19.88)
	0.23 – 0.34	159	-34.13 (-75.35, 7.10)	82	-59.77 (-106.92, -12.62)	0.28 – 0.50	299	-3.22 (-36.61, 30.17)	196	2.90 (-37.67, 43.47)
	>0.35	158	-37.51 (-80.40, 5.38)	41	-23.26 (-73.70, 27.19)	>0.50	298	-2.07 (-39.11, 34.96)	209	6.36 (-37.81, 50.53)
FEV_1 **(mL)**	<0.14	160	Ref	140	Ref	<0.17	299	Ref	174	Ref
	0.14 – 0.23	159	-46.12 (-82.05, -10.19)	107	-70.24 (-112.35, -28.13)	0.17 – 0.28	296	-36.96 (-66.22, -7.70)	179	-21.95 (-57.53, 13.62)
	0.23 – 0.34	159	-53.61 (-89.87, -17.35)	82	-68.78 (-110.55, -27.01)	0.28 – 0.50	299	-7.90 (-38.33, 22.53)	196	-9.14 (-45.48, 27.19)
	>0.35	158	-42.37 (-80.09, -4.65)	41	-27.52 (-72.21, 17.18)	>0.50	298	-6.87 (-40.61, 26.88)	209	-4.55 (-44.11, 35.01)
FEV_1/FVC (%)	<0.14	160	Ref	140	Ref	<0.17	299	Ref	174	Ref
	0.14 – 0.23	159	-1.43 (-2.90, 0.05)	107	-1.11 (-2.69, 0.46)	0.17 – 0.28	296	-0.11 (-1.17, 0.95)	179	-0.34 (-1.62, 0.94)
	0.23 – 0.34	159	-2.01 (-3.51, -0.52)	82	-1.29 (-2.86, 0.27)	0.28 – 0.50	299	-0.27 (-1.37, 0.83)	196	-0.76 (-2.07, 0.55)

>0.35	158	-0.82 (-2.38, 0.73)	41	-0.72 (-2.39, 0.96)	>0.50	298	-0.20 (-1.42, 1.02)	209	-0.51 (-1.94, 0.91)

[a] Models adjusted for children's region of residence, sex, age, and height at the time of the lung function assessment, maternal age at delivery, maternal pre-pregnancy BMI, maternal educational level, maternal social class, maternal country of birth, parity, maternal smoking during pregnancy, maternal diet, and maternal alcohol consumption. Values represent mL for FVC and FEV_1, and % for FEV_1/FVC.

Supplementary Methods 1. Calculation of cohort-specific conversion factors of maternal serum to cord serum OCs concentrations.

OCs were measured in two biological matrices: maternal serum and cord serum. Gipuzkoa and Valencia had both cord and maternal serum measurements, and Sabadell only maternal serum measurements. Since cord blood is considered the best proxy of prenatal OCs exposure,[9] we estimated cord serum OCs from maternal serum concentrations. Both maternal and cord serum measures were available in most of the participants from Gipuzkoa and Valencia regions. Conversion factors were therefore calculated from paired mother-child OCs measurements in these regions of residence of the INMA cohort. We used non-lipid adjusted OCs concentrations. Values above 0.5 ng/mL were considered as outliers and were excluded. Each compound specific conversion factor was calculated as the mean of the four slopes obtained from linear regression models with cord and maternal serum.[38]

regress cord_*p,p'*-DDE matserum_*p,p'*-DDE

regress cord_*p,p'*-DDE matserum_*p,p'*-DDE, noconstant

regress matserum_*p,p'*-DDE cord_ *p,p'*-DDE

regress matserum_ *p,p'*-DDE cord_ *p,p'*-DDE, noconstant

Where:

- cord_*p,p'*-DDE: non-lipid adjusted *p,p'*-DDE cord serum levels in ng/mL

- matserum_*p,p'*-DDE: non-lipid adjusted *p,p'*-DDE maternal serum levels in ng/mL

Supplementary Methods 2. Description of the imputation procedure.

- Imputations were performed separately for each region of residence (Gipuzkoa, Sabadell, and Valencia).
- Software used and key setting: STATA 14 software (Stata Corporation, College Station, Texas). Multiple Imputation by Chained Equations (ICE) command. Ten cycles performed.
- Variables included in the imputation procedure:

  - Maternal variables: lipids; cord and maternal serum *p,p'*-DDE, HCB, PCB-138, PCB-153, PCB-180; age; BMI; education; social class; smoking during pregnancy; parity; history of asthma, allergy or eczema; mode of delivery; dairy, meat, fish, vegetables, and alcohol consumption during pregnancy.

  - Child's variables: sex; gestational age; birth weight; ethnicity; ever asthma; breastfeeding; height and weight at the time of the lung function; and day-care attendance.
- Procedure for non-normally distributed variables: log-transformation
- Procedure for binary/categorical variables: logistic, ordinal, and multinomial models.

5.3. Paper III

Abellan A, Mensink-Bout SM, Garcia-Esteban R, Beneito A, Chatzi L, Duarte-Salles T, Fernandez MF, Garcia-Aymerich J, Granum B, Iñiguez C, Jaddoe VWV, Kannan K, Lertxundi A, Lopez-Espinosa MJ, Philippat C, Sakhi AK, Santos S, Siroux V, Sunyer J, Trasande L, Vafeiadi M, Vela-Soria F, Yang TC, Zabaleta C, Vrijheid M, Duijts L, Casas M.

In utero **exposure to bisphenols and asthma, wheeze, and lung function in school-age children: A prospective meta-analysis of 8 European birth cohorts**

Under review

In utero exposure to bisphenols and asthma, wheeze, and lung function in school-age children: A prospective meta-analysis of 8 European birth cohorts

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Conflicts of interest

All authors declare they have no conflicts of interest to disclose.

KEY POINTS

Question: Is *in utero* exposure to bisphenols, chemicals of concern present in many daily life products, associated with asthma, wheeze, and lung function in school-age children? Does this association differ by sex?

Findings: In this prospective meta-analysis of 3007 mother-child pairs from 8 European birth cohorts, *in utero* exposure to bisphenol A was prevalent and increased the risk of asthma and wheeze among school-age girls. There was no evidence of an association with lung function at school-age nor wheezing patterns across childhood.

Meaning: Findings imply reduction of bisphenol A exposure to optimize long term respiratory health.

ABSTRACT

Importance: Bisphenols are chemical pollutants widely used in consumer products. *In utero* exposure to bisphenols may alter lung development and increase the risk of respiratory morbidity in the offspring. However, evidence is scarce and mostly focused on bisphenol A (BPA) only.

Objective: To examine the associations of *in utero* exposure to BPA, bisphenol F (BPF), and bisphenol S (BPS) with asthma, wheeze, and lung function in school-age children, and whether these associations differ by sex.

Design: A prospective meta-analysis on individual participant data from 8 population-based birth cohorts established between 1999 and 2010.

Setting: This study included participants from 8 birth cohorts from 6 European countries: Generation R, the Netherlands; INMA Sabadell, INMA Gipuzkoa, INMA Valencia, Spain; BiB, United Kingdom; EDEN, France; MoBa, Norway; and RHEA, Greece.

Participants: We included 3,007 mother-child pairs with available information on maternal urinary bisphenols and respiratory outcomes in the offspring during childhood.

Exposures: Bisphenols concentrations were determined in single or a two spot maternal urine samples collected during pregnancy.

Main Outcomes: Between 7 and 11 years of age, current asthma and wheeze were assessed from questionnaires and lung function by spirometry. Wheezing patterns were constructed from questionnaires across childhood.

Results: Exposure was prevalent with 90% of maternal samples containing concentrations above detection limits. BPF and BPS were found in 27% and 49% of samples, respectively. *In utero* exposure to BPA tended to be associated with increased odds of wheeze in the overall population (OR=1.05, 95%CI=0.97, 1.13), but not with asthma, wheezing patterns or lung function. Among girls, BPA was associated with increased odds of current asthma (OR=1.13, 95%CI=1.01, 1.27) and wheeze (OR=1.14, 95%CI=1.01, 1.30) (p-interaction sex=0.01). We did not observe consistent associations of BPF and BPS with respiratory outcomes.

Conclusions and Relevance: This study suggests that *in utero* BPA exposure is prevalent and is associated with increased odds of asthma and wheeze among school-age girls. Findings imply reduction of bisphenol A exposure to optimize long term respiratory health.

Keywords

Bisphenol A, pregnancy, asthma, wheezing, lung function, mother-child cohort

Abbreviations

BMI: body mass index

BPA: bisphenol A

BPF: bisphenol F

BPS: bisphenol S

CI: confidence interval

DAG: directed acyclic graph

FEV_1: forced expiratory volume in 1s

$FEF_{25-75\%}$: mid-expiratory flow

FVC: forced vital capacity

GLI: Global Lung Function Initiative

IPD: individual participant data

ISAAC: International Study on Asthma and Allergy in Childhood

LOD: limit of detection

OR: odds ratio

INTRODUCTION

Impaired development of the respiratory and immune systems resulting from adverse environments *in utero* might predispose individuals to respiratory morbidity later in life [1–7]. There is growing concern over the role that chemical pollutants play in the development of respiratory diseases during early life [8–11], specifically over bisphenols, due to their mass production [12], widespread human exposure[13,14], and their ability to cross the placental barrier [15]. Bisphenol A (BPA), used in many consumer products, is the most commonly used bisphenol. BPA production is restricted in some countries [16], which has resulted in the emergence of substitutes such as bisphenol F (BPF) and bisphenol S (BPS), with suspected similar toxicity [17,18]. Individual prospective cohort studies investigated *in utero* exposure to BPA in relation to asthma-related symptoms and lung function, but showed inconsistent results [19–27]. Because bisphenols can interfere with sex hormones [28], their potential health effects may be sex-dependent. However, previous studies reported inconsistent results [19,24–27]. Limited sample size of previous studies may have hindered consistent and sex-specific effect estimations. Additionally, no study has investigated the influence of *in utero* exposure to bisphenols other than BPA on respiratory health.

Using large population-based cohorts, we aimed to investigate whether *in utero* exposure to bisphenols is associated with current asthma, wheeze, and lung function at school-age, wheezing patterns through childhood, and whether these associations differ by sex.

METHODS

Study population

We included 3,007 mother-child pairs from 8 European population-based birth cohorts: Generation R, the Netherlands [29]; INMA (INfancia y Medio Ambiente) Sabadell, INMA Gipuzkoa, INMA Valencia, Spain [30]; BiB (Born in Bradford), UK [31]; EDEN (Etude des Déterminants pré et post natals du développement et de la santé de l'Enfant), France [32]; MoBa (Norwegian Mother, Father and Child Cohort Study), Norway [33,34]; and RHEA (Mother-Child Cohort in Crete), Greece [35]. Cohorts recruited the population between 1999 and 2010 (Table 1). Five of these were part of the HELIX (Human Early Life Exposome) Project and followed common standardised protocols [36]. Participants with bisphenols measurements during pregnancy and information on asthma, wheeze or lung function during childhood were included (Figure S1). All cohorts received approval from the ethics committees of the centres involved and written informed consent was obtained from all participants.

Bisphenol exposure assessment

Concentrations of BPA, BPF and BPS were determined in a single or a two spot maternal urine samples during pregnancy (Table 2). BPA was measured in all included cohorts; BPF and BPS were measured in Generation R and in the three INMA cohorts. Bisphenols concentrations were adjusted for creatinine to correct for urine dilution. See Supplementary Methods 2 for complete details.

Asthma, wheeze, and lung function

We defined current asthma [37] as a positive answer to two of the following: i) ever asthma diagnosis; ii) wheezing in the last year; iii) asthma medication in the last year. This information was collected from parental-administered questionnaires adapted from the International Study on Asthma and Allergy in Childhood (ISAAC) [38]. Information on wheezing was collected from 1 to 11 years (Table S1) and was used to create wheezing patterns: i) never wheezing; ii) early wheezing, ≤4 years; iii) late wheezing, >4 years; and iv) persistent wheezing, ≤4 and >4 years. Lung function was assessed by spirometry at school-age following the ATS/ERS guidelines [39]. All children with an acceptable manoeuvre were included. Lung function parameters selected for the study were forced vital capacity (FVC), FEV_1, FEV_1/FVC and mid-expiratory flow ($FEF_{25-75\%}$). We standardised lung function parameters into z-scores based on the Global Lung Function Initiative (GLI) reference equations [40].

Covariates

Relevant information on maternal and child characteristics was obtained from questionnaires, medical records, antenatal healthcare visits and physical measurements during the cohort's follow-ups. Covariates were selected from literature and summarised in a directed acyclic graph (DAG) (Figure S2) to depict the known and hypothesised causal relations between variables.

Statistical analyses

We imputed missing values in covariates and BPA concentrations below the limit of detection (LOD) using multiple imputation by chained equations methods (Supplementary Methods 1). BPA concentrations were log_2 transformed to reach normality. Since the number of samples with BPF and BPS concentrations below the LOD was very high (>50%), these two compounds were dichotomized (<LOD – reference category, ≥LOD).

To assess the associations between bisphenols and respiratory outcomes, we performed 1-stage meta-analysis pooling individual participant data (IPD) from all cohorts using mixed-effects models taking into account clustering of participants within cohorts (random intercepts). We performed multivariable logistic models for the associations of bisphenols with current asthma, wheeze, and wheezing patterns, and multivariable linear models for the associations with lung function. We used the 1-stage approach to stratify analyses by sex, testing for interaction ($p \leq 0.1$) with greater power [41]. Sex-stratified models between BPF, BPS and wheezing patterns could not be performed because numbers in each category were too small. Current asthma and wheeze models were adjusted for maternal education, age, pre-pregnancy BMI, smoking during pregnancy, and child's sex, age, and ethnicity. Lung function models were adjusted for the same set as the above models except child's sex and age. Although ethnicity was accounted for in the z-scores, we additionally adjusted for it to avoid any residual confounding [40].

We performed a 2-stage meta-analysis to assess the associations between BPA and current asthma, wheeze, and lung function to account for between and within cohort heterogeneity. We estimated the effect estimates in each cohort separately and calculated the combined estimate by a random effects meta-analysis model. We tested for heterogeneity between effect estimates by using I^2 values [42]. We did not perform 2-stage meta-analysis for neither BPF and BPS, only available in 4 cohorts, nor for wheezing patterns, because of the low number of individuals in each wheezing category. All models were adjusted for the same set of covariates as the 1-stage meta-analysis.

To test the robustness of our results, we performed various sensitivity analyses using the 1-stage meta-analysis models. We ran the models using the complete case dataset and further repeated them excluding one cohort at a time to determine the influences of any particular cohort in the overall estimates. To test whether results were not explained by birth outcomes, we repeated the analyses excluding preterm (<37 weeks) and low birth weight (<2500g) babies. To overcome exposure misclassification due to the high variability of bisphenols, we ran lung function models correcting for measurement error applying regression calibration methods considering a reliability coefficient for BPA of 0.14 (Supplementary Methods 3). We also performed lung function models including only children with at least two acceptable and reproducible spirometry manoeuvres and excluding children with an asthma diagnosis.

RESULTS

Characteristics of study population

Table 1 shows the main maternal and child characteristics in each cohort. There were no differences in main characteristics between the observed and imputed datasets (Table S2). Median BPA concentrations ranged from 1.56 (Generation R) to 9.54 (MoBa) μg/g. Overall, BPA was detected in 90% of the samples. BPF and BPS were detected in fewer samples except in Generation R, where 40% and 70% of samples had detectable levels of BPF and BPS, respectively (Table 2).

Bisphenols and asthma and wheeze

Results from the 1-stage meta-analysis showed that overall, *in utero* BPA exposure tended to be associated with increased odds of wheeze at school-age (OR=1.05, 95%CI=0.97, 1.13 per doubling of BPA concentration) but not with current asthma nor wheezing patterns (Table 3). Associations with asthma and wheeze at school-age were modified by child's sex (p-interaction=0.01). Stratified analyses showed that for each doubling in BPA concentration during pregnancy, girls had higher odds of having current asthma (OR=1.13, 95%CI=1.01, 1.27) and wheeze (OR=1.14, 95%CI=1.01, 1.30) (Table 3). We did not observe sex differences with wheezing patterns. We did not observe associations with BPF. Children whose mothers had BPS levels above the LOD seemed to have lower odds of presenting wheeze in the last year (OR=0.64, 95%CI=0.40, 1.04), late (OR=0.43, 95%CI=0.19, 1.00) and persistent wheeze (OR=0.56,

95%CI=0.35, 0.90). The 2-stage meta-analysis of the associations between BPA and current asthma and wheeze showed similar results than the 1-stage meta-analysis with heterogeneity across cohorts (Figure 1). BPA estimates with asthma and wheeze (in the overall population, girls, and boys) were similar when excluding preterm and low birth weight babies (Table S3), and in complete case analysis (Table S4). When excluding cohort by cohort, results did not show notable changes (Table S5). Associations between BPS and wheezing patterns did not change substantially in complete case analysis (Table S4).

Bisphenols and lung function

The 1-stage meta-analysis showed small or no effects of increasing *in utero* BPA concentrations with lung function parameters. In the overall population, each doubling in BPA concentrations was associated with 0.02 (95% CI=0.00, 0.04) z-score increase in FEV_1 and $FEF_{25-75\%}$ (Table 3). No associations were observed with FVC and FEV_1/FVC. Associations with FEV_1 and $FEF_{25-75\%}$ were modified by child's sex (p-interaction<0.10). In sex-stratified analysis, we observed increased FEV_1 associated with BPA exposure among boys (β=0.03, 95%CI=0.00, 0.06). Among children exposed to BPF levels above the LOD, FEV_1 increased by 0.12 z-scores (95% CI=0.01, 0.23) and $FEF_{25-75\%}$ by 0.13 z-scores (95% CI=0.01, 0.25) (Table 3). Associations between BPF and FVC and FEV_1 were modified by child's sex, observing increased FVC (β=0.17, 95%CI=0.02, 0.32) and FEV_1 (β=0.15, 95%CI=-0.00, 0.29) among girls (p-interaction<0.10). No associations were observed with BPS

and lung function parameters (Table 3). Results from the 2-stage meta-analysis showed overall null effects in the associations between *in utero* BPA exposure and lung function with low heterogeneity between cohorts (Figure 1). Results remained similar when excluding preterm and low birth weight babies (Table S3), in complete case analysis (Table S4), when including only children with at least two acceptable and reproducible spirometry manoeuvres, and when excluding children with asthma (Table S6). The estimates of the association between BPA and FEV_1 shifted towards the null when we excluded Generation R from the model (Table S5). The association between BPA and FEV_1 also disappeared when we repeated the models applying regression calibration (data not shown). Associations observed for BPF diluted and disappeared when including children with at least two acceptable and reproducible spirometry manoeuvres, and when excluding children with asthma (Table S6).

DISCUSSION

Results from this prospective IPD meta-analysis of 3,007 mother-child pairs showed that *in utero* exposure to BPA was associated with increased odds of asthma and wheeze at school-age among girls. The associations did not seem to be explained by lung function adaptations, as results did not show consistent associations of *in utero* BPA exposure with lung function. Substitute bisphenols were associated with increased FEV_1 and $FEF_{25-75\%}$ (BPF) and decreased risk of late and persistent wheezing (BPS).

Comparison with previous studies

Our findings support the hypothesis that *in utero* BPA increases the risk of asthma-related symptoms during childhood reported in some [19–21,24–26] but not all [27,43,44] of previous studies. Our study adds evidence for potential sex-dependent effects of BPA exposure, only assessed in few studies that yielded contradicting results [24–26]. Two studies reported higher risk in girls, one showed increased odds of infant allergic diseases (including wheeze and eczema) at 6 months of age [25], and another reported increased risk of asthma at 7 years [24] without statistical evidence that BPA effects differed between sexes. One study reported increased odds of asthma diagnosis at 7 years among boys [26]. A cohort study that involved only boys also reported a tendency of increased risk of asthma at 5 years, but not with wheeze at the same age, from *in utero* BPA exposure [21]. The lack of consistency of sex-specific effects of previous studies might be partly explained by their small sample size. In our study population, with a large sample size after stratification, we observed that *in utero* exposure to BPA increased the odds of current asthma and wheeze at school-age in girls. Further, most previous studies have focused on children of ages ≤7 years; only one study [44] assessed current asthma until 12 years of age and reported no associations in relation to *in utero* BPA; in that study sex-dependent effects were not examined. To our knowledge, there is no previous study assessing *in utero* BPA exposure on wheezing patterns across childhood, preventing comparison of our findings of no associations between increasing BPA exposure with any wheezing pattern.

Literature on *in utero* BPA exposure and lung function is scarce. Only one cohort study associated *in utero* BPA exposure with a decrease in %FEV_1 at 4 years but this association disappeared at 5 years of age [20]. This association was not observed in three other cohorts that assessed lung function from 5 to 12 years [21–23]. In our study, BPA was positively associated with lung function from 6 to 12 years of age only among boys, but estimates were small and generally towards the null. Such associations disappeared in sensitivity analyses when using the complete case dataset and when excluding Generation R from the analyses, the cohort with the largest sample size. Further studies are warranted to study the role of BPA on lung function growth.

To our knowledge, this is the first study to assess the associations between *in utero* BPF and BPS exposure and respiratory health. In our study we observed increased FEV_1 and $FEF_{25-75\%}$ among children whose mothers had detectable BPF levels and decreased odds of late and persistent wheezing among those with detectable BPS. BPA substitutes were detected in a low percentage of the study participants, except in Generation R. This might indicate that the introduction of BPA substitutes in the Netherlands was earlier than in other countries, as previously reported [45]. In other countries where these bisphenols were not as present in the market as they are currently, we expect current population's exposure to be higher, which guarantees the need for further investigation.

Interpretation of findings

Our results suggest that *in utero* exposure to BPA might increase the risk of asthma and wheeze at school-age through immunomodulatory mechanisms without notable lung function adaptations. This could be explained by the ability of bisphenols to cross the placenta [15] and interfere with the developing respiratory and immune systems by binding to a number of receptors related to inflammatory and oxidative stress pathways [46–49]. Immunomodulatory alterations from exposure to BPA have been observed in *in vitro*, animal, and human studies and include the increase in serum immunoglobulin-E, eosinophilic inflammation in the airways, stimulation of pro-inflammatory cytokines, T helper (Th) 1/Th2 cell shifts, and alterations in Th17 and β-cell counts [48–50]. Although animal studies have observed deleterious effects on the structural development of the lung after *in utero* exposure to BPA [51,52], we could not confirm that in our study. We hypothesise that on a population level, children without a current asthma exacerbation or with adequately controlled asthma, might present spirometry results within the healthy standards.

Sexually dimorphic effects of bisphenols on diverse health outcomes have been reported [53–55]. Fluctuations of sex hormones and their consequences on immune functions can play a role in asthma pathogenesis. The sexual dimorphism of asthma is especially observed in hormonally changing periods such as puberty, pregnancy, and menopause [56]. Observational, clinical, and animal studies have highlighted changes in oestrogen and testosterone levels

to influence incidence and severity of asthma [57–60]. Given the endocrine disrupting capacity of bisphenols, they can alter key hormone-signalling pathways and thus induce changes in sex hormones, which may partly explain the results found in this study.

Strengths and limitations

The strengths of this study rely on its prospective IPD meta-analysis design with multiple bisphenols, harmonized and objective respiratory outcomes, and large sample size, which enabled us to assess the subtle effects usually associated with exposure to environmental hazards [61] and the potential sex-dependent effects. By performing prospective IPD meta-analyses, we increased the strength of evidence without relying on published data and thus limited potential publication bias and reviewer selection bias, common limitations of retrospective meta-analysis [62].

This study has however several limitations. First, exposure to bisphenols was determined in 1 or 2 spot urine samples, which given the short biological half-life of bisphenols, might have led to exposure misclassification. This is likely to have underestimated our results, biasing the estimates towards the null, without increasing the risk of bias towards false-positive results [63]. The high within-day and between-week variability also limited the assessment of critical windows of exposure in our study. Further studies including a number of repeated bisphenol measurements during several days of the week in the three trimesters of pregnancy are needed [64,65]. Second, due to the low number of samples with detectable levels of BPF and

BPS, we were not able to consider the exposure as continuous and categorised them into detected and undetected. In our population, mothers among the detected group tended to smoke less and had a higher educational level compared to those among the undetected group (data not shown). We suspect mothers with higher education and a healthier lifestyle tend to use more BPA-free products which in turn may contain BPA substitutes, as observed for parabens [66]. Although we adjusted our models for lifestyle and socioeconomic factors, residual confounding cannot be ruled out. Third, bisphenols were analysed in different laboratories with some showing poor correlations (Supplementary Methods 2). However, after excluding the cohorts that conducted the analyses in these laboratories from the analyses, results did not change. Fourth, although we could construct childhood wheezing phenotypes, the data on wheezing episodes available across ages differed between cohorts (Supplementary Table S1). We were able to create four patterns, which might not be sufficient to capture effects on a specific age. Fifth, although lung function parameters were obtained following the ATS/ERS criteria, these estimates need to be carefully interpreted since the spirometry measurement error might be greater than the expected estimates. Finally, the study population included higher educated, less deprived and more Caucasian origin families. Although our results might not be generalised to the general population, this study encompasses the largest number of participants from different regions of Europe to date, and it is unlikely this affected the internal validity of the study.

Conclusions

Our study suggests that *in utero* exposure to BPA increases the risk of asthma and wheeze among school-age girls. Identification of early determinants of respiratory health in childhood is of utmost importance, given their long-term effect on disease throughout life. Further research is needed on the assessment of temporal variabilities in exposure in relation to health outcomes, in order to improve current EU chemical legislation. Current regulation is focused on BPA, obviating analogues with suspected similar toxicity [67], and needs to move forward by avoiding entire chemical classes instead of individual compounds.

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TABLES AND FIGURES

Table 1. Maternal and child characteristics of participating cohorts (n=3007).

	Generation R (n=1151)	**INMA Gipuzkoa** (n=280)	**INMA Sabadell** (n=379)	**INMA Valencia** (n=331)	**BiB**[a] (n=204)	**EDEN**[a] (n=197)	**MoBa**[a] (n=268)	**RHEA**[a] (n=197)
Years of recruitment	2002-2006	2006-2008	2004-2006	2004-2005	2007-2010	2003-2006	1999-2009	2007-2008
Maternal characteristics								
Maternal age (years), mean (SD)	31.0 (4.6)	32.8 (3.1)	32.0 (4.1)	31.9 (3.9)	28.7 (5.8)	30.6 (4.9)	32.7 (3.7)	30.8 (4.8)
Pre-pregnancy BMI (kg/m^2), mean (SD)	24.3 (4.3)	23.0 (3.6)	23.7 (4.6)	23.4 (4.1)	28.4 (5.4)	23.3 (4.2)	22.6 (3)	24.0 (4.2)
Maternal education, No. (%)								
≤ Primary	75 (6.7)	31 (11.1)	81 (21.5)	92 (27.8)	86 (47.8)	12 (6.2)	0 (0)	9 (4.6)
Secondary	439 (39.4)	100 (35.8)	160 (42.6)	134 (40.5)	32 (17.8)	72 (36.9)	54 (20.9)	109 (55.9)
University	601 (53.9)	148 (53.1)	135 (35.9)	105 (31.7)	62 (34.4)	111 (56.9)	204 (79.1)	77 (39.5)
Smoking during pregnancy, No. (%)	140 (13.4)	37 (13.7)	54 (14.4)	75 (22.7)	25 (13.8)	46 (23.4)	9 (3.5)	42 (21.4)
Child characteristics at birth								
Child ethnicity, No. (%)								
Caucasian	896 (78.2)	261 (99.2)	306 (97.5)	306 (97.3)	83 (43.5)	195 (99.5)	252 (96.5)	197 (100)
African/American	79 (6.9)	0 (0)	1 (0.3)	0 (0)	5 (2.6)	0 (0)	0 (0)	0 (0)

Asian	74 (6.5)	0 (0)	0 (0)	0 (0)	95 (49.7)	1 (0.5)	7 (2.7)	0 (0)
Other or Mixed	96 (8.4)	2 (0.8)	7 (2.2)	12 (4)	8 (4.2)	0 (0)	2 (0.8)	0 (0)
Child sex, No. (%) girls	566 (49.2)	140 (50)	180 (47.5)	158 (47.7)	80 (47.1)	82 (45.6)	126 (48.7)	78 (43.3)
Preterm birth, No. (%) yes	27 (2.4)	9 (3.2)	8 (2.1)	9 (2.7)	8 (3.9)	11 (5.6)	12 (4.6)	20 (10.2)
Low birth weight, No. (%) yes	28 (2.4)	14 (5)	15 (4)	12 (3.6)	14 (6.9)	6 (3.1)	8 (3.1)	6 (3.1)
Child characteristics at respiratory outcomes assessment								
Child age (years), mean (SD)	9.7 (0.2)	7.9 (0.2)	6.8 (0.4)	7.5 (0.2)	6.6 (0.2)	10.8 (0.6)	8.5 (0.5)	6.5 (0.3)
Child height (cm), mean (SD)	141.1 (6.4)	127.9 (5.5)	121.6 (5.7)	126 (5.1)	119.8 (5)	143.6 (7.4)	133.4 (6.1)	120.4 (4.8)
Current asthma, No. (%) yes	66 (6.7)	22 (7.9)	33 (8.8)	24 (7.3)	45 (22.1)	29 (14.7)	21 (7.8)	20 (10.2)
Wheeze in the last year, No. (%) yes	41 (4.1)	24 (8.7)	40 (10.6)	38 (11.5)	52 (25.5)	25 (12.7)	20 (7.5)	15 (7.6)
Wheezing patterns, No. (%)								
Never	350 (49.7)	134 (51.3)	159 (43.7)	156 (47.9)	31 (46.3)	117 (69.7)	176 (81.8)	167 (84.8)
Early	261 (37.1)	104 (39.9)	167 (45.9)	133 (40.8)	16 (23.9)	16 (9.5)	21 (9.8)	15 (7.6)
Late	20 (2.8)	9 (3.4)	2 (0.5)	5 (1.5)	9 (13.4)	22 (13.1)	12 (5.6)	11 (5.6)
Persistent	73 (10.4)	14 (5.4)	36 (9.9)	32 (9.8)	11 (16.4)	13 (7.7)	6 (2.8)	4 (2)
FVC z-score, mean (SD)	0.16 (0.94)	0.63 (0.96)	0.32 (0.96)	0.26 (0.91)	0.28 (1.37)	-0.65 (0.82)	0.11 (0.91)	0.15 (1.31)
FEV_1 z-score, mean (SD)	0.12 (0.99)	0.26 (0.94)	-0.00 (0.97)	0.26 (0.94)	-0.36 (1.22)	-0.79 (0.92)	-0.22 (0.95)	-0.06 (1.03)

FEV_1/FVC z-score, mean (SD)	-0.10 (0.98)	-0.61 (0.97)	-0.55 (0.95)	-0.01 (0.93)	-1.07 (1.00)	-0.19 (0.95)	-0.58 (0.83)	-0.51 (0.95)
$FEF_{25-75\%}$ z-score, mean (SD)	0.52 (1.08)	-0.24 (1.07)	-0.36 (1.01)	-0.12 (0.90)	-6.43 (1.58)	-7.38 (1.54)	-6.86 (1.59)	-6.53 (1.52)

[a] Subsample included in the HELIX Project.
Abbreviations: BMI: body mass index; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1s; $FEF_{25-75\%}$: mid expiratory flow; SD: standard deviation.

Table 2. Creatinine adjusted bisphenol levels (µg/g) and percentage of bisphenol samples above the LOD.

	Overall	**Generation R**	**INMA Gipuzkoa**	**INMA Sabadell**	**INMA Valencia**	**BiB**[a]	**EDEN**[a]	**MoBa**[a]	**RHEA**[a]
Trimester measurement	-	1st	1st and 3rd	1st and 3rd	1st and 3rd	3rd	3rd	2nd	1st
BPA									
LOD BPA (ng/ml)	-	0.15	0.12	0.10	0.03	0.03	0.40	0.03	0.03
BPA, No. (%) >LOD	2697 (90)	901 (78)	225 (80)	376 (99)	331 (100)	202 (99)	197 (100)	268 (100)	197 (100)
BPA µg/g, median (IQR)	2.50 (3.70)	1.56 (3.03)	2.95 (4.23)	2.51 (2.25)	3.51 (3.99)	1.59 (1.58)	2.50 (2.31)	9.54 (13.22)	1.98 (2.77)
BPF									
LOD BPF (ng/ml)	-	0.18	0.06	0.07	0.10	-	-	-	-
BPF, No. (%) >LOD	521 (27)	459 (40)	29 (10)	15 (8)	18 (5)	-	-	-	-
BPS									
LOD BPS (ng/ml)	-	0.05	0.05	0.10	0.33	-	-	-	-
BPS, No. (%) >LOD	961 (49)	81 (71)	83 (30)	33 (17)	33 (10)	-	-	-	-

[a] Subsample included in the HELIX Project.
Abbreviations: BPA: bisphenol A; BPS: bisphenol F; BPS: bisphenol S; IQR: interquartile range; LOD: limit of detection.

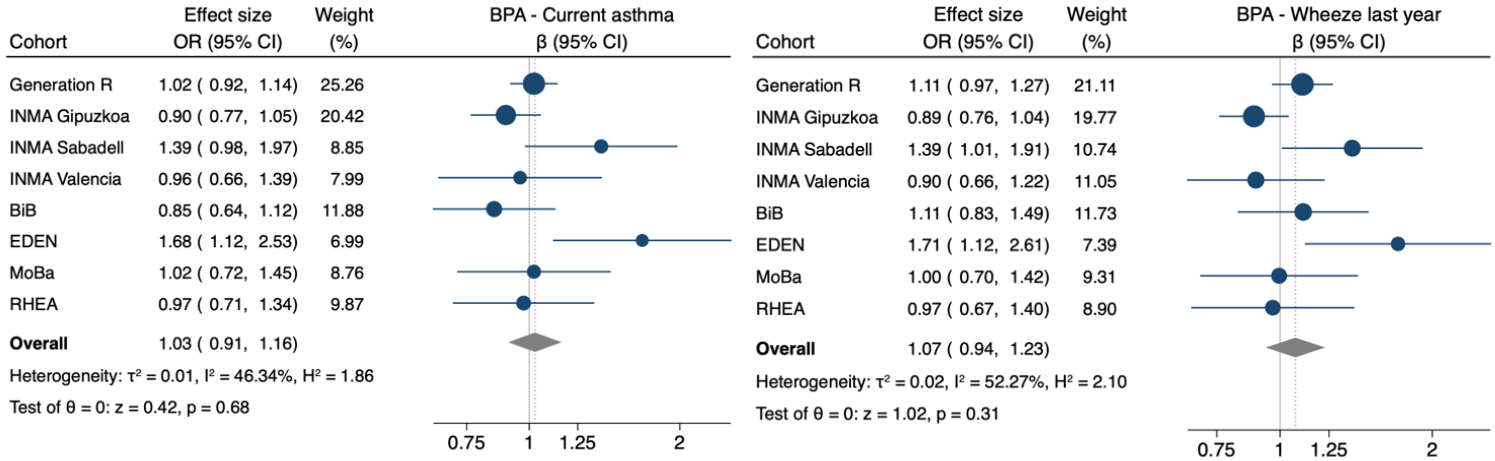
Table 3. Overall and sex-stratified associations between creatinine adjusted bisphenols and respiratory outcomes from 1-stage meta-analysis.

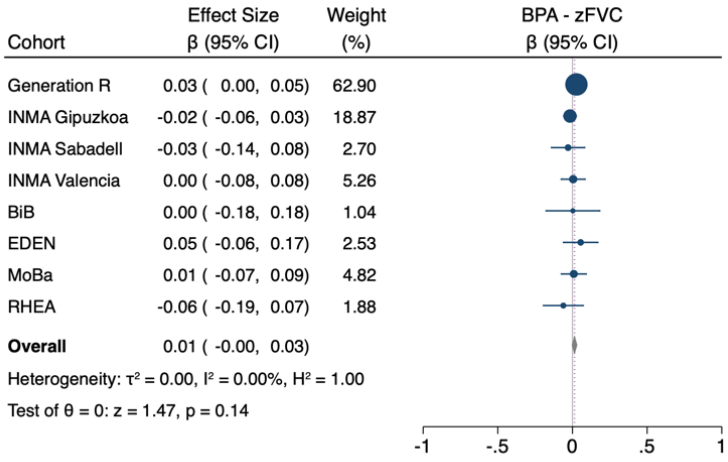
	$N_{Overall}$	Overall	$P_{interaction}$	N_{Girls}	Girls	N_{Boys}	Boys
BPA (µg/g)							
Asthma and wheeze, OR (95% CI)[a]							
Current asthma	2831	1.01 (0.94, 1.09)	0.009	1323	1.13 (1.01, 1.27)	1431	0.95 (0.87, 1.03)
Wheeze in the last year	2846	1.05 (0.97, 1.13)	0.013	1327	1.14 (1.01, 1.30)	1442	0.99 (0.90, 1.09)
Wheezing patterns, OR (95% CI)[a,c]							
Early	2023	0.99 (0.94, 1.04)	0.572	962	0.97 (0.90, 1.04)	1026	1.00 (0.93, 1.08)
Late	1378	1.02 (0.90, 1.15)	0.463	665	1.06 (0.90, 1.25)	658	0.95 (0.80, 1.12)
Persistent	1474	0.97 (0.89, 1.06)	0.288	702	1.00 (0.87, 1.16)	741	0.94 (0.84, 1.05)
Lung function, z-score (95% CI)[b]							
FVC	2677	0.01 (-0.01, 0.03)	0.397	1292	0.01 (-0.01, 0.04)	1385	0.02 (-0.01, 0.04)
FEV_1	2729	0.02 (0.00, 0.04)	0.044	1318	0.01 (-0.01, 0.04)	1411	0.03 (0.00, 0.06)
FEV_1/FVC	2662	0.00 (-0.01, 0.02)	0.111	1288	-0.00 (-0.03, 0.03)	1374	0.01 (-0.02, 0.04)
$FEF_{25-75\%}$	2741	0.02 (-0.00, 0.04)	0.000	1321	0.01 (-0.01, 0.04)	1420	0.02 (-0.01, 0.04)
BPF (≥LOD vs <LOD)							
Asthma and wheeze, OR (95% CI)[a]							
Current asthma	1776	1.09 (0.69, 1.73)	0.029	871	0.44 (0.17, 1.14)	905	1.55 (0.89, 2.67)
Wheeze in the last year	1791	1.12 (0.66, 1.90)	0.024	875	0.46 (0.15, 1.45)	916	1.59 (0.87, 2.94)
Wheezing patterns, OR (95% CI)[a,c]							
Early	1302	0.98 (0.73, 1.31)	0.495	-	-	-	-
Late	757	1.12 (0.48, 2.62)	0.938	-	-	-	-
Persistent	851	0.94 (0.57, 1.56)	0.011	-	-	-	-
Lung function, z-score (95% CI)[b]							

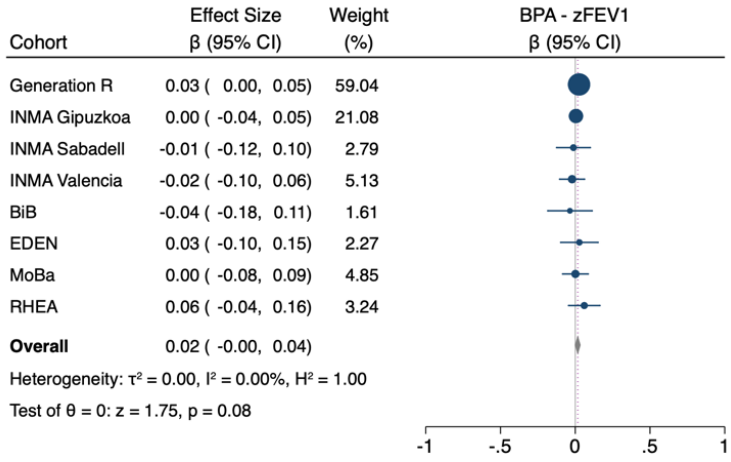
FVC	1808	0.10 (-0.01, 0.20)	0.035	892	0.17 (0.02, 0.32)	916	0.01 (-0.14, 0.16)
FEV_1	1806	0.12 (0.01, 0.23)	0.099	891	0.15 (-0.00, 0.29)	915	0.08 (-0.07, 0.24)
FEV_1/FVC	1806	0.02 (-0.09, 0.13)	0.256	891	-0.09 (-0.24, 0.06)	915	0.13 (-0.03, 0.29)
$FEF_{25-75\%}$	1806	0.13 (0.01, 0.25)	0.934	891	0.14 (-0.02, 0.31)	915	0.14 (-0.03, 0.31)
BPS (≥LOD vs <LOD)							
Asthma and wheeze, OR (95% CI)[a]							
Current asthma	1776	0.73 (0.47, 1.14)	0.405	871	1.08 (0.51, 2.27)	905	0.61 (0.36, 1.05)
Wheeze in the last year	1791	0.64 (0.40, 1.04)	0.382	875	0.66 (0.29, 1.50)	916	0.69 (0.38, 1.25)
Wheezing patterns, OR (95% CI)[a,c]							
Early	1302	1.01 (0.77, 1.32)	0.928	-	-	-	-
Late	757	0.43 (0.19, 1.00)	0.624	-	-	-	-
Persistent	851	0.56 (0.35, 0.90)	0.440	-	-	-	-
Lung function, z-score (95% CI)[b]							
FVC	1808	0.05 (-0.05, 0.16)	0.216	892	0.06 (-0.08, 0.20)	916	0.04 (-0.10, 0.19)
FEV_1	1806	0.09 (-0.02, 0.19)	0.084	891	0.10 (-0.03, 0.23)	915	0.09 (-0.06, 0.24)
FEV_1/FVC	1806	0.06 (-0.05, 0.16)	0.846	891	0.01 (-0.14, 0.15)	915	0.11 (-0.04, 0.26)
$FEF_{25-75\%}$	1806	0.02 (-0.09, 0.14)	0.870	891	0.02 (-0.14, 0.18)	915	0.04 (-0.12, 0.21)

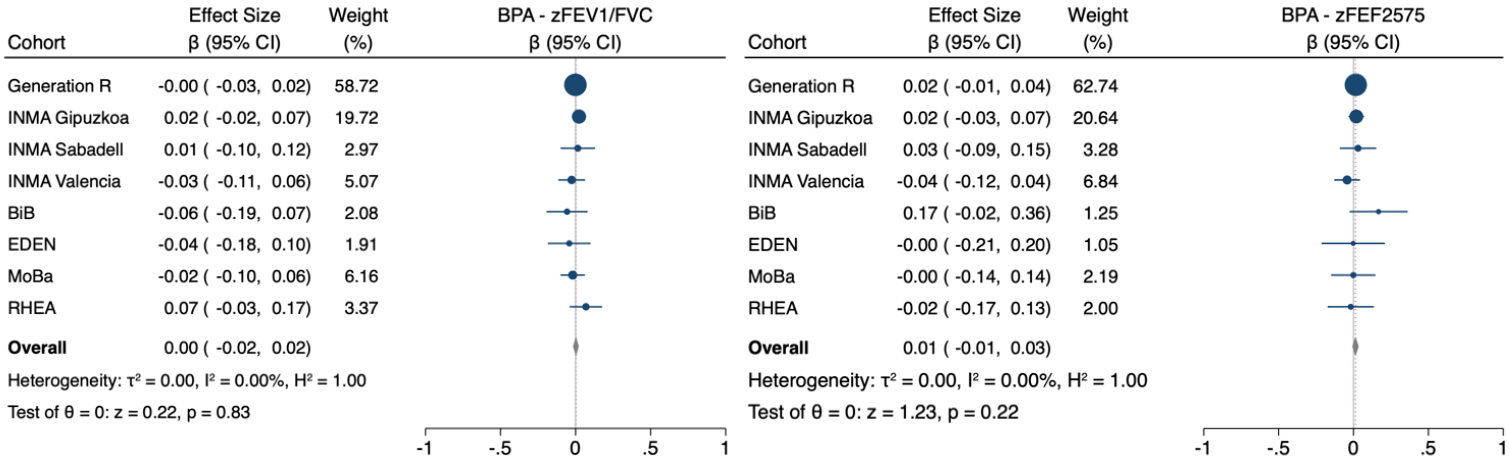
[a] Models adjusted for maternal age, education, pre-pregnancy BMI, smoking during pregnancy, and child's age, sex and ethnicity.
[b] Models adjusted for maternal age, education, pre-pregnancy BMI, smoking during pregnancy, and child's ethnicity.
[c] Reference base category: Never wheezers.
Note: discrepancies between the overall N and sex-stratified N are due to missing values in sex. Overall N in each wheezing pattern includes also never wheezers.
Abbreviations: BPA: bisphenol A; BPF: bisphenol F; BPS: bisphenol S; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1s; $FEF_{25-75\%}$: mid expiratory flow; LOD: limit of detection; OR: odds ratio; CI: confidence interval.

Figure 1. Overall associations between creatinine adjusted bisphenol A (µg/g) and respiratory outcomes from 2-stage meta-analysis [a].




Cohort
Effect Size β (95% CI)
Weight (%)
BPA - zFVC β (95% CI)
Generation R 0.03 (0.00, 0.05) 62.90
INMA Gipuzkoa -0.02 (-0.06, 0.03) 18.87
INMA Sabadell -0.03 (-0.14, 0.08) 2.70
INMA Valencia 0.00 (-0.08, 0.08) 5.26
BiB 0.00 (-0.18, 0.18) 1.04
EDEN 0.05 (-0.06, 0.17) 2.53
MoBa 0.01 (-0.07, 0.09) 4.82
RHEA -0.06 (-0.19, 0.07) 1.88
Overall 0.01 (-0.00, 0.03)
Heterogeneity: τ² = 0.00, I² = 0.00%, H² = 1.00
Test of θ = 0: z = 1.47, p = 0.14
-1
-.5
0
.5
1


Cohort
Effect Size β (95% CI)
Weight (%)
BPA - zFEV1 β (95% CI)
Generation R 0.03 (0.00, 0.05) 59.04
INMA Gipuzkoa 0.00 (-0.04, 0.05) 21.08
INMA Sabadell -0.01 (-0.12, 0.10) 2.79
INMA Valencia -0.02 (-0.10, 0.06) 5.13
BiB -0.04 (-0.18, 0.11) 1.61
EDEN 0.03 (-0.10, 0.15) 2.27
MoBa 0.00 (-0.08, 0.09) 4.85
RHEA 0.06 (-0.04, 0.16) 3.24
Overall 0.02 (-0.00, 0.04)
Heterogeneity: τ² = 0.00, I² = 0.00%, H² = 1.00
Test of θ = 0: z = 1.75, p = 0.08
-1
-.5
0
.5
1



[a] Values represent OR (95%CI) in associations between bisphenol A and current asthma and wheeze; and z-score (95% CI) in associations between BPA and lung function parameters. Current asthma and wheeze models were adjusted for maternal education, age, pre-pregnancy BMI, smoking during pregnancy, and child's sex, age, and ethnicity. Lung function models were adjusted for maternal education, age, pre-pregnancy BMI, smoking during pregnancy, and child's ethnicity. Abbreviations: BPA: bisphenol A; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1s; $FEF_{25-75\%}$: mid expiratory flow; OR: odds ratio; CI: confidence interval.

SUPPLEMENTARY MATERIAL

Figure S1. Flowchart of included population.

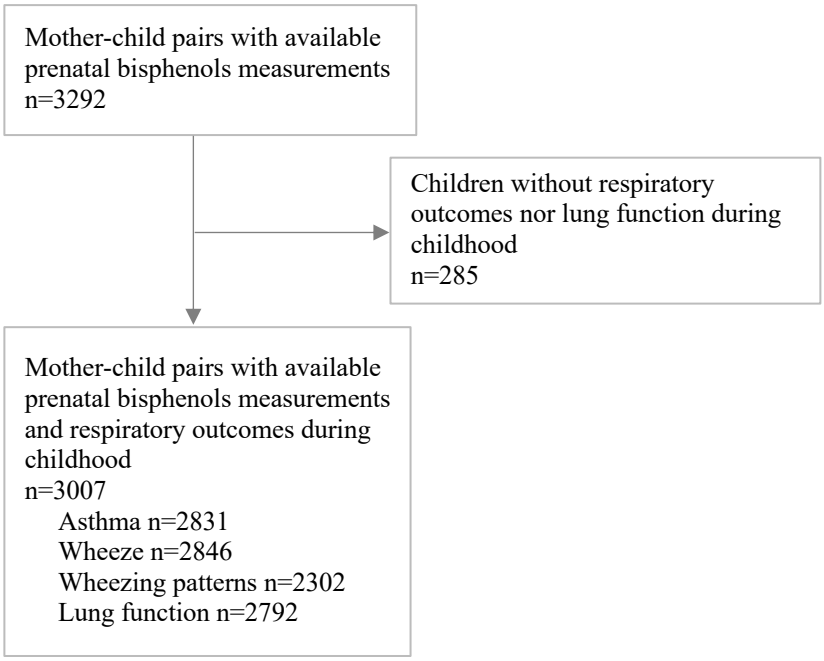
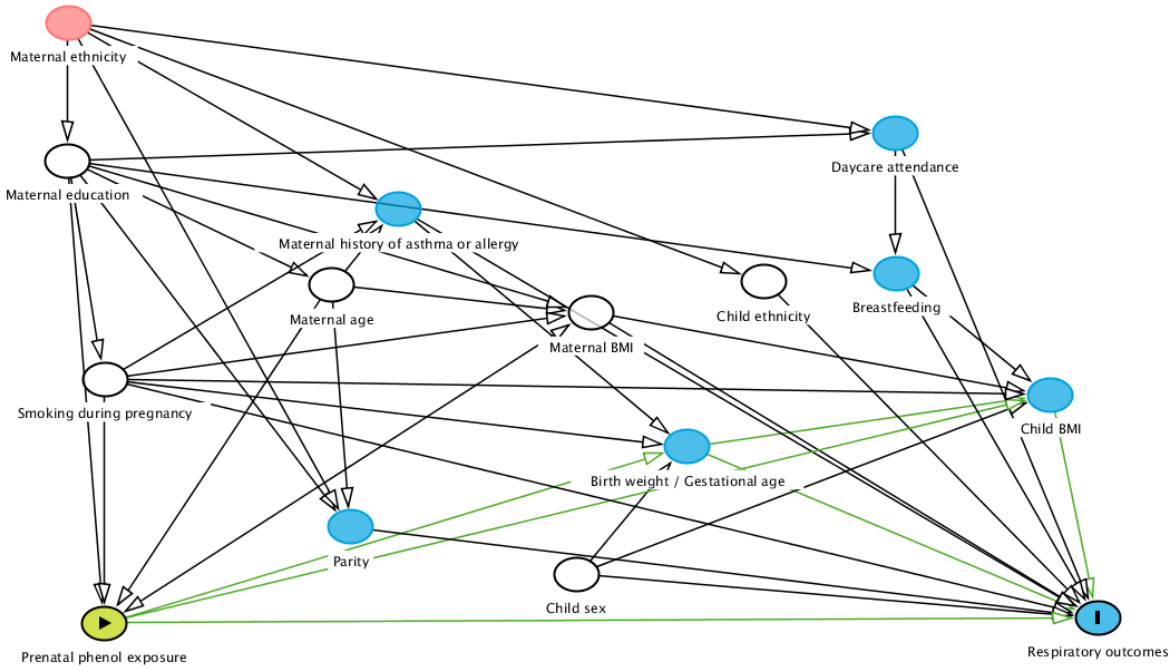


Figure S2. Directed Acyclic Graph.



Green node represents exposure, blue-black node outcome, blue nodes ancestors of outcome, red node unadjusted ancestor of exposure and outcome, and white nodes adjusted ancestors of exposure and outcome variables. Red arrows (not present) represent biasing paths, black arrows represent non-biasing paths, and green arrows represent causal paths.

Table S1. Wheezing information available in each cohort.

		Generation R	INMA Gipuzkoa	INMA Sabadell	INMA Valencia	BiB	EDEN	MoBa	RHEA
Early	1 year	x	x	x	x	x		x	x
	2 years	x		x	x	x		x	x
	3 years	x	x	x	x	x		x	x
	4 years	x	x	x	x	x	x	x	x
Late	5 years	x					x		
	6 years			x		x			x
	7 years		x		x				
	8 years							x	
	9-11 years	x					x		

Table S2. Maternal and child characteristics in observed and imputed datasets (n=3007).

		% Missing	Observed	Imputed	p-value
Maternal age (years), mean (SD)		0.40	31.3 (4.5)	31.3 (4.5)	0.977
Maternal pre-pregnancy BMI (kg/m2), mean (SD)		0.76	24.0 (4.4)	24.1 (4.4)	0.913
Maternal education, %	≤ Primary	2.59	13.2	13.5	0.860
	Secondary		37.6	37.6	
	University		49.3	48.9	
Smoking during pregnancy, %		5.12	15.0	15.2	0.736
Child ethnicity, %	Caucasian	4.06	86.5	86.7	0.996
	African American		3.0	2.9	
	Asian		6.1	6.0	
	Other or Mixed		4.4	4.4	
Child sex, % female		2.56	48	48	0.901
Child age (years), mean (SD)		14.00	8.4 (1.4)	8.5 (1.4)	0.131
Child height (cm), mean (SD)		16.63	131.8 (10.6)	132.4 (17.3)	0.082
Preterm birth, % yes		0.30	3.5	3.5	0.984
Low birth weight, % yes		0.33	3.4	3.4	0.989

Abbreviations: BMI: body mass index; SD: standard deviation.

Table S3. Overall and sex-stratified associations between creatinine adjusted bisphenol A (µg/g) and respiratory outcomes from 1-stage meta-analysis excluding preterm and low birth weight babies.

	$N_{Overall}$	Overall	N_{Girls}	Girls	N_{Boys}	Boys
Excluding preterm births (N=2894)						
Asthma and wheeze, OR (95% CI)[a]						
Current asthma	2720	1.01 (0.94, 1.09)	1306	1.17 (1.03, 1.32)	1394	0.95 (0.87, 1.03)
Wheeze in the last year	2737	1.06 (0.98, 1.15)	1312	1.20 (1.05, 1.36)	1405	1.00 (0.90, 1.10)
Wheezing patterns, OR (95% CI)[a,c]						
Early	1949	0.99 (0.94, 1.04)	-	-	-	-
Late	1331	1.03 (0.91, 1.17)	-	-	-	-
Persistent	1427	0.97 (0.88, 1.06)	-	-	-	-
Lung function, z-score (95% CI)[b]						
FVC	2577	0.01 (-0.01, 0.03)	1249	0.01 (-0.01, 0.04)	1328	0.01 (-0.02, 0.04)
FEV_1	2626	0.02 (0.00, 0.04)	1272	0.01 (-0.01, 0.04)	1354	0.03 (-0.00, 0.05)
FEV_1/FVC	2563	0.00 (-0.02, 0.02)	1245	-0.00 (-0.03, 0.02)	1318	0.01 (-0.02, 0.04)
$FEF_{25-75\%}$	2637	0.02 (-0.00, 0.04)	1275	0.02 (-0.01, 0.04)	1362	0.02 (-0.01, 0.04)
Excluding low birth weight babies (N=2894)						
Asthma and wheeze, OR (95% CI)[a]						
Current asthma	2723	1.01 (0.95, 1.09)	1296	1.15 (1.02, 1.29)	1408	0.95 (0.87, 1.04)
Wheeze in the last year	2739	1.05 (0.97, 1.14)	1301	1.17 (1.03, 1.33)	1419	1.00 (0.91, 1.10)
Wheezing patterns, OR (95% CI)[a,c]						

Early	1955	0.99 (0.94, 1.04)	-	-	-	-
Late	1341	1.02 (0.90, 1.15)	-	-	-	-
Persistent	1433	0.97 (0.89, 1.06)	-	-	-	-
Lung function, z-score (95% CI)[b]						
FVC	2573	0.01 (-0.01, 0.03)	1235	0.01 (-0.01, 0.04)	1338	0.01 (-0.02, 0.04)
FEV_1	2623	0.02 (0.00, 0.04)	1259	0.01 (-0.01, 0.04)	1364	0.03 (-0.00, 0.05)
FEV_1/FVC	2560	0.00 (-0.02, 0.02)	1231	-0.00 (-0.03, 0.03)	1329	0.01 (-0.02, 0.04)
$FEF_{25-75\%}$	2633	0.02 (-0.00, 0.04)	1262	0.01 (-0.01, 0.04)	1371	0.02 (-0.00, 0.04)

[a] Models adjusted for maternal age, education, pre-pregnancy BMI, smoking during pregnancy, and child's age, sex and ethnicity.
[b] Models adjusted for maternal age, education, pre-pregnancy BMI, smoking during pregnancy, and child's ethnicity.
[c] Reference base category: Never wheezers.
Note: discrepancies between the overall N and sex-stratified N are due to missing values in sex. Overall N in each wheezing pattern includes also never wheezers.
Abbreviations: BPA: bisphenol A; BPS: bisphenol F; BPS: bisphenol S; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1s; $FEF_{25-75\%}$: mid expiratory flow; LOD: limit of detection; OR: odds ratio; CI: confidence interval

Table S4. Overall and sex-stratified associations between creatinine adjusted bisphenols and respiratory outcomes from 1-stage meta-analysis in complete case datasets.

	N	Overall	Girls	Boys
BPA (µg/g)				
Asthma and wheeze, OR (95% CI)[a]				
Current asthma	2093	0.99 (0.90, 1.09)	1.09 (0.95, 1.26)	0.92 (0.82, 1.05)
Wheeze in the last year	2097	1.04 (0.94, 1.15)	1.11 (0.94, 1.30)	1.00 (0.88, 1.14)
Wheezing patterns, OR (95% CI)[a,c]				
Early	1583	0.97 (0.90, 1.05)	0.97 (0.88, 1.08)	0.97 (0.87, 1.08)
Late	1080	1.03 (0.87, 1.22)	1.09 (0.87, 1.35)	0.96 (0.75, 1.23)
Persistent	1152	0.94 (0.82, 1.07)	0.94 (0.76, 1.16)	0.94 (0.79, 1.11)
Lung function, z-score (95% CI)[b]				
FVC	2224	0.00 (-0.03, 0.03)	-0.01 (-0.05, 0.03)	0.01 (-0.03, 0.05)
FEV_1	2273	0.01 (-0.02, 0.04)	-0.01 (-0.04, 0.03)	0.03 (-0.01, 0.07)
FEV_1/FVC	2209	0.01 (-0.01, 0.04)	0.00 (-0.03, 0.04)	0.02 (-0.02, 0.06)
$FEF_{25-75\%}$	2285	0.03 (-0.01, 0.06)	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)
BPF (≥LOD vs <LOD)				
Asthma and wheeze, OR (95% CI)[a]				
Current asthma	1422	1.17 (0.68, 2.00)	0.59 (0.22, 1.60)	1.61 (0.84, 3.09)
Wheeze in the last year	1428	1.40 (0.77, 2.56)	0.69 (0.21, 2.29)	2.02 (0.98, 4.14)
Wheezing patterns, OR (95% CI)[a,c]				
Early	1095	0.91 (0.65, 1.27)	-	-
Late	629	1.28 (0.49, 3.34)	-	-
Persistent	707	0.92 (0.52, 1.63)	-	-

Lung function, z-score (95% CI)[b]				
FVC	1682	0.13 (0.02, 0.24)	0.19 (0.04, 0.35)	0.03 (-0.13, 0.19)
FEV_1	1680	0.14 (0.03, 0.26)	0.17 (0.02, 0.32)	0.10 (-0.06, 0.27)
FEV_1/FVC	1680	0.01 (-0.10, 0.13)	-0.10 (-0.26, 0.06)	0.14 (-0.03, 0.31)
$FEF_{25-75\%}$	1680	0.14 (0.01, 0.26)	0.18 (0.01, 0.36)	0.13 (-0.06, 0.31)
BPS (≥LOD vs <LOD)				
Asthma and wheeze, OR (95% CI)[a]				
Current asthma	1422	0.62 (0.38, 1.03)	0.83 (0.36, 1.89)	0.53 (0.28, 1.01)
Wheeze in the last year	1428	0.64 (0.37, 1.12)	0.59 (0.23, 1.50)	0.73 (0.37, 1.44)
Wheezing patterns, OR (95% CI)[a,c]				
Early	1095	1.07 (0.79, 1.44)	-	-
Late	629	0.52 (0.20, 1.34)	-	-
Persistent	707	0.61 (0.36, 1.04)	-	-
Lung function, z-score (95% CI)[b]				
FVC	1682	0.04 (-0.07, 0.15)	0.06 (-0.09, 0.20)	0.02 (-0.13, 0.17)
FEV_1	1680	0.07 (-0.04, 0.18)	0.11 (-0.03, 0.24)	0.05 (-0.11, 0.21)
FEV_1/FVC	1680	0.04 (-0.07, 0.15)	0.02 (-0.13, 0.17)	0.08 (-0.08, 0.24)
$FEF_{25-75\%}$	1680	0.01 (-0.11, 0.13)	0.04 (-0.12, 0.20)	0.02 (-0.16, 0.19)

[a] Models adjusted for maternal age, education, pre-pregnancy BMI, smoking during pregnancy, and child's age, sex and ethnicity.
[b] Models adjusted for maternal age, education, pre-pregnancy BMI, smoking during pregnancy, and child's ethnicity.
[c] Reference base category: Never wheezers.
Abbreviations: BPA: bisphenol A; BPS: bisphenol F; BPS: bisphenol S; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1s; $FEF_{25-75\%}$: mid expiratory flow; LOD: limit of detection; OR: odds ratio; CI: confidence interval.

Table S5. Overall associations between creatinine adjusted bisphenol A (µg/g) and main respiratory outcomes from 1-stage random effects meta-analysis excluding one cohort at a time.

	Excl Generation R		Excl INMA Gipuzkoa		Excl INMA Sabadell		Excl INMA Valencia		Excl BiB		Excl EDEN		Excl MoBa		Excl RHEA	
	N	Estimate	N	Estimate	N	Estimate	N	Estimate	N	Estimate	N	Estimate	N	Estimate	N	Estimate
Current asthma, OR *(95% CI)*[a]	1386	0.99 (0.88, 1.11)	2553	1.04 (0.96, 1.12)	2453	1.00 (0.93, 1.08)	2500	1.02 (0.95, 1.09)	2627	1.03 (0.96, 1.10)	2634	0.99 (0.92, 1.06)	2563	1.01 (0.94, 1.09)	2634	1.01 (0.94, 1.09)
Wheeze in the last year, OR *(95% CI)*[a]	1385	1.00 (0.90, 1.11)	2569	1.10 (1.01, 1.20)	2470	1.04 (0.96, 1.12)	2515	1.06 (0.98, 1.15)	2642	1.05 (0.97, 1.14)	2649	1.04 (0.96, 1.12)	2578	1.05 (0.97, 1.14)	2649	1.05 (0.97, 1.14)
Wheezing patterns, OR (95% CI)[a,c]	1412	1.02 (0.95, 1.11)	1785	0.98 (0.92, 1.04)	1697	0.98 (0.93, 1.03)	1734	0.98 (0.93, 1.04)	1976	0.99 (0.94, 1.04)	1890	0.98 (0.94, 1.04)	1826	0.98 (0.93, 1.04)	1841	0.98 (0.93, 1.03)
Early	644	1.00 (0.81, 1.22)	1235	1.09 (0.95, 1.25)	1217	1.02 (0.90, 1.15)	1217	1.02 (0.90, 1.16)	1338	1.01 (0.90, 1.15)	1239	0.97 (0.86, 1.11)	1190	1.00 (0.88, 1.14)	1200	1.03 (0.90, 1.17)
Late	703	1.04 (0.91, 1.19)	1326	0.98 (0.89, 1.08)	1279	0.95 (0.87, 1.05)	1286	0.98 (0.89, 1.07)	1432	0.98 (0.89, 1.07)	1344	0.97 (0.89, 1.06)	1292	0.98 (0.90, 1.07)	1303	0.96 (0.87, 1.04)
Persistent	1671	0.01 (-0.02, 0.04)	2466	0.02 (0.00, 0.04)	2415	0.02 (0.00, 0.04)	2411	0.02 (0.00, 0.04)	2562	0.02 (0.00, 0.04)	2552	0.02 (0.00, 0.04)	2472	0.02 (0.00, 0.04)	2554	0.02 (0.00, 0.04)
FEV_1, z-score *(95% CI)*[b]	1412	1.02 (0.95, 1.11)	1785	0.98 (0.92, 1.04)	1697	0.98 (0.93, 1.03)	1734	0.98 (0.93, 1.04)	1976	0.99 (0.94, 1.04)	1890	0.98 (0.94, 1.04)	1826	0.98 (0.93, 1.04)	1841	0.98 (0.93, 1.03)

[a] Models adjusted for maternal age, education, pre-pregnancy BMI, smoking during pregnancy, and child's age, sex and ethnicity.
[b] Models adjusted for maternal age, education, pre-pregnancy BMI, smoking during pregnancy, and child's ethnicity.
[c] Reference base category: Never wheezers.
Abbreviations: BPA: bisphenol A; BPS: bisphenol F; BPS: bisphenol S; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1s; $FEF_{25-75\%}$: mid expiratory flow; LOD: limit of detection; OR: odds ratio; CI: confidence interval.

Table S6. Overall associations between creatinine adjusted bisphenols and lung function from 1-stage random meta-analysis excluding children with non-reproducible spirometry and children with current asthma[a].

	Excluding non-reproducible		Excluding children with current asthma	
	N	z-score (95% CI)	N	z-score (95% CI)
BPA (µg/g)				
FVC	2315	0.02 (0.00, 0.03)	2395	0.02 (0.00, 0.04)
FEV_1	2358	0.02 (0.00, 0.04)	2439	0.02 (0.00, 0.04)
FEV_1/FVC	2314	0.00 (-0.02, 0.02)	2383	0.00 (-0.02, 0.02)
$FEF_{25-75\%}$	2357	0.03 (0.00, 0.05)	2449	0.02 (0.00, 0.05)
BPF (≥LOD vs <LOD)				
FVC	1566	0.09 (-0.02, 0.20)	1634	0.10 (-0.02, 0.21)
FEV_1	1565	0.10 (-0.01, 0.21)	1632	0.11 (-0.01, 0.22)
FEV_1/FVC	1565	0.00 (-0.11, 0.12)	1632	0.01 (-0.11, 0.13)
$FEF_{25-75\%}$	1565	0.13 (0.01, 0.25)	1632	0.13 (0.00, 0.25)
BPS (≥LOD vs <LOD)				
FVC	1566	0.05 (-0.06, 0.16)	1634	0.06 (-0.05, 0.17)
FEV_1	1565	0.07 (-0.04, 0.18)	1632	0.10 (-0.02, 0.21)
FEV_1/FVC	1565	0.03 (-0.08, 0.14)	1632	0.06 (-0.05, 0.17)
$FEF_{25-75\%}$	1565	-0.04 (-0.16, 0.08)	1632	0.03 (-0.09, 0.15)

[a] Models adjusted for maternal age, education, pre-pregnancy BMI, smoking during pregnancy, and child's ethnicity.

Abbreviations: BPA: bisphenol A; BPS: bisphenol F; BPS: bisphenol S; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1s; $FEF_{25-75\%}$: mid expiratory flow; LOD: limit of detection; CI: confidence interval

Supplementary Methods 1. Multiple imputation by chained equations.

We performed Multiple Imputation by Chained Equations using the *ice* command. We used STATA 14 software (Stata Corporation, College Station, Texas).

Missingness in covariates ranged between 0.4% and 14% (see Supplementary Table S2). Overall, 80% of the sample had complete information on all covariates. Over the 20% with missing data, most of them had missing in only one covariate. Child age and smoking were the most common variables with missing values. We assume, given the distribution of missingness, that our data is either MCAR or MAR. However, we are aware that in epidemiological research data, MCAR is rarely the case. Multiple imputation by chained equations is an appropriate approach to handle missingness in both cases when data is MCAR or MAR (1).

Multiple imputation was performed in each cohort separately, with twenty-five cycles ran in each. A total of 25 complete datasets of each cohort were generated. The following maternal and children variables were included in the imputation procedure:

- Maternal urinary BPA concentrations: BPA concentrations below the limit of detection (LOD) (between 0% and 22%) on a defined range between 0 and the corresponding LOD in each cohort (Table 2).
- Maternal variables: urinary creatinine concentrations; age; BMI; smoking; alcohol; socioeconomic status; education; pet keeping during pregnancy; parity; type of delivery; history of asthma or allergy.

- Children variables: birth weight; gestational age; breastfeeding; daycare attendance; ethnicity; sex; age at the last follow up; weight and height at the spirometry measurement.

Non-normally distributed variables were transformed using the most suitable transformation in each case (cubic, square, identity, square root, log, $\log_2$, 1/square root, inverse, 1/square, 1/cubic). When normality could not be reached through transformation, variables were included in the imputation command using predictive mean matching.

All subsequent analyses were performed applying the STATA 14 *mim* command.

Supplementary Methods 2. Urinary bisphenols determination and interlaboratory comparison.

Concentrations were determined in a single spot urine sample collected throughout pregnancy in all cohorts except in the INMA cohorts where two spot urine samples were available (1st and 3rd trimester): in INMA Sabadell and Valencia, bisphenols were quantified in each spot sample and we used the average of both measurements and in INMA Gipuzkoa bisphenols were quantified in a pool of both urines.

For Generation R, bisphenols were measured in Wadsworth Centre, New York, USA (2). In INMA Gipuzkoa, bisphenols were determined at Instituto de Investigación Biosanitaria ibs.GRANADA, Spain. In INMA Sabadell, the concentrations were determined in the Department of Analytical Chemistry, University of Cordoba, Spain (3). In INMA Valencia, BiB, MoBa, and RHEA, bisphenols were measured at Norwegian Institute for Public Health (NIPH), Norway, as part of the HELIX (Human Early Life Exposome) Project (4). For EDEN, BPA was determined at the National Center for Environmental Health laboratory, CDC, Atlanta, Georgia, USA (5). In order to ensure comparability of results between cohorts, we conducted interlaboratory comparisons for BPA with 10 urines. Correlations of urinary concentrations of BPA between pairs of laboratories ranged from 0.36 to 0.90 (Pearson). The poorest correlations were observed between Granada and Córdoba labs in relation to the other laboratories. The coefficient of variation of BPA for the different laboratories ranged from 35% (Granada) to 80% (NIPH), indicating high dispersion of BPA within laboratories.

Supplementary Methods 3. Calculation of the reliability coefficient of bisphenol A.

The reliability coefficient of a spot urine sample collected during pregnancy was calculated by estimating whether this sample was a good measure of the average pregnancy concentration. To do that, we used data from the HELIX Pregnancy Panel Study of 52 pregnant women from Barcelona, Spain (6). Pregnant women were recruited between 2014 and 2015. Criteria for inclusion were a) singleton pregnancy; b) age≥18 years at the time of start of pregnancy; c) first visit to be conducted before 20 weeks of pregnancy; and d) residence in the study area covered by the cohort. Pregnant women were followed during a normal working week in the 2nd (mean: 16.3 gestational weeks, standard deviation (SD): 2.0) and 3rd (mean: 31.4, SD: 2.0) pregnancy trimesters. Urine samples were collected three times per day: first morning, afternoon, and bedtime voids. Details on collection and processing of urine samples can be found elsewhere (6). We made within-subject pools of all urines collected in a week by taking 0.5 ml from each aliquot. BPA was determined in the two weekly pools of each trimester and the average was used as the gold standard. We also determined BPA in a spot urine sample selected at random from all the first morning voids of the two trimesters. We selected the first morning void because this was the sample mostly collected in the cohorts participating in the present study. We then computed the square correlations between the spot urine and the gold standard (7). The reliability coefficient for BPA was 0.14. This reliability coefficient is similar to the interclass correlation

coefficients obtained in other variability studies in pregnant women (8–10).

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5.4. Paper IV

Abellan A, Warembourg C, Mensink-Bout SM, Ambros A, de Castro M, Fossati S, Guxens M, Jaddoe VWV, Nieuwenhuijsen MJ, Vrijheid M, Santos S, Casas M, Duijts L.

Urban environment during pregnancy and lung function, wheeze, and asthma in school-age children. The Generation R Study.

In preparation

Urban environment during pregnancy and lung function, wheeze, and asthma in school-age children. The Generation R Study.

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Contribution of authors to the study

AA, CW, SM, SS, MC, LD contributed to the conception and design, acquisition of data, analyses and interpretation of the data, drafted the article, revised it critically for important intellectual content, and gave final approval of the version to be published.
AA, MdC, SF, MG, VJ, MN, MV contributed to the conception and design, acquisition of data, revised the drafted manuscript critically for important intellectual content, and gave final approval of the version to be published.

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ABSTRACT

Background: Many early life environmental exposures are associated with the development of lower lung function and risk of asthma in childhood. Evidence on the many exposures related to the urban environment during pregnancy and especially the concurrence of them in relation to children's respiratory health is lacking.

Objective: To examine the associations of exposure to the full urban environment during pregnancy, including air pollution, noise, traffic, green and blue spaces, and built environment with lung function, wheeze, and asthma during childhood.

Methods: We included 5,624 mother-child pairs participating in the Generation R birth cohort. We estimated a total of 44 urban environment exposures including air pollution, road traffic noise, traffic, green spaces, blue spaces, and built environment during pregnancy. At 10 years of age, lung function was measured by spirometry and asthma from questionnaires. Wheezing patterns were constructed from questionnaires across childhood. We performed single, multiple exposure and cluster analyses to assess the associations.

Results: higher maternal exposure to NO_2 and $PM_{2.5}$ during pregnancy was associated with lower lung function only in mid-to-small airways in childhood (z-score for $PM_{2.5}$=-0.10, 95%CI=-0.15, -0.05). Higher levels of $PM_{2.5}$ increased FEV_1 and FVC, but these associations were not observed when considering the urban environment as a whole in cluster

analyses. High road traffic noise was associated with increased asthma at 10 years, and increasing size of the nearest major blue spaces with higher lung function (FEV_1 z-score=0.02, 95%CI=0.00, 0.04) and lower odds of asthma (OR=0.92, 95%CI=0.84, 1.00). Cluster analyses revealed that living during pregnancy in an urban environment with higher levels of air pollution, noise, urbanisation and lower levels of natural spaces may contribute to lower lung function in mid-to-small airways (z-score=-0.08, 95%CI=-0.17, 0.01), and to increased odds of early and late wheeze during childhood (OR early=1.23, 95%CI=1.00, 1.51).

Conclusion: this study shows that the urban environment during pregnancy is of relevance to the offspring's respiratory health through childhood. Air pollution, road traffic noise, and blue spaces were the main determinants of childhood respiratory health.

INTRODUCTION

Lung function and asthma development during childhood are key determinants for respiratory health into adult life (1). Many early life environmental exposures are associated with the development of lower lung function and risk of asthma in childhood (2). Air pollution exposure after birth is suggested to be associated with lower lung function (3–5), increased risk of wheeze and asthma (6–11), and exacerbating both pre-existing conditions (12) during childhood. Some studies examined the role of maternal exposure to air pollution during pregnancy on child's respiratory health and reported mixed findings (13–26). Some studies found that maternal exposure to air pollution during pregnancy was associated with lower lung function (13,14,19–22), and increased risks of wheeze (23) and asthma in children (23–26), while others did not (15–18,22). The potential adverse health effects of other exposures related to the urban environment such as noise (17,27), lack of green (17,28) and blue spaces (17,29), increased built environment (17), and the concurrence of these exposures (17) on respiratory health is limited and inconsistent (30). Only one study has assessed maternal exposure to the full urban environment during pregnancy and observed no association with child's lung function but did not study wheeze or asthma as outcomes (17).

Examining the full urban environment in relation to respiratory health is important since half of the global population currently lives in urban settings, and this proportion is expected to increase up to 70% by 2050 (31). Despite that urban living offers opportunities for a healthy living

including access to services and goods, employment and social interaction, it may also imply increasing exposure to a harmful environment (32,33). Exploring the urban environment as a whole in relation to respiratory health, especially in vulnerable developing periods of life, may help to identify the most harmful urban exposures to ultimately identify effective prevention strategies to reduce them.

Therefore, we aimed to examine the associations of exposure to the full urban environment during pregnancy, including air pollution, noise, traffic, green and blue spaces, and built environment with lung function, wheeze, and asthma among 5,624 children participation in a population-based prospective cohort study.

METHODS

Design The present study was embedded in the Generation R Study, a population-based prospective birth cohort study in Rotterdam, the Netherlands Pregnant women were recruited during the whole pregnancy at their health care centers between 2002 and 2006 (34). The study wss approved by the Medical Ethical Committee of the Erasmus MC, University Medical Centre in Rotterdam. Written informed consent was obtained from all participants. A total of 5,624 mother-child pairs were included in the current study (Figure S1).

Urban environment during pregnancy The full range of urban environment variables was estimated at the home address during pregnancy using geographic information system (GIS) modelling (Table

1). The same variables were also estimated for the year prior to the outcomes assessment for a posterior sensitivity analysis. We included a total of 44 exposures from the following exposure groups: air pollution, road traffic noise, traffic, green spaces, blue spaces, and built environment. Air pollution was estimated using land use regression (LUR) models developed within the European Study of Cohort for Air Pollution Effects (ESCAPE) framework (35,36), and were temporally adjusted following the ESCAPE guidelines (37). Noise was assessed as road traffic noise from the European road traffic noise maps generated under EC Directive 2002/49/EC (38). Traffic was estimated using the Dutch National Database of Road Traffic Data (Nationale Databank Wegverkeersgegevens). Green and blue spaces were estimated from LANDSAT 4-5, 7, 8 (39) and Urban Atlas maps (40) in buffers of 100 m, 300 m, or 500 m from the home address. Built environment exposures were estimated in buffers of 100 m or 300 m from the home address from several sources including the Global and European Human Settlement maps (41,42), NAVTEQ (43), Urban Atlas (40), and Open Street Maps (44). Complete details on the exposures assessed, distributions, and data sources are fully described in Supplementary Table S1 and Pearson correlations in Figure S2.

Child's respiratory outcomes Lung function was measured at a mean age of 9.79 years (standard deviation (SD)=0.35 years) by spirometry test following the American Thoracic Society and European Respiratory Society guidelines (45). We measured forced expiratory volume in 1s (FEV_1), forced vital capacity (FVC), FEV_1/FVC, and mid-expiratory flow ($_{FEF25-75\%}$) and converted these into age-, height-, sex-, and ethnicity-

adjusted z-scores (46). Information on acceptability and reproducibility of spirometry tests was collected. Children with at least one acceptable manoeuvre were included in the analyses. Information on wheeze and asthma was collected through parental-reported questionnaires adapted from the International Study on Asthma and Allergy in Childhood (ISAAC) (47). Wheezing in the past 12 months (yes, no) was annually asked at the ages of 1 to 4 years, and at 6 and 9 years. Wheezing patterns were defined as 'never', 'early' (≤3 years only), 'late' (>3-9 years only), and 'persistent' (≤3 years and >3-9 years) wheezing. At a mean age of 9.79 years, current asthma was defined as ever-physician-diagnosed asthma with either wheezing or asthma medication use in the past 12 months.

Covariates Maternal characteristics were obtained from questionnaires during pregnancy and included educational level, age at pregnancy, pre-pregnancy body mass index (BMI), smoking during pregnancy, and history of asthma and atopy. Additionally, information on area-level SES was obtained from the Social and Cultural Planning Office of The Netherlands. Area-level SES was assessed as quintiles of the neighbourhood deprivation, considering education, income, and position on the labour market of the neighbourhood inhabitants. Child's sex, gestational age, and birth weight information was collected from midwife and hospital records at birth. Child's height was measured at the time of the lung function assessment and ethnicity information was obtained from questionnaires and assessed from the parental ethicnity. Covariates were selected from literature and summarised in a directed acyclic graph

(DAG) to depict the known and hypothesised causal relations between variables and avoid adjusting for mediators or colliders (Figure S3).

Statistical analysis We performed a non-response analysis comparing mothers and children included in the analyses to those lost to follow up at the age of 10 years using chi-square tests for categorical variables and independent samples t-tests for continuous variables. Multiple imputation by chained equations was performed for missing values in exposures and selected covariates (48). All variables not following a normal distribution were transformed to approach normality. A total of 10 imputed datasets were generated and Rubin's rules were applied to summarize effect estimates (48). Later, we standardised by the interquartile range (IQR) all continuous exposures to ensure comparability of exposure estimates. Therefore, all continuous exposure estimates are expressed as IQR increase.

We examined associations of urban environmental exposures during pregnancy with lung function, wheezing patterns, and asthma using three complementary approaches. First, we performed single exposure analyses through an exposome-wide association study (ExWAS) method. This method assesses exposure by exposure associations with the outcome through multivariable linear or logistic regression models and is afterwards corrected for multiple hypothesis testing using an adapted version of the Bonferroni correction that takes into account the correlation structure of the data (corrected p-value=0.002) (49). Second, we performed multiple exposure models to evaluate possible confounding between the multiple urban exposures. We built a DAG to account for the

complex interrelationships between exposures (Figure S4). We selected one indicator of each family of exposures, the most commonly used in the literature, and adjusted the models accordingly to the selection from the DAG. Third, we applied a hierarchical clustering on principal components (HCPC) analysis to identify children sharing similar urban exposure patterns. Because area-level SES may play a substantial role in the association between urban exposure patterns and childhood respiratory outcomes, we first standardised all exposures by the mean exposure level in each quintile of area-level SES to reduce any residual confounding. We applied a principal component analysis (PCA) to reduce the data dimension and retained the components that explained at least 90% of the variance. Then we applied an ascending hierarchical classification based on the PCA components to identify clusters of exposure. We used the identified clusters as the independent variable in the regression models. Models from all approaches were adjusted for a common set of covariates selected from the DAG (Figure S2). Lung function models were adjusted for maternal education, pre-pregnancy BMI, age, smoking during pregnancy, history of asthma or atopy, area-level SES, and child's ethnicity and season of birth. Wheeze and asthma models were additionally adjusted for child's sex.

Further sensitivity analyses were performed to assess the robustness of the results. Lung function models in ExWAS, multipollutant, and HCPC analyses were repeated including only children with 3 acceptable and reproducible manoeuvres from spirometry test. Since previous studies have found associations of urban environmental postnatal exposures with child's respiratory health, we repeated the single-exposure analyses of the main exposures of each family of exposures adjusting the models for their

corresponding postnatal exposures. We further repeated the cluster analyses excluding children born preterm or with low birth weight. We described the levels of urban exposures and distribution of outcomes in each area-level SES strata. Statistical analyses were performed using R Statistical Software.

RESULTS

Subject characteristics Maternal and child characteristics are shown in Table 2. Median (IQR) pregnancy air pollution levels (NO_2=38.48 (7.48) $\mu g/m^3$, PM_{10}= 31.36 (6.14) $\mu g/m^3$, $PM_{2.5}$= 19.72 (3.83) $\mu g/m^3$) were slightly below the current EU regulations (40 $\mu g/m^3$ for NO_2 and PM_{10}, and 25 $\mu g/m^3$ for $PM_{2.5}$) (Table S1). Of all mothers, 47% and 37% were exposed to daily noise and night noise levels, respectively, above the current EU regulations (Lden≥55 dB; Ln≥50 dB). Most of the population had access to a major green (80%) and blue space (54%) in a buffer of 300 m from the home address. Median (IQR) population density was 4136 (215) inhabitants/km^2. Of all children (n=5,624), 26%, 5%, and 11% were early, late, and persistent wheezers, respectively. Six percent had current asthma at a mean age of 9.8 (SD=0.35) years. Non-response analyses showed that children included in the analyses had mothers who were older, with lower pre-pregnancy BMI, higher education, and living in less deprived neighbourhoods. Children included in the analyses were less likely to be born preterm or with low birth weight and were less ethnically diverse (Table S2). There were no differences in maternal and child characteristics between the complete case and the imputed datasets (Table S3).

Urban environment during pregnancy and child's respiratory outcomes

Single exposure (ExWAS) analyses In single-exposure analyses, higher NO_2 exposure during pregnancy was associated with lower $FEF_{25-75\%}$ (z-score -0.05 [95%CI=-0.10, 0.00]) (Tables S4). Higher exposure to PM_{10}, $PM_{2.5}$, and PM_{coarse} was related to higher FEV_1 (e.g., 0.08, [0.03, 0.13] for $PM_{2.5}$) and FVC (0.09 [0.05, 0.14] for $PM_{2.5}$) but lower $FEF_{25-75\%}$ (-0.10 [-0.15, -0.05] for $PM_{2.5}$). Higher size of the nearest major blue space was associated with higher FEV_1 (0.02 [0.00, 0.04]) and less clearly with higher FVC and $FEF_{25-75\%}$ (Table S4). Some characteristics of the building environment were associated with higher or lower lung function parameters. No associations were observed of traffic and green spaces and lung function. After correction for multiple testing (p-value=0.002), only the associations of PM_{10} and $PM_{2.5}$ remained statistically significant. Regarding respiratory symptoms, higher NO_x was associated with higher odds of early wheeze (1.11 [1.00, 1.22]). Higher NO_2, PM_{10}, $PM_{2.5}$, $PM_{2.5abs}$, and PM_{coarse} was associated with lower offs of persistent wheeze (e.g., Odds Ratio (OR) for $PM_{2.5}$=0.81 [95%CI [0.67, 0.97]) but not with other wheezing patterns nor with asthma (Table S5). Higher road traffic noise, particularly at night, was associated with higher odds of early and persistent wheeze and current asthma at 10 years (e.g.,1.69 [1.01, 2.83] for current asthma). Higher size of the nearest blue space was related to higher odds of persistent wheeze (1.08 [1.01, 1.15]) but lower odds of current asthma (0.92 [0.84, 1.00]). Traffic and green spaces were not associated with any of the respiratory outcomes. After correction for multiple testing, none of the abovementioned associations remained statistically significant (Table S5).

Multiple exposure analyses After adjusting for other urban exposures, estimates remained similar to those from single-exposure analyses. NO_2 exposure during pregnancy remained associated with lower $FEF_{25-75\%}$ (-0.05 [-0.10, 0.00]) while increasing $PM_{2.5}$ remained associated with higher FEV_1 and FVC (0.09 [0.04, 0.14] for FEV_1), but lower $FEF_{25-75\%}$ (-0.10 [-0.15, -0.05]) (Figure 1 and Table S6). Associations of higher size of the closest blue space with higher FEV_1, FVC and $FEF_{25-75\%}$ remained after adjustment for the other urban exposures. Regarding respiratory symptoms, the association of NO_2 with persistent wheezing disappeared (Figure 2 and Table S7) but the association of $PM_{2.5}$ with lower odds of persistent wheeze remained. Higher noise levels at night remained associated with higher odds of early wheeze and current asthma (1.71 [0.99, 2.97]). Increasing size of the nearest blue space was also associated with higher odds of persistent wheeze (1.10 [1.02, 1.17]) and lower odds of current asthma (0.92 [0.85, 1.01]). Higher building density in a 300 m buffer showed an association with higher odds of persistent wheeze (OR=1.19, 95%CI=0.99, 1.42) (Figure 2 and Table S7).

Cluster analyses We identified 3 clusters of urban exposures. Figure 3 compares exposure levels in each cluster to the average exposure level in the study population (mean=0). Cluster 1 (n=1,533) identified mother-child pairs exposed to lower levels of air pollution, road traffic noise, traffic, and urbanisation, and to higher levels of green and blue spaces. We selected this cluster as the reference cluster in our analyses because it had the lowest levels of a priori harmful exposures. Cluster 2 (n=2,907) identified mother-child pairs exposed to lower levels of most air pollutants, although not as low as Cluster 1, and to lower levels of noise,

green, and blue spaces, and increased levels of traffic and urbanisation. Cluster 3 (n=1,184) identified mother-child pairs who were exposed to higher air pollution, noise, and urbanisation, and lower levels of traffic, green, and blue spaces. Mother-child pairs between clusters differed in terms of maternal education, season of birth, and ethnicity (Table S8). Mothers in Cluster 3 were higher educated and slightly more likely to be Caucasian, compared to the other clusters. Compared to Cluster 1, children belonging to the other two clusters tended to have lower lung function, particularly $FEF_{25-75\%}$ (-0.08 [-0.17, 0.01] for children in Cluster 3) and higher odds of early wheezing (1.23 [1.00, 1.51] for Cluster 3), late wheezing (1.48 [0.99, 2.25] for Cluster 3), and current asthma (1.14 [0.85, 1.54] for Cluster 2) but lower odds of persistent wheezing (0.75 [0.56, 1.01] for Cluster 3) (Table 3).

Sensitivity analyses The observed associations remained materially similar when we included only children with reproducible spirometry measurements (Table S9-S11).

When we adjusted the main single-exposure models for their corresponding postnatal exposures, some of the observed associations with lung function and current asthma remained similar (e.g., PM2.5 and blue spaces with lung function and noise at night with asthma) (Table S12-S13) while the association of blue spaces and current asthma became stronger (0.90 [0.82, 0.99]) (Table S13). When we repeated the cluster analyses excluding preterm and low birth weight born children, the associations remained similar (Table S14). We did not observe the most harmful environments in the most deprived areas (Table S15). The highest median levels of NO2 in was observed in quintiles 4 (highly

deprived) and 2 (low deprived). Highest levels of noise were observed in the two most deprived quintiles. Quintiles 1 (least deprived) and 3 (medium deprived) presented the highest levels of green spaces, and quintiles 1, 2, and 4, the highest levels of blue spaces.

DISCUSSION

Main results

This study, one of the first that systematically analyse multiple urban exposures in relation to child's respiratory health, showed that higher maternal exposure to NO_2 and $PM_{2.5}$ during pregnancy was associated with lower lung function only in mid-to-small airways ($FEF_{25-75\%}$) in the child. Higher levels of $PM_{2.5}$ increased FEV_1 and FVC, but these associations were no longer observed when considering the urban environment as a whole in cluster analyses. We also observed that high road traffic noise levels were associated with increased asthma at 10 years of age, and increasing size of the nearest major blue spaces with higher lung function and lower odds of asthma. Cluster analyses revealed that living during pregnancy in an urban environment characterised by higher levels of air pollution, noise, urbanisation and lower levels of natural spaces may contribute to lower lung function in mid-to-small airways, and to increased odds of early and late wheeze during childhood.

Comparison with previous studies

Our single and multi-exposure study confirms the adverse effects of air pollution on mid-to-small airways observed in previous studies (13,19,21). The association of air pollutants with lung function did not

follow the same pattern between lung function parameters. In our study, we observed that higher $PM_{2.5}$, PM_{10}, and PM_{coarse} was associated with higher FEV_1 and FVC, and higher NO_2, $PM_{2.5}$, PM_{10}, PM_{coarse}, and $PM_{2.5abs}$ with lower $FEF_{25-75\%}$ but only the associations with PM_{10} and $PM_{2.5}$ remained after correction for multiple testing. A single exposure study from the PARIS (Pollution and Asthma Risk: an Infant Study) birth cohort reported that higher prenatal NO_x was not associated with FEV_1, FVC or FEV_1/FVC, but was with lower $FEF_{25-75\%}$ (21), highlighting the relevance of $FEF_{25-75\%}$ as an early marker of small airways impairment (50). Some single exposure studies reported that higher exposure during pregnancy to $PM_{2.5}$ and PM_{10} was associated with lower FEV_1, FVC or FEV_1/FVC (13,14,20,22) while others reported no associations (15–17,22). Our contradictory results of higher $PM_{2.5}$ with higher FEV_1 and FVC should be further explored before any strong conclusions could be made. Regarding the outcomes wheezing and asthma, a recent systematic review showed an association between higher exposure to NO_2 during pregnancy and increased odds of wheeze in early childhood and between higher prenatal PM_{10} and NO_2 and increased odds of asthma in young children (23). Two later studies found that higher prenatal exposure to NO_2 (24) and $PM_{2.5}$ (24,25) were associated with increased odds of asthma in early childhood. Contrarily, such associations were not observed in a recent meta-analysis of five birth cohorts covering seven areas in Europe (18). In our study, in multiple exposure analysis, we also observed some counterintuitive results because a higher exposure to $PM_{2.5}$ was associated with decreased risk of persistent wheeze and no association was observed with asthma at 10 years. Differences in results between studies may partly be due to spatial and source differences that

lead to diverse pollutant composition and hence, of toxicity of the different pollutants.

Regarding road traffic noise, we observed an association of high levels of noise, especially at night, with increased risk of early wheeze and asthma at age 10 years. Although this association did not pass the multiple testing correction, the increased asthma risk was observed across different noise levels and remained after adjusting for other urban exposures and for postnatal exposure to noise. Only two studies have explored noise exposure during pregnancy and reported null associations (17,27). A study from over 4000 participants from the BAMSE birth cohort in Sweden explored the role of prenatal and postnatal exposure to residential noise, with objective noise measurements, and wheeze and asthma until adolescence and did not find consistent associations in relation to asthma and wheeze until adolescence (27). However, they observed a tendency of increased risk of ever suffering from asthma until 16 years of age. Considering the prevalent high noise exposure (Lden≥55 dB; Ln≥50 dB) and its potential health risks, early life and long-term exposure to noise in relation to child's respiratory health merits further investigation.

We did not observe any association with traffic nor with green spaces exposure. Overall null associations of both indicators with lung function were also reported in a previous exposome study from the HELIX birth cohort (17). A recent study that assessed green spaces exposure at birth found that greenness in a 100 m buffer from the home and the presence of urban green spaces in a 300 m buffer was associated with higher lung function up to 24 years of age, independently of air pollution and asthma

(28). Only two studies have assessed prenatal or at birth green spaces exposure in relation to lung function. One reported a small increase in lung function up to 24 years of age (28) and another no association (17). Such differences could be explained by differences according to the type and composition of green spaces, seasonal variability, and climate differences (51). Further, in our study setting median levels of green space was similar to the two previous studies but the range of levels was very narrow (NDVI 300 m buffer=0.39 (0.14), which may have hindered to detect associations.

Regarding blue spaces, this is the first study to report an association of increasing size of the nearest major blue space with higher lung function and lower odds of asthma at age 10 years. Only one study previously assessed prenatal exposure to blue spaces in relation to lung function and reported null associations (17). However, the levels of blue spaces in that study were very low and were only assessed as presence or absence of blue spaces in a 300 m buffer (17). A study that evaluated postnatal blue spaces exposure in relation to wheeze and asthma at school age also reported no associations (29). Again, in that study the proportion of blue spaces was extremely low. The Rotterdam area is a unique setting with very high proportion of blue spaces (including a river, lakes, canals, and an extensive industrial port), compared to other cities. Given the potential health benefits of blue spaces, but at the same time the risks of the industrial port, further research is guaranteed.

We observed inconsistent associations across the many built environment exposures. Up to now, only one study has assessed the influence of the

built environment during pregnancy and childhood respiratory health (17) and reported no associations with lung function.

When we considered the totality of the urban exposures to create clusters of exposure, we observed that compared to mother-child pairs living in less polluted, less noisy, more natural, and less urban areas, those living in areas with higher air pollution, noise, urbanization, and lower levels of green and blue spaces had lower $FEF_{25-75\%}$ and increased risk of early and late wheeze but lower risk of persistent wheeze. To our knowledge, only one study had previously considered a wide range of urban exposures during pregnancy in relation to lung function (17), but not with wheeze and asthma during childhood. In that study they did not find any association with lung function in single or multiple-exposure analysis. They only reported a positive association with inverse distance to the nearest road, which they considered a spurious finding. In that study, the statistical power was limited to assess the multiplicity of the exposures included with the small effect on lung function that was expected (17). In our study we assessed a wide range of urban exposures during pregnancy in relation to multiple respiratory health outcomes during childhood with a relatively large sample size.

Interpretation of results

Air pollutants inhaled by the mother during pregnancy can cross the placenta and thus have a direct effect on the fetus development (52). The mechanisms underlying the effects of air pollution, especially prenatal, on lung function and asthma development are not fully understood. Both PM and gases can induce inflammation and oxidative stress at various levels

(i.e. trachea, bronchi, bronchioli, alveoli) depending on its deposition site according to their size (53). Oxidative stress, inflammation processes, and also epigenetic changes are suspected to play an important role on the respiratory effects of air pollution (20,54). However, mechanisms could be specific per pollutant. Air pollution could directly affect lung development by disturbing organogenesis due to the aforementioned mechanisms, and indirectly affect it through adverse birth outcomes. In our study the observed effects of air pollution on mid-to-small airways remained after we excluded children who were born preterm or with low birth weight, is in favour of a direct effect on lung organogenesis.

Noise acts as a physiological and psychological stressor. It is suggested to promote stress responses through the hypothalamic-pituitary-adrenal axis leading to the release of stress hormones such as cortisol (55). This was supported in studies that reported children exposed to higher levels of noise at night (56) and those with higher annoyance to noise (57) showed increased saliva cortisol levels. Further, maternal psychological distress during pregnancy has been associated with pre-school wheezing (58), and lower lung function and asthma at school-age (59). Stress mechanisms may also disrupt sleep and night-time recovery of the immune system, leading to inflammatory processes also in the respiratory system (55). Further, chronic stress-reactions and sleep disturbances may lead to alterations in the endocrine system that could impair critical developmental processes of the lung and immune system (55,60–62).

There are several potential mechanisms by which green and blue spaces may affect lung and asthma development (63). First, green and blue

spaces could act as mitigators of harmful exposures such as air pollution (64). Second, by promoting healthier lifestyle and reducing stress (e.g., increasing physical activity, promoting social cohesion) (65). Third, by influencing individual's microbiome as postulated by the biodiversity hypothesis (66,67). This hypothesis holds that urban environments are usually associated with a lack of natural exposures and biodiversity that affect the human microbiome and its immunoregulatory capacity necessary for a healthy development (68).

The features of the built environment have the potential to influence the health of its inhabitants through the influence on the magnitude of environmental stressors and on health-related behaviors (69). Although in The Netherlands the use of active transportation (i.e. cycling) is common, more efforts can be put into developing an even more pedestrian focused design in detriment of traffic. That would lead to less traffic-related air pollution and increased physical activity in the population, that consequently would provide beneficial health effects. For example, lack of physical activity during pregnancy has been associated with increased risk of wheeze, asthma, and allergic rhinitis in the offspring (70).

Strengths and limitations

The strengths of this study were its population-based design with a large sample size, its assessment with multiple and complementary approaches to assess the complexity of a wide range of urban environment exposures on childhood respiratory health, considering also multiple outcomes across childhood. This wide exposure approach might help avoid publication bias and provided a better adjustment for confounding

exposures, compared to single pollutant approaches. To our knowledge, this is the first study to comprehensively assess a wide range of urban environment exposures on wheeze and asthma in addition to lung function outcomes in children, thus, careful interpretation of our results is needed. In addition, we assessed lung function with spirometry following the ATS/ERS guidelines and performed by trained research staff, which provides an objective and reliable measurement.

Some methodological limitations need to be addressed. First, results for $FEF_{25-75\%}$ need to be interpreted with caution as it is a parameter with high variability, especially in children. However, in sensitivity analyses, when we included only children with reproducible spirometries according to the ATS/ERS guidelines, results remained robust. Second, despite we covered a broad range of urban exposures, we did not have information on other exposures that might be relevant for respiratory health (e.g., volatile compounds, pollen). Third, in approaches examining a broad range of exposure, measurement error bias of exposures is unavoidable and is present in a different magnitude across exposures. Thus, the comparison of each exposure associations with respiratory health should be interpreted cautiously. Fourth, some associations with non-time varying exposures such as blue spaces could be at risk of reverse causation and some highly correlated could not reflect purely the exposure during pregnancy. To minimise that, and to ensure the observations referred to exposures during pregnancy, we further adjusted our analyses by postnatal exposures measured the year prior to the outcomes assessment, and most results remained robust, only associations that seem to go into the unexpected directions were diluted or disappeared

after adjusting for postnatal exposures. In addition, the protective effects of blue spaces on asthma were strengthened when adjusted for postnatal exposures, highlighting the potential benefit of such exposures during the pregnancy period. Fifth, non-response resulted in a selection toward a healthier and more affluent group. Last, the way how SES influences the associations of urban exposures with health is not clear. While in the US studies have shown an association of higher deprivation with more harmful environments, in the EU findings have been mixed (71). In our study population levels of exposure across SES quintiles was mixed. Although we thoughtfully adjusted our models for the relevant SES covariates and we further standardised the exposures by area-level SES in cluster analyses, the presence of residual confounding can't be excluded. Exploring the role of SES in associations of the urban environment with respiratory health merits further investigation.

CONCLUSION

In conclusion, this study shows that the urban environment during pregnancy is of relevance to the offspring's respiratory health through childhood. Air pollution, road traffic noise, and blue spaces were the main determinants of childhood respiratory health. Thus, improving the urban design in order to minimise harmful and maximise beneficial urban exposures, along with reducing social inequalities related, is key to ensure a healthy respiratory development in childhood, that in turn, will result in a reduction of respiratory morbidity later in life.

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TABLES AND FIGURES

Table 1. Summary of urban environment exposures

Exposure family	Exposure
Air pollution	NO_2, NO_x, PM_{10}, $PM_{2.5}$, $PM_{2.5absorbance}$, PM_{coarse}, $PM_{2.5}$ elemental components (Cu, Fe, K, Ni, S, Si, V, Zn)
Noise	Lden, Ln
Traffic	Inverse distance to the nearest road
Green spaces	NDVI (within 100m, 300m, 500m buffer), Size of nearest major green space (>5000 m²), availability of a major green space (>5000 m²) within a 300m buffer, distance to the nearest major green space (>5000 m²)
Blue spaces	Size of the nearest major blue space (>5000 m²), availability of a major blue space (>5000 m²) within a 300m buffer, distance to the nearest major blue space (>5000 m²)
Built environment	Population density, building density (within 100m, 300m, 500m buffer), street connectivity within 300m buffer, facilities density within 300m buffer, facilities richness within 300m buffer, Land Use Mix (Shannon's Evenness Index) within 300m buffer, Walkability within 300m buffer, public bus lines (within 100m, 300m, 500m buffer), public bus stops (within 100m, 300m, 500m buffer), unhealthy food environment within 300m buffer

*Estimated using geographic information system within PostgreSQL (copyright © 1996-2017 The PostgreSQL Global Development Group), PostGIS (Creative Commons Attribution-Share Alike 3.0 License http://postgis.net), Python (Python Software Foundation. Python Language Reference, version 2.7. Available at http://www.python.org) and R (R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/) platforms

Table 2. Maternal and child characteristics.

	Population for analysis (n=5,624)
Maternal characteristics	
Age (years)	31.00 (4.92)
Pre-pregnancy BMI (kg/m2)	23.47 (4.10)
Education, low	2550 (49%)
Smoking	
Never	3784 (77%)
Before pregnancy	429 (9%)
During pregnancy	727 (15%)
History of asthma or atopy, yes,	1804 (37%)
Area-level SES	
Quintile 1, least deprived	593 (11%)
Quintile 2	646 (12%)
Quintile 3	663 (12%)
Quintile 4	616 (11%)
Quintile 5, most deprived	3087 (55%)
Child characteristics	
Sex, female,	2822 (50%)
Gestational age at birth (weeks)	40.14 (1.86)
Preterm birth	260 (5%)
Birth weight (g)	3450.00 (690.00)
Low birth weight	239 (4%)
Season of birth	
Spring	1333 (24%)
Summer	1516 (27%)
Autumn	1541 (27%)
Winter	1234 (22%)
Ethnicity	
Caucasian	4355 (79%)
African/American	402 (7%)
South East Asian	195 (4%)
North East Asian	137 (2%)
Other/Mixed	406 (7%)
Age (years)	9.79 (0.35)
Height (cm)	141.52 (6.60)
FEV_1 (z-score)	0.16 (0.98)

FVC (z-score)	0.20 (0.94)
FEV_1/FVC (z-score)	-0.10 (0.96)
$FEF_{25-75\%}$ (z-score)	0.45 (1.09)
Wheezing patterns	
Never	2422 (58%)
Early	1109 (26%)
Late	213 (5%)
Persistent	453 (11%)
Current asthma	268 (6%)

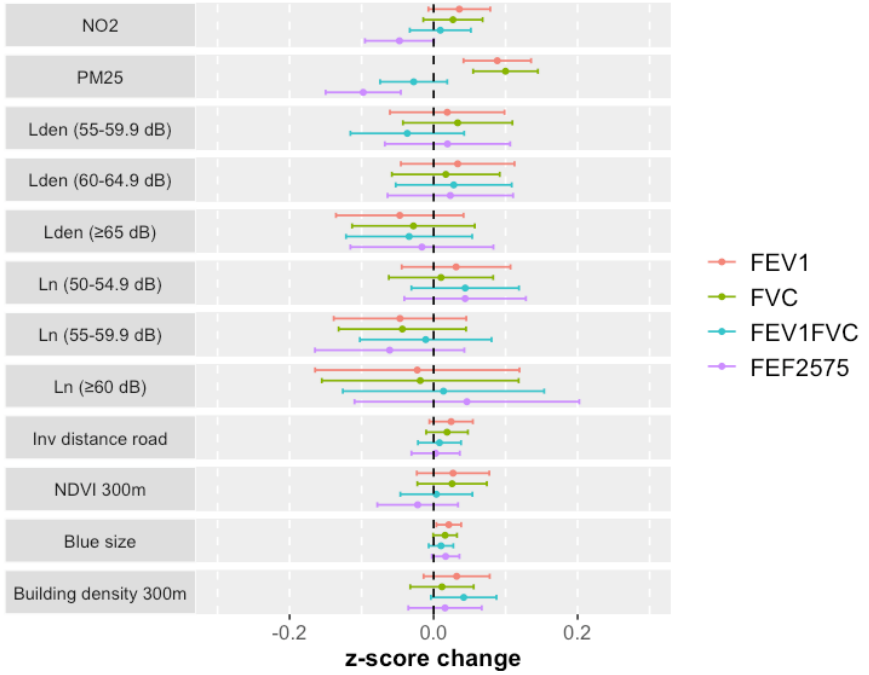
*Values are mean (SD) for continuous variables and N (%) for categorical variables.
Abbreviations: BMI: body mass index; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1s; $FEF_{25-75\%}$: mid expiratory flow; SD: standard deviation.

Table 3. Associations of maternal exposure to the full urban environment in clusters and lung function, wheezing patterns, and asthma in school-age children[a].

	Cluster 2 (n=2907)		Cluster 3 (n=1184)	
	Estimate (95%CI)	p-value	Estimate (95%CI)	p-value
Lung function (z-score change)				
FEV_1	-0.03 (-0.10, 0.03)	0.294	-0.04 (-0.12, 0.04)	0.278
FVC	-0.02 (-0.08, 0.04)	0.470	-0.03 (-0.10, 0.05)	0.519
FEV_1/FVC	-0.01 (-0.07, 0.05)	0.791	-0.03 (-0.11, 0.04)	0.393
$FEF_{25-75\%}$	-0.03 (-0.10, 0.04)	0.425	-0.08 (-0.17, 0.01)	0.069
Wheezing patterns and asthma (OR)				
Early wheezing	1.13 (0.95, 1.36)	0.164	1.23 (1.00, 1.51)	0.054
Late wheezing	1.22 (0.86, 1.76)	0.282	1.48 (0.99, 2.25)	0.059
Persistent wheezing	0.85 (0.67, 1.07)	0.166	0.75 (0.56, 1.01)	0.060
Asthma	1.14 (0.85, 1.54)	0.388	0.98 (0.67, 1.42)	0.907

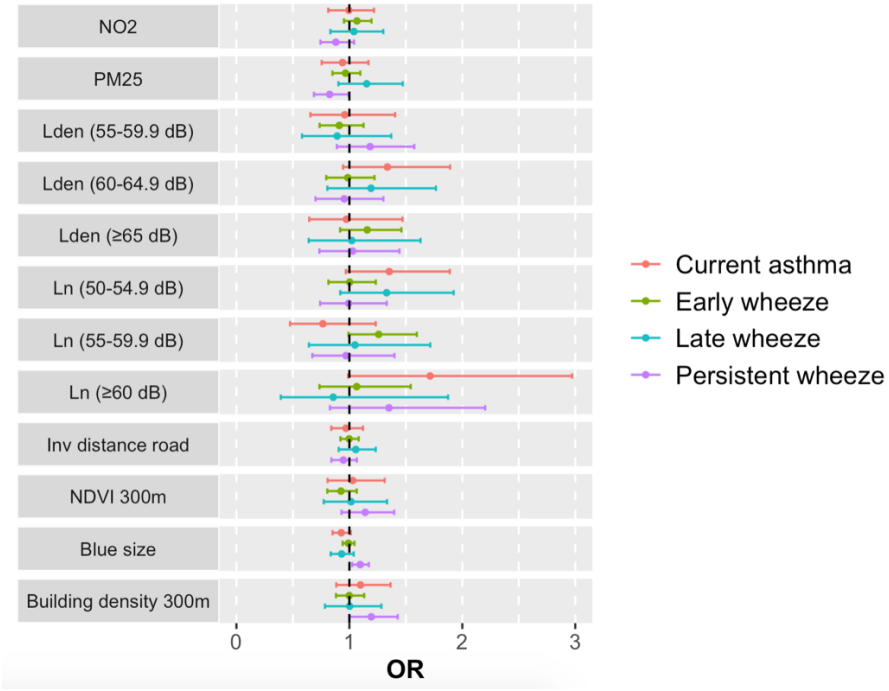
[a] Associations are compared to the reference Cluster 1 (n=1533).
Lung function models were adjusted for maternal education, pre-pregnancy BMI, age, smoking during pregnancy, history of asthma or atopy, and child's ethnicity and season of birth. Wheezing and asthma models were additionally adjusted for child's sex.
Abbreviations: FEV_1: forced expiratory volume in 1s; FVC: forced vital capacity; $FEF_{25-75\%}$: mid expiratory flow; OR: odds ratio; CI: confidence interval.

Figure 1. Associations of maternal exposure to the full urban environment and lung function in school-aged children.



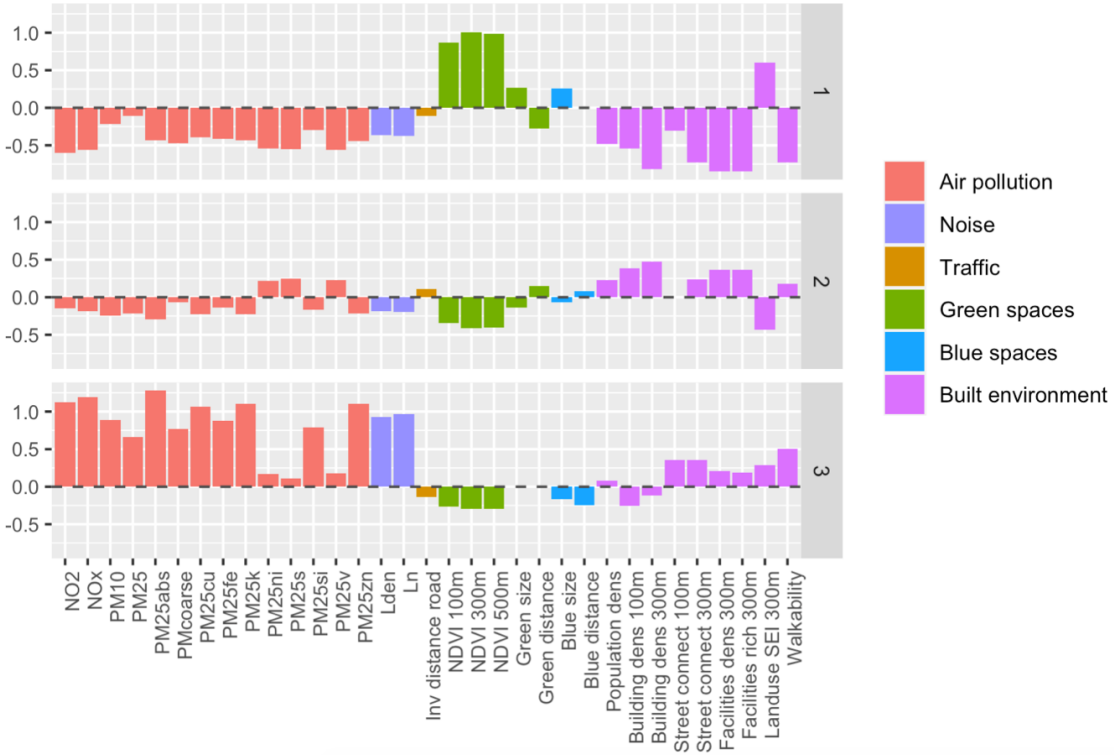
Air pollution models were adjusted for noise, traffic, green spaces, blue spaces and built environment. Noise models were adjusted for air pollution, traffic, green spaces, blue spaces and built environment. Traffic, green spaces, blue spaces and built environment models were adjusted for each other. All models were additionally adjusted for maternal education, pre-pregnancy BMI, age, smoking during pregnancy, history of asthma or atopy, area-level SES, and child's ethnicity and season of birth.

Figure 2. Associations of maternal exposure to the full urban environment and wheezing patterns and asthma in school-aged children.



Air pollution models were adjusted for noise, traffic, green spaces, blue spaces and built environment. Noise models were adjusted for air pollution, traffic, green spaces, blue spaces and built environment. Traffic, green spaces, blue spaces and built environment models were adjusted for each other. All models were additionally adjusted for maternal education, pre-pregnancy BMI, age, smoking during pregnancy, history of asthma or atopy, area-level SES, and child's sex, ethnicity, and season of birth.

Figure 3. Description of the clusters of urban exposures.



Bars represent the level of exposure of each urban exposure in the cluster compared to the mean level of exposure in the overall population (mean=0).

SUPPLEMENTARY MATERIAL

Figure S1. Flowchart of the study population.

Table S1. Urban environmental exposures description.

Figure S2. Correlation matrix of continuous urban exposures during pregnancy.

Figure S3. Directed Acyclic Graph (DAG) between urban environment during pregnancy and childhood respiratory health.

Figure S4. Directed Acyclic Graph (DAG) between urban environment specific exposures and childhood respiratory health.

Table S2. Maternal and child characteristics in the included and excluded populations.

Table S3. Maternal and child characteristics in the complete case and imputed populations.

Table S4. Single-exposure associations between *in utero* urban environment exposures and lung function (n=4968).

Table S5. Single-exposure associations of *in utero* urban environment exposures with wheezing patterns (n=4197) and asthma (n=4698).

Table S6. Multiple-exposure associations of *in utero* urban environment exposures with lung function (n=4968).

Table S7. Multiple-exposure associations of *in utero* urban environment exposures with wheezing patterns (n=4197) and asthma (n=4698).

Table S8. Maternal and child characteristics in the clusters of urban exposures.

Table S9. Single-exposure associations between *in utero* urban environment exposures and lung function including only children with reproducible spirometries (n=4734).

Table S10. Multiple-exposure associations of *in utero* urban environment exposures with lung function including only children with reproducible spirometries (n=4734).

Table S11. Associations of *in utero* urban exposure clusters with lung function including only children with reproducible spirometries.

Table S12. Single-exposure associations of *in utero* urban environment exposures with lung function adjusted for postnatal urban environment exposures (n=4968).

Table S13. Single-exposure associations of *in utero* urban environment exposures with asthma adjusted for postnatal urban environment exposures (n=4698).

Table S14. Associations of *in utero* urban exposure clusters with lung function, wheezing patterns, and asthma excluding preterm and low birth weight born children.

Figure S1. Flowchart of the study population.

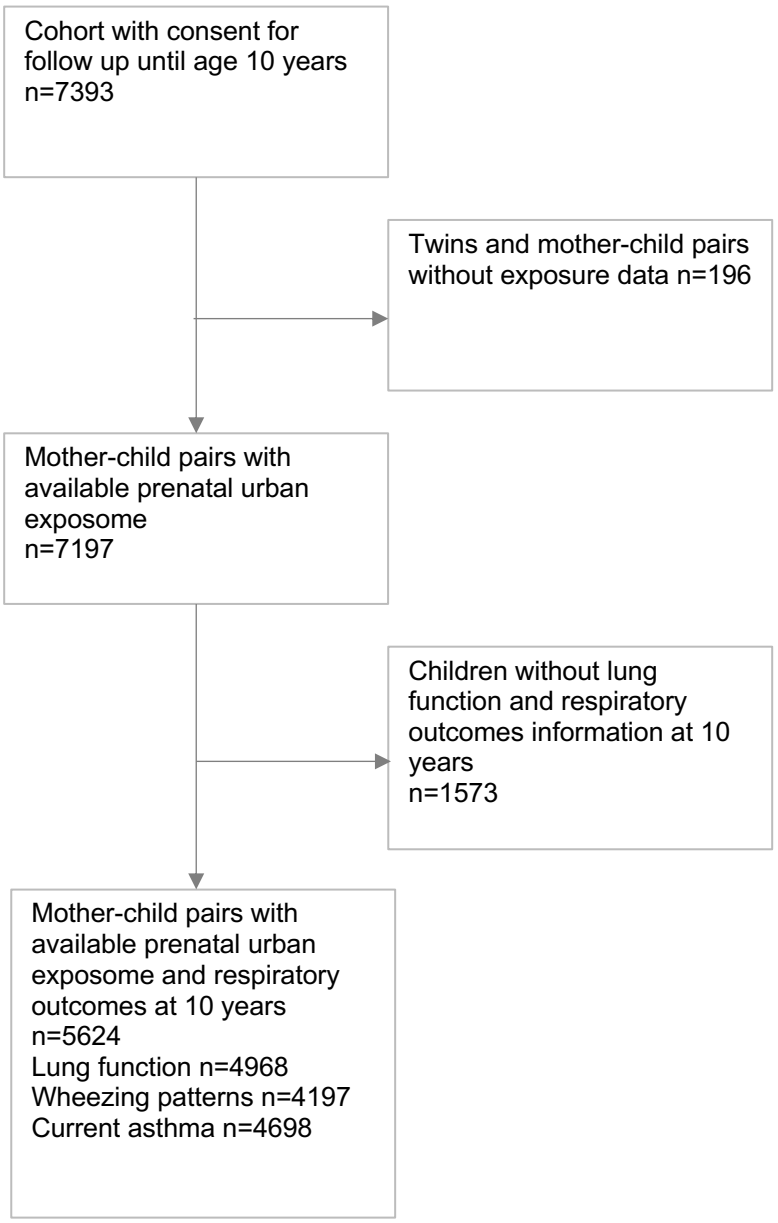


Table S1. Urban environmental exposures description.

Exposure	Unit	Median (IQR) or N (%)	Missing, N (%)	Transformation	Data source
Air pollution					
NO_2	$\mu g/m^3$	38.48 (7.48)	12 (0.21)		
NO_x	$\mu g/m^3$	57.23 (21.84)	12 (0.21)		
PM_{10}	$\mu g/m^3$	31.36 (6.14)	12 (0.21)		
$PM_{2.5}$	$\mu g/m^3$	19.72 (3.83)	12 (0.21)		
PM_{25abs}	10^{-5} m^{-1}	1.69 (0.39)	12 (0.21)		
PM_{coarse}	$\mu g/m^3$	11.53 (2.42)	12 (0.21)		
$PM_{2.5cu}$	ng/m^3	4.64 (0.53)	0 (0)	Natural log	Land Use Regression (LUR) models (European Study of Cohorts for Air Pollution Effects (ESCAPE))
$PM_{2.5fe}$	ng/m^3	119.67 (14.87)	0 (0)		
$PM_{2.5k}$	ng/m^3	110.57 (5.95)	0 (0)		
$PM_{2.5ni}$	ng/m^3	3.08 (0.72)	0 (0)		
$PM_{2.5s}$	ng/m^3	930.16 (19.44)	0 (0)		
$PM_{2.5si}$	ng/m^3	88.78 (2.75)	0 (0)		
$PM_{2.5v}$	ng/m^3	5.08 (1.65)	0 (0)		

$PM_{2.5zn}$	ng/m^3	18.87 (3.50)	0 (0)		
Noise					
Day evening and night noise, Lden	dB	54 (13)	220 (3.91)	Natural log	European road traffic noise maps (European Noise Directive)
Night noise, Ln	dB	45 (13)	220 (3.91)	Natural log	European road traffic noise maps (European Noise Directive)
Day evening and night noise, Lden	dB		220 (3.91)	None	European road traffic noise maps (European Noise Directive)
	<55	2843 (52.61)			
	55-59.9	890 (16.47)			
	60-64.9	890 (16.47)			
	≥65	781 (14.45)			
Night noise, Ln	dB		220 (3.91)	None	European road traffic noise maps (European Noise Directive)
	<50	3647 (67.49)			
	50-54.9	909 (16.82)			
	55-59.9	604 (11.18)			
	≥60	244 (4.52)			
Traffic					
Inverse distance to the nearest road	m^{-1}	0.09 (0.05)	16 (0.28)	Natural log	Nationale Databank Wegverkeersgegevens
Green spaces					

NDVI within 100 m buffer	0 to 1	0.37 (0.14)	16 (0.28)	None	LANDSAT 4-5 Thematic Mapper, LANDSAT 7 Enhanced Thematic Mapper Plus, and LANDSAT 8 Operational Land Imager/Thermal infrared Sensor (OLI/TIRS) with 30 m x 30 m resolution
NDVI within 300 m buffer	0 to 1	0.39 (0.14)	16 (0.28)	None	LANDSAT 4-5 Thematic Mapper, LANDSAT 7 Enhanced Thematic Mapper Plus, and LANDSAT 8 Operational Land Imager/Thermal infrared Sensor (OLI/TIRS) with 30 mx30 m resolution
NDVI within 500 m buffer	0 to 1	0.39 (0.15)	16 (0.28)	None	LANDSAT 4-5 Thematic Mapper, LANDSAT 7 Enhanced Thematic Mapper Plus, and LANDSAT 8 Operational Land Imager/Thermal infrared Sensor (OLI/TIRS) with 30 m x 30 m resolution
Size of the nearest major green space (>5000 m²)	m²	$1.38 \cdot 10^{10}$ ($1.51 \cdot 10^{10}$)	21 (0.37)	Natural log	Urban Atlas (European Environmental Protection Agency)
Major green space (>5000 m²) within a 300 m buffer			21 (0.37)	None	Urban Atlas (European Environmental Protection Agency)
	Yes	4480 (79.96)			
	No	1123 (20.04)			
Distance to the nearest major green space (>5000 m²)	m	$1.45 \cdot 10^{8}$ ($1.94 \cdot 10^{8}$)	21 (0.37)	Square root	Urban Atlas (European Environmental Protection Agency)
Blue spaces					

Size of the nearest major blue space (>5000 m²)	m²	$2.31 \cdot 10^{10}$ ($5.08 \cdot 10^{10}$)	21 (0.37)	Natural log	Urban Atlas (European Environmental Protection Agency)
Major blue space (>5000 m²) within a 300 m buffer			21 (0.37)	None	Urban Atlas (European Environmental Protection Agency)
	Yes	3035 (54.17)			
	No	2568 (45.83)			
Distance to the nearest major blue space (>5000 m²)	m	$2.43 \cdot 10^{8}$ ($3.51 \cdot 10^{8}$)	21 (0.37)	Square root	Urban Atlas (European Environmental Protection Agency)
Built environment					
Population density	number of inhabitants/km²	4136.41 (213.23)	17 (0.3)	None	Global Human Settlement layer
Building density within 100 m buffer	area occupied by buildings (m²)/km²	529787.00 (178552.00)	16 (0.28)	Squared	European Settlement Map 2017
Building density within 300 m buffer	area occupied by buildings (m²)/km²	463273.50 (154424.50)	16 (0.28)	Squared	European Settlement Map 2017
Street connectivity within 100 m buffer	number of intersections/km²	224.26 (160.18)	81 (1.44)	Square root	NAVTEQ
Street connectivity within 300 m buffer	number of intersections/km²	217.14 (121.03)	18 (0.32)	Square root	NAVTEQ

Facilities density within 300 m buffer	number of facilities/km²*	45.98 (113.18)	16 (0.28)	Cube root	NAVTEQ
Facilities richness within 300 m buffer	number of different facility types present/maximum potential number of facility types	0.12 (0.14)	16 (0.28)	None	NAVTEQ
Land Use Mix (Shannon's Evenness Index) within 300 m buffer**	0 to 1	0.47 (0.13)	21 (0.37)	None	Urban Atlas (European Environmental Protection Agency)
Walkability within 300 m buffer***	0 to 1	0.35 (0.08)	23 (0.41)	Log2	Global Human Settlement layer, NAVTEQ, Urban Atlas (European Environmental Protection Agency)
Public bus lines within 100 m buffer	Number of bus lines/km²		19 (0.34)	Categorisation	Open Street Maps
	None	4101 (73.22)			
	≥1	1501 (26.78)			
Public bus lines within 300 m buffer	Number of bus lines/km²		19 (0.34)	Categorisation	Open Street Maps
	None	1932 (34.47)			
	≥1	3673 (65.53)			

Public bus lines within 500 m buffer	Number of bus lines/km²		19 (0.34)	Categorisation	Open Street Maps
	None	993 (17.72)			
	≥1	4612 (82.28)			
Public bus stops within 100 m buffer	Number of bus stops/km²		19 (0.34)	Categorisation	Open Street Maps
	None	4871 (86.90)			
	≥1	734 (13.10)			
Public bus stops within 300 m buffer	Number of bus stops/km²		19 (0.34)	Categorisation	Open Street Maps
	None	1646 (29.37)			
	≥1	3959 (70.63)			
Public bus stops within 500 m buffer	Number of bus stops/km²		19 (0.34)	Categorisation	Open Street Maps
	None	351 (6.26)			
	≥1	5254 (93.74)			
Unhealthy food environment within 300 m buffer	Number of unhealthy food facilities/km²		16 (0.28)	Categorisation	NAVTEQ
	None	1292 (23.04)			
	1 to 10	974 (17.37)			
	≥10	3342 (59.59)			

* Facilities considering all points of daily life activities (e.g., schools, medical centres, shops, restaurants, libraries).

** Indicates the diversity and evenness of land uses. Index ranges from 0 to 1, with higher values indicating more even distribution of different types of land use.
*** Quantifies how walkable is a buffer of 300 m considering four components of the built environment: Land Use Mix (Shannon's Evenness Index), facilities richness, population density, and connectivity index. Walkability was calculated for the LifeCycle project based on previously used methods (1).

Figure S2. Correlation matrix of continuous urban exposures during pregnancy.

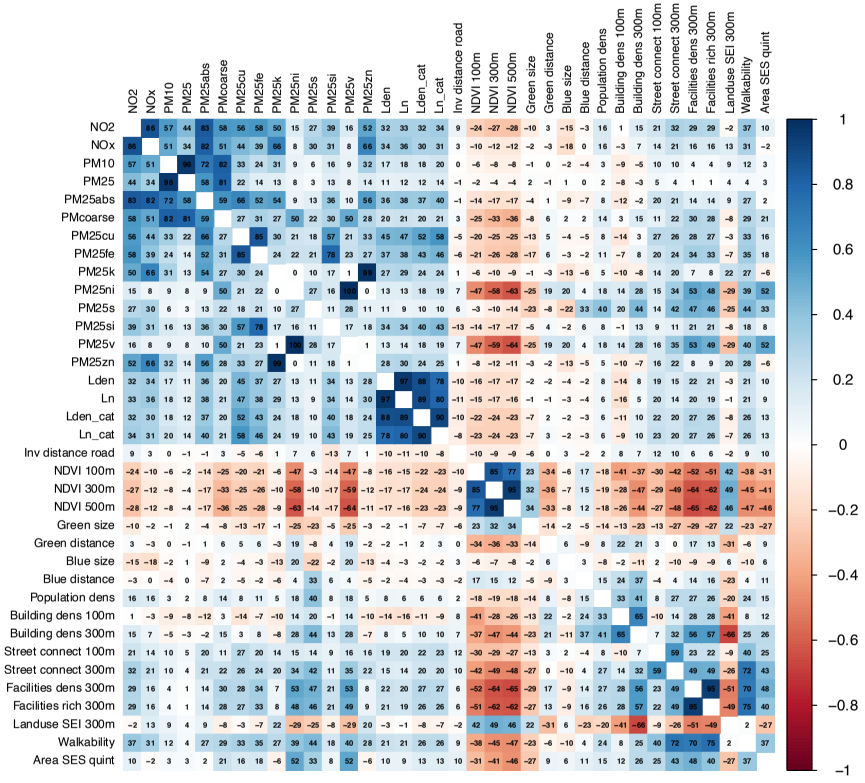
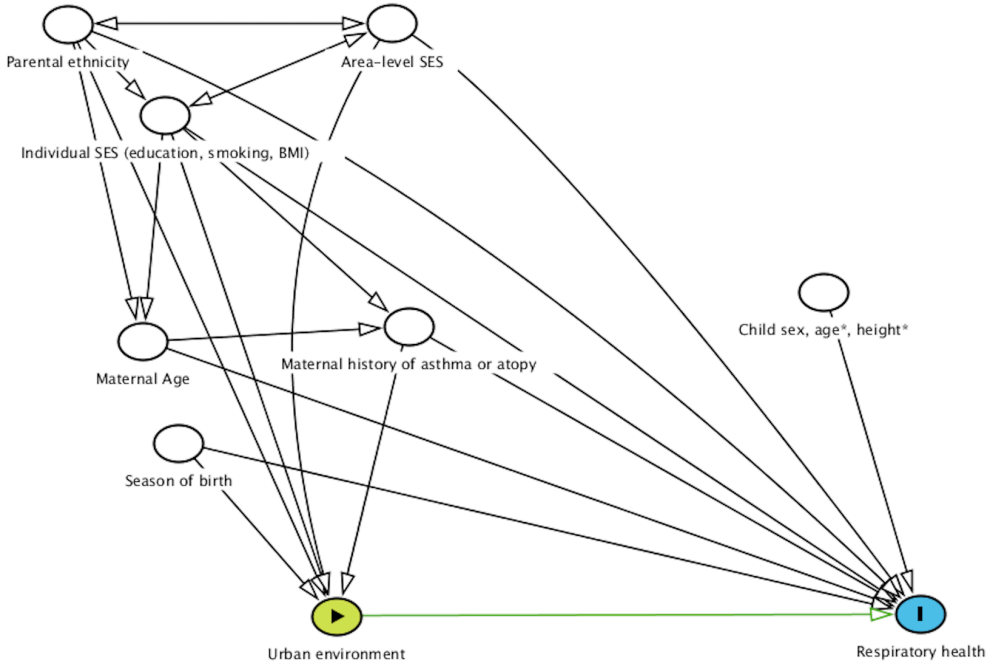


Figure S3. Directed Acyclic Graph (DAG) between urban environment during pregnancy and childhood respiratory health.



*Child's age and height were only accounted for the construction of z-scores of lung function.

Figure S4. Directed Acyclic Graph (DAG) between urban environment specific exposures and childhood respiratory health.

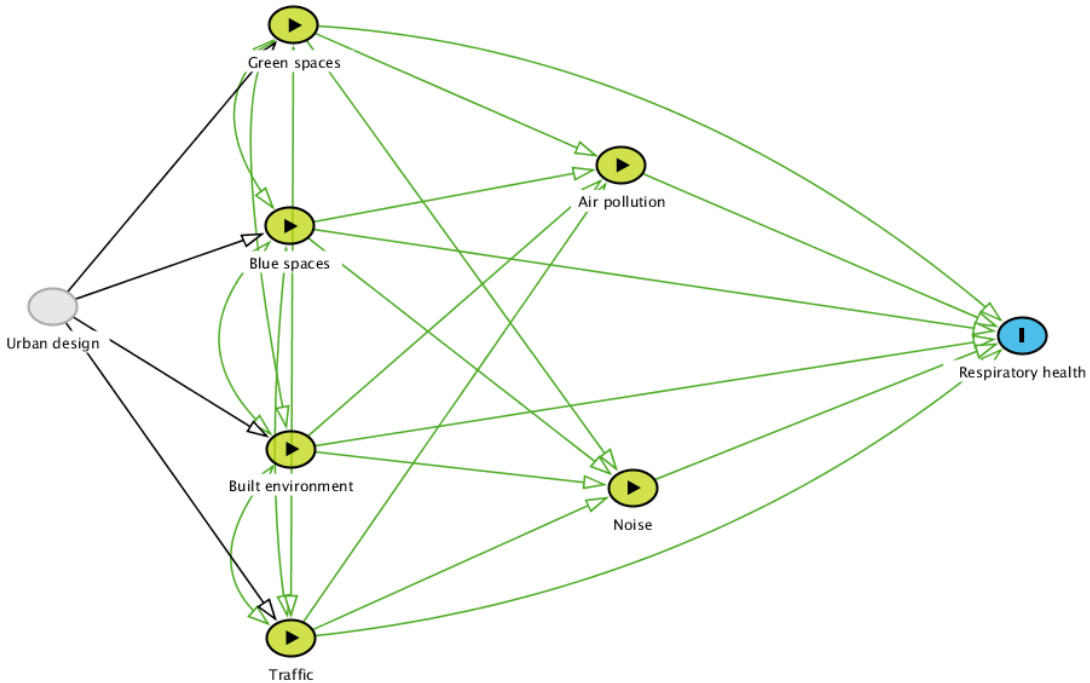


Table S2. Maternal and child characteristics in the included and excluded populations.

	Included (n=5624)	Excluded (n=1766)	p-value
Maternal characteristics			
Age (years)	31.00 (4.92)	29.29 (5.43)	<0.001
Pre-pregnancy BMI (kg/m2)	23.47 (4.10)	23.88 (4.51)	<0.001
Education, low	2550 (49%)	974 (64%)	<0.001
Smoking			
Never	3784 (77%)	450 (75%)	0.635
Before pregnancy	429 (9%)	57 (9%)	
During pregnancy	727 (15%)	94 (16%)	
History of asthma or atopy, yes	1804 (37%)	221 (38%)	0.655
Area-level SES (quintiles)			
Quintile 1, least deprived	593 (11%)	119 (7%)	<0.001
Quintile 2	646 (12%)	147 (8%)	
Quintile 3	663 (12%)	199 (11%)	
Quintile 4	616 (11%)	168 (10%)	
Quintile 5, most deprived	3087 (55%)	1106 (64%)	
Child characteristics			
Sex, female	2822 (50%)	356 (49%)	0.711
Gestational age at birth (weeks)	40.14 (1.86)	39.86 (2.29)	<0.001

Preterm birth	260 (5%)	179 (10%)	<0.001
Birth weight (g)	3450.00 (690.00)	3360.00 (780.00)	<0.001
Low birth weight	239 (4%)	170 (10%)	<0.001
Season of birth			
Spring	1333 (24%)	405 (24%)	0.896
Summer	1516 (27%)	441 (26%)	
Autumn	1541 (27%)	468 (28%)	
Winter	1234 (22%)	359 (21%)	
Ethnicity			
Caucasian	4355 (79%)	1241 (76%)	0.010
African/American	402 (7%)	149 (9%)	
SE Asian	195 (4%)	54 (3%)	
NE Asian	137 (2%)	44 (3%)	
Other/Mixed	406 (7%)	152 (9%)	

* Differences were calculated using Student's t test for continuous normally distributed variables, Mann-Whitney U test for continuous non-normally distributed variables, and chi-square test for categorical variables.

Table S3. Maternal and child characteristics in the complete case and imputed populations.

	Missing	Included (n=5624)	Excluded (n=1766)	p-value
Maternal characteristics				
Age (years)	0 (0%)	31.00 (4.92)	29.29 (5.43)	<0.001
Pre-pregnancy BMI (kg/m2)	1359 (24.2%)	23.47 (4.10)	23.88 (4.51)	<0.001
Education, low	420 (7.5%)	2550 (49%)	974 (64%)	<0.001
Smoking	684 (12.2%)			
Never		3784 (77%)	450 (75%)	0.635
Before pregnancy		429 (9%)	57 (9%)	
During pregnancy		727 (15%)	94 (16%)	
History of asthma or atopy, yes	760 (13.5%)	1804 (37%)	221 (38%)	0.655
Area-level SES (quintiles)	19 (0.3%)			
Quintile 1, least deprived		593 (11%)	119 (7%)	<0.001
Quintile 2		646 (12%)	147 (8%)	
Quintile 3		663 (12%)	199 (11%)	
Quintile 4		616 (11%)	168 (10%)	
Quintile 5, most deprived		3087 (55%)	1106 (64%)	
Child characteristics				
Sex, female	0 (0%)	2822 (50%)	356 (49%)	0.711

Gestational age at birth (weeks)	33 (0.6%)	40.14 (1.86)	39.86 (2.29)	<0.001
Preterm birth	7 (0.1%)	260 (5%)	179 (10%)	<0.001
Birth weight (g)	0 (0%)	3450.00 (690.00)	3360.00 (780.00)	<0.001
Low birth weight	0 (0%)	239 (4%)	170 (10%)	<0.001
Season of birth	33 (0.6%)			
Spring		1333 (24%)	405 (24%)	0.896
Summer		1516 (27%)	441 (26%)	
Autumn		1541 (27%)	468 (28%)	
Winter		1234 (22%)	359 (21%)	
Ethnicity	129 (2.3%)			
Caucasian		4355 (79%)	1241 (76%)	0.010
African/American		402 (7%)	149 (9%)	
SE Asian		195 (4%)	54 (3%)	
NE Asian		137 (2%)	44 (3%)	
Other/Mixed		406 (7%)	152 (9%)	

* Differences were calculated using Student's t test for continuous normally distributed variables, Mann-Whitney U test for continuous non-normally distributed variables, and chi-square test for categorical variables.

Table S4. Single-exposure associations between *in utero* urban environment exposures and lung function (n=4968).

	FEV_1 z-score change (95%CI)	p-value	**FVC** z-score change (95%CI)	p-value	**FEV_1/FVC** z-score change (95%CI)	p-value	**$FEF_{25-75\%}$** z-score change (95%CI)	p-value
Air pollution								
NO_2	0.02 (-0.02, 0.06)	0.367	0.01 (-0.02, 0.05)	0.519	0.00 (-0.03, 0.04)	0.856	-0.05 (-0.09, 0.00)	0.038
NO_x	0.01 (-0.03, 0.05)	0.644	0.00 (-0.03, 0.04)	0.812	0.00 (-0.04, 0.04)	0.906	-0.02 (-0.06, 0.02)	0.407
PM_{10}	0.06 (0.02, 0.11)	0.004	0.08 (0.04, 0.12)	<0.001	-0.03 (-0.08, 0.01)	0.120	-0.10 (-0.15, -0.05)	<0.001
$PM_{2.5}$	0.08 (0.03, 0.13)	0.001	0.09 (0.05, 0.14)	<0.001	-0.03 (-0.08, 0.01)	0.164	-0.10 (-0.15, -0.05)	<0.001
PM_{25abs}	0.02 (-0.01, 0.06)	0.224	0.03 (-0.01, 0.06)	0.115	-0.02 (-0.05, 0.02)	0.354	-0.04 (-0.08, 0.00)	0.036
PM_{coarse}	0.05 (0.01, 0.09)	0.009	0.05 (0.02, 0.09)	0.005	-0.01 (-0.05, 0.03)	0.733	-0.04 (-0.09, 0.00)	0.051
$PM_{2.5cu}$	0.00 (-0.02, 0.02)	0.735	0.00 (-0.02, 0.02)	0.643	0.00 (-0.02, 0.02)	0.987	-0.01 (-0.04, 0.01)	0.310
$PM_{2.5fe}$	0.00 (-0.03, 0.03)	0.998	-0.01 (-0.03, 0.02)	0.478	0.01 (-0.01, 0.04)	0.260	-0.01 (-0.03, 0.02)	0.703
$PM_{2.5k}$	-0.02 (-0.04, 0.01)	0.176	-0.01 (-0.03, 0.01)	0.420	-0.02 (-0.04, 0.01)	0.219	-0.02 (-0.05, 0.01)	0.133
$PM_{2.5ni}$	-0.03 (-0.09, 0.02)	0.240	-0.03 (-0.09, 0.02)	0.231	0.01 (-0.05, 0.06)	0.848	0.05 (-0.01, 0.12)	0.100
$PM_{2.5s}$	0.02 (-0.01, 0.05)	0.140	0.02 (-0.01, 0.05)	0.212	0.01 (-0.02, 0.04)	0.557	-0.01 (-0.05, 0.02)	0.425
$PM_{2.5si}$	0.00 (-0.01, 0.01)	0.851	0.00 (-0.01, 0.00)	0.373	0.01 (0.00, 0.01)	0.061	0.00 (0.00, 0.01)	0.317
$PM_{2.5v}$	-0.03 (-0.09, 0.02)	0.246	-0.03 (-0.09, 0.02)	0.235	0.01 (-0.05, 0.06)	0.832	0.05 (-0.01, 0.12)	0.102
$PM_{2.5zn}$	-0.02 (-0.05, 0.01)	0.205	-0.01 (-0.04, 0.02)	0.522	-0.02 (-0.05, 0.01)	0.185	-0.03 (-0.06, 0.01)	0.117

Noise								
Lden	0.01 (-0.04, 0.06)	0.680	0.00 (-0.05, 0.04)	0.861	0.02 (-0.02, 0.06)	0.389	0.00 (-0.05, 0.05)	0.986
Day evening and night noise, Lden (<55 Ref), dB								
55-59.9	0.02 (-0.06, 0.10)	0.624	0.03 (-0.04, 0.11)	0.367	-0.04 (-0.12, 0.04)	0.336	0.01 (-0.08, 0.09)	0.864
60-64.9	0.03 (-0.05, 0.11)	0.438	0.01 (-0.06, 0.09)	0.711	0.03 (-0.05, 0.11)	0.497	0.02 (-0.07, 0.10)	0.731
≥65	-0.03 (-0.12, 0.05)	0.409	-0.02 (-0.10, 0.06)	0.588	-0.03 (-0.11, 0.05)	0.524	-0.04 (-0.13, 0.05)	0.391
Night noise, Ln	0.00 (-0.04, 0.05)	0.885	-0.01 (-0.05, 0.04)	0.741	0.02 (-0.03, 0.06)	0.513	0.00 (-0.05, 0.06)	0.898
Night noise, Ln (<50 Ref), dB								
50-54.9	0.03 (-0.05, 0.10)	0.447	0.01 (-0.06, 0.08)	0.829	0.04 (-0.03, 0.12)	0.258	0.04 (-0.05, 0.12)	0.389
55-59.9	-0.04 (-0.13, 0.05)	0.352	-0.04 (-0.13, 0.04)	0.350	-0.01 (-0.10, 0.08)	0.810	-0.07 (-0.17, 0.03)	0.148
≥60	-0.01 (-0.14, 0.13)	0.933	-0.01 (-0.14, 0.12)	0.911	0.02 (-0.12, 0.15)	0.811	0.00 (-0.15, 0.14)	0.973
Traffic								
Inverse distance to nearest road	0.03 (0.00, 0.06)	0.101	0.02 (-0.01, 0.05)	0.205	0.01 (-0.02, 0.04)	0.516	0.00 (-0.03, 0.04)	0.791
Green spaces								
NDVI within 100 m buffer	0.02 (-0.02, 0.06)	0.290	0.02 (-0.02, 0.06)	0.224	-0.01 (-0.05, 0.03)	0.714	0.01 (-0.04, 0.05)	0.721
NDVI within 300 m buffer	0.01 (-0.04, 0.06)	0.672	0.02 (-0.03, 0.06)	0.423	-0.02 (-0.06, 0.03)	0.515	-0.03 (-0.08, 0.02)	0.249
NDVI within 500 m buffer	0.00 (-0.05, 0.05)	0.990	0.01 (-0.04, 0.06)	0.759	-0.01 (-0.07, 0.04)	0.590	-0.02 (-0.08, 0.04)	0.492

Size of nearest major green space (>5000 m²)	0.00 (-0.02, 0.03)	0.758	0.00 (-0.02, 0.02)	0.943	0.01 (-0.01, 0.03)	0.458	0.00 (-0.03, 0.02)	0.800
Major green space (>5000 m²) in a 300 m buffer, yes	0.00 (-0.07, 0.06)	0.898	-0.02 (-0.08, 0.05)	0.566	0.02 (-0.05, 0.09)	0.588	0.02 (-0.06, 0.09)	0.622
Distance nearest major green space (>5000 m²)	-0.02 (-0.05, 0.02)	0.413	0.00 (-0.04, 0.03)	0.934	-0.02 (-0.06, 0.02)	0.294	0.00 (-0.04, 0.05)	0.863
Blue spaces								
Size of nearest major blue space (>5000 m²)	0.02 (0.00, 0.04)	0.028	0.02 (0.00, 0.03)	0.069	0.01 (-0.01, 0.02)	0.372	0.02 (0.00, 0.03)	0.097
Major green space (>5000 m²) in a 300 m buffer, yes	-0.01 (-0.07, 0.04)	0.695	0.01 (-0.04, 0.06)	0.687	-0.05 (-0.10, 0.01)	0.091	-0.02 (-0.08, 0.04)	0.531
Distance nearest major blue space (>5000 m²)	0.00 (-0.04, 0.04)	0.916	0.00 (-0.04, 0.04)	0.910	0.01 (-0.03, 0.05)	0.690	0.01 (-0.03, 0.06)	0.597
Built environment								
Population density	0.01 (0.00, 0.01)	0.207	0.00 (-0.01, 0.01)	0.915	0.01 (0.00, 0.02)	0.021	0.00 (-0.01, 0.01)	0.789
Building density within 100 m buffer	-0.02 (-0.06, 0.02)	0.381	-0.02 (-0.06, 0.02)	0.416	0.01 (-0.03, 0.05)	0.724	0.00 (-0.05, 0.04)	0.863
Building density within 300 m buffer	0.02 (-0.03, 0.06)	0.440	0.00 (-0.04, 0.04)	0.923	0.04 (0.00, 0.08)	0.078	0.02 (-0.03, 0.06)	0.464
Street connectivity within 300 m buffer	-0.01 (-0.05, 0.03)	0.561	-0.01 (-0.04, 0.03)	0.677	-0.01 (-0.05, 0.02)	0.538	0.01 (-0.03, 0.05)	0.629

Street connectivity within 100 m buffer	-0.05 (-0.09, 0.00)	0.037	-0.03 (-0.07, 0.01)	0.192	-0.03 (-0.08, 0.01)	0.125	0.00 (-0.05, 0.05)	0.982
Facilities density within 300 m buffer	0.01 (-0.03, 0.05)	0.610	0.00 (-0.04, 0.04)	0.999	0.02 (-0.02, 0.06)	0.273	-0.02 (-0.06, 0.03)	0.430
Facilities richness within 300 m buffer	0.01 (-0.04, 0.05)	0.751	0.00 (-0.05, 0.04)	0.878	0.03 (-0.02, 0.07)	0.261	-0.02 (-0.07, 0.03)	0.393
Land Use Mix (Shannon's Evenness Index) within 300 m buffer	0.02 (-0.02, 0.06)	0.411	0.03 (0.00, 0.07)	0.084	-0.04 (-0.08, 0.00)	0.038	0.02 (-0.03, 0.06)	0.480
Walkability	-0.01 (-0.04, 0.03)	0.721	0.00 (-0.04, 0.03)	0.974	-0.01 (-0.05, 0.02)	0.435	0.00 (-0.05, 0.04)	0.822
Public bus lines within 100 m buffer, ≥1	0.00 (-0.06, 0.07)	0.887	0.00 (-0.06, 0.06)	0.962	0.00 (-0.06, 0.06)	0.908	-0.02 (-0.09, 0.04)	0.491
Public bus lines within 300 m buffer, ≥1	-0.01 (-0.07, 0.04)	0.652	0.01 (-0.05, 0.06)	0.808	-0.03 (-0.09, 0.02)	0.228	-0.01 (-0.07, 0.06)	0.810
Public bus lines within 500 m buffer, ≥1	-0.02 (-0.09, 0.05)	0.525	-0.01 (-0.08, 0.06)	0.793	-0.02 (-0.09, 0.05)	0.536	-0.02 (-0.10, 0.06)	0.646
Public bus stops within 100 m buffer, ≥1	-0.01 (-0.09, 0.07)	0.896	0.01 (-0.07, 0.09)	0.772	-0.04 (-0.12, 0.04)	0.314	0.02 (-0.07, 0.11)	0.611
Public bus stops within 300 m buffer, ≥1	-0.06 (-0.12, 0.00)	0.041	-0.05 (-0.10, 0.01)	0.101	-0.02 (-0.08, 0.04)	0.529	-0.01 (-0.08, 0.05)	0.712

Public bus stops within 500 m buffer, ≥1	-0.06 (-0.17, 0.05)	0.293	-0.06 (-0.16, 0.05)	0.320	0.00 (-0.11, 0.12)	0.941	0.04 (-0.08, 0.17)	0.490
Unhealthy food environment within 300 m buffer								
1 to 10	0.01 (-0.09, 0.10)	0.906	0.03 (-0.06, 0.12)	0.513	-0.06 (-0.15, 0.03)	0.213	-0.02 (-0.13, 0.08)	0.690
≥10	0.02 (-0.07, 0.10)	0.645	0.01 (-0.07, 0.09)	0.866	0.02 (-0.06, 0.10)	0.649	0.00 (-0.10, 0.09)	0.958

*Models adjusted for maternal education, pre-pregnancy BMI, age, smoking during pregnancy, history of asthma or atopy, area-level SES, and child's ethnicity and season of birth.

Table S5. Single-exposure associations of *in utero* urban environment exposures with wheezing patterns (n=4197) and asthma (n=4698).

	Early wheeze		**Late wheeze**		**Persistent wheeze**		**Current asthma**	
	OR (95%CI)	p-value	OR (95%CI)	p-value	OR (95%CI)	p-value	OR (95%CI)	p-value
Air pollution								
NO_2	1.10 (0.99, 1.22)	0.069	1.07 (0.88, 1.30)	0.499	0.84 (0.72, 0.98)	0.026	1.03 (0.86, 1.23)	0.787
NO_x	1.11 (1.00, 1.22)	0.048	1.06 (0.88, 1.29)	0.530	0.90 (0.78, 1.04)	0.150	1.10 (0.92, 1.31)	0.281
PM_{10}	1.01 (0.90, 1.14)	0.842	1.13 (0.90, 1.41)	0.283	0.84 (0.71, 0.99)	0.037	0.96 (0.78, 1.17)	0.681
$PM_{2.5}$	0.99 (0.87, 1.12)	0.882	1.14 (0.90, 1.45)	0.269	0.81 (0.67, 0.97)	0.023	0.93 (0.75, 1.16)	0.527
PM_{25abs}	1.07 (0.98, 1.18)	0.136	1.09 (0.90, 1.30)	0.377	0.86 (0.75, 0.99)	0.033	1.05 (0.89, 1.24)	0.549
PM_{coarse}	1.03 (0.93, 1.14)	0.594	1.16 (0.95, 1.42)	0.151	0.80 (0.69, 0.93)	0.004	0.97 (0.80, 1.16)	0.718
$PM_{2.5cu}$	1.03 (0.98, 1.09)	0.275	0.98 (0.88, 1.10)	0.721	0.96 (0.89, 1.05)	0.380	1.04 (0.94, 1.14)	0.444
$PM_{2.5fe}$	1.02 (0.96, 1.09)	0.491	0.96 (0.84, 1.11)	0.590	0.93 (0.84, 1.03)	0.181	1.03 (0.91, 1.16)	0.687
$PM_{2.5k}$	1.03 (0.96, 1.09)	0.424	1.06 (0.94, 1.18)	0.365	0.95 (0.87, 1.05)	0.313	1.07 (0.97, 1.19)	0.182
$PM_{2.5ni}$	1.04 (0.88, 1.22)	0.656	1.09 (0.80, 1.47)	0.585	0.98 (0.79, 1.21)	0.821	1.09 (0.84, 1.41)	0.530
$PM_{2.5s}$	1.07 (0.98, 1.17)	0.120	1.08 (0.90, 1.29)	0.424	0.96 (0.85, 1.09)	0.562	1.11 (0.96, 1.29)	0.161
$PM_{2.5si}$	1.00 (0.98, 1.02)	0.993	0.98 (0.94, 1.03)	0.462	0.98 (0.95, 1.01)	0.198	1.02 (0.98, 1.05)	0.342
$PM_{2.5v}$	1.05 (0.89, 1.23)	0.579	1.10 (0.81, 1.49)	0.537	0.97 (0.78, 1.20)	0.775	1.09 (0.84, 1.42)	0.517
$PM_{2.5zn}$	1.04 (0.96, 1.11)	0.335	1.07 (0.93, 1.22)	0.339	0.94 (0.85, 1.05)	0.254	1.08 (0.95, 1.22)	0.225

Noise								
Lden	1.07 (0.95, 1.21)	0.254	1.08 (0.85, 1.37)	0.534	0.93 (0.78, 1.10)	0.390	1.11 (0.90, 1.36)	0.350
Day evening and night noise, Lden (<55 Ref), dB								
55-59.9	0.92 (0.75, 1.14)	0.437	0.91 (0.60, 1.40)	0.675	1.14 (0.86, 1.51)	0.377	0.97 (0.67, 1.42)	0.884
60-64.9	1.00 (0.81, 1.24)	0.975	1.20 (0.81, 1.76)	0.365	0.91 (0.67, 1.23)	0.530	1.36 (0.96, 1.91)	0.082
≥65	1.22 (0.99, 1.51)	0.067	1.03 (0.67, 1.59)	0.891	0.94 (0.69, 1.29)	0.719	0.99 (0.68, 1.46)	0.972
Night noise, Ln	1.10 (0.97, 1.25)	0.145	1.08 (0.84, 1.38)	0.572	0.93 (0.77, 1.11)	0.401	1.09 (0.87, 1.36)	0.444
Night noise, Ln (<50 Ref), dB								
50-54.9	1.02 (0.83, 1.26)	0.822	1.34 (0.93, 1.92)	0.118	0.94 (0.71, 1.26)	0.693	1.36 (0.98, 1.90)	0.066
55-59.9	1.30 (1.04, 1.64)	0.024	1.05 (0.65, 1.68)	0.852	0.91 (0.64, 1.29)	0.588	0.76 (0.48, 1.21)	0.250
≥60	1.14 (0.81, 1.62)	0.443	0.90 (0.43, 1.88)	0.774	1.12 (0.71, 1.77)	0.622	1.69 (1.01, 2.83)	0.044
Traffic								
Inverse distance to nearest road	1.00 (0.92, 1.09)	0.973	1.06 (0.91, 1.23)	0.489	0.94 (0.84, 1.06)	0.347	0.97 (0.84, 1.12)	0.686
Green spaces								
NDVI within 100 m buffer	0.95 (0.84, 1.06)	0.358	0.96 (0.77, 1.20)	0.742	1.08 (0.92, 1.27)	0.367	1.01 (0.83, 1.23)	0.899
NDVI within 300 m buffer	0.93 (0.82, 1.06)	0.264	1.01 (0.79, 1.30)	0.928	1.05 (0.88, 1.26)	0.590	1.02 (0.82, 1.26)	0.886
NDVI within 500 m buffer	0.90 (0.78, 1.04)	0.151	0.99 (0.75, 1.30)	0.927	1.03 (0.84, 1.26)	0.773	1.05 (0.82, 1.34)	0.687

Size of nearest major green space (>5000 m²)	0.99 (0.93, 1.06)	0.824	1.11 (0.98, 1.26)	0.098	1.02 (0.93, 1.11)	0.676	1.08 (0.96, 1.21)	0.202
Major green space (>5000 m²) in a 300 m buffer, yes	0.92 (0.76, 1.10)	0.359	1.22 (0.83, 1.80)	0.307	0.86 (0.67, 1.11)	0.239	1.19 (0.86, 1.65)	0.297
Distance nearest major green space (>5000 m²)	1.04 (0.94, 1.16)	0.430	0.97 (0.80, 1.19)	0.771	1.10 (0.96, 1.27)	0.185	1.03 (0.87, 1.23)	0.712
Blue spaces								
Size of nearest major blue space (>5000 m²)	0.99 (0.94, 1.05)	0.781	0.93 (0.84, 1.04)	0.184	1.08 (1.01, 1.15)	0.021	0.92 (0.84, 1.00)	0.047
Major green space (>5000 m²) in a 300 m buffer, yes	0.97 (0.83, 1.12)	0.651	0.77 (0.58, 1.02)	0.070	1.04 (0.84, 1.28)	0.716	0.90 (0.70, 1.16)	0.425
Distance nearest major blue space (>5000 m²)	1.00 (0.90, 1.12)	0.958	1.15 (0.93, 1.42)	0.209	1.01 (0.86, 1.19)	0.890	1.07 (0.89, 1.29)	0.466
Built environment								
Population density	0.99 (0.97, 1.02)	0.463	1.00 (0.95, 1.05)	0.935	1.00 (0.96, 1.03)	0.897	1.00 (0.96, 1.05)	0.900
Building density within 100 m buffer	0.99 (0.89, 1.11)	0.853	1.00 (0.81, 1.24)	0.996	1.14 (0.98, 1.33)	0.096	1.05 (0.87, 1.28)	0.600
Building density within 300 m buffer	1.02 (0.91, 1.15)	0.683	1.02 (0.82, 1.28)	0.841	1.09 (0.93, 1.28)	0.292	1.11 (0.91, 1.35)	0.295
Street connectivity within 300 m buffer	0.97 (0.88, 1.07)	0.547	1.00 (0.83, 1.21)	0.973	0.93 (0.81, 1.07)	0.315	1.07 (0.90, 1.26)	0.453

Street connectivity within 100 m buffer	1.03 (0.91, 1.16)	0.622	1.09 (0.86, 1.38)	0.471	0.91 (0.77, 1.08)	0.294	1.00 (0.81, 1.22)	0.964
Facilities density within 300 m buffer	1.03 (0.92, 1.15)	0.659	1.12 (0.91, 1.38)	0.275	0.97 (0.83, 1.13)	0.681	1.12 (0.94, 1.35)	0.202
Facilities richness within 300 m buffer	1.04 (0.91, 1.18)	0.578	1.13 (0.89, 1.44)	0.311	0.94 (0.78, 1.12)	0.484	1.16 (0.94, 1.43)	0.162
Land Use Mix (Shannon's Evenness Index) within 300 m buffer	1.00 (0.90, 1.11)	0.982	1.03 (0.84, 1.27)	0.742	1.02 (0.88, 1.19)	0.752	0.91 (0.76, 1.09)	0.283
Walkability	1.04 (0.94, 1.15)	0.400	1.16 (0.95, 1.41)	0.150	0.93 (0.81, 1.08)	0.351	1.05 (0.88, 1.25)	0.595
Public bus lines within 100 m buffer, ≥1	0.90 (0.76, 1.07)	0.222	0.80 (0.57, 1.13)	0.209	0.72 (0.57, 0.93)	0.011	1.01 (0.76, 1.34)	0.936
Public bus lines within 300 m buffer, ≥1	1.03 (0.89, 1.20)	0.689	1.09 (0.81, 1.47)	0.557	1.07 (0.86, 1.33)	0.532	1.03 (0.79, 1.35)	0.807
Public bus lines within 500 m buffer, ≥1	0.99 (0.82, 1.19)	0.910	1.12 (0.78, 1.63)	0.540	1.22 (0.93, 1.61)	0.147	1.29 (0.91, 1.84)	0.153
Public bus stops within 100 m buffer, ≥1	1.05 (0.84, 1.30)	0.691	0.54 (0.31, 0.95)	0.031	0.94 (0.68, 1.29)	0.690	1.05 (0.73, 1.52)	0.788
Public bus stops within 300 m buffer, ≥1	1.10 (0.93, 1.29)	0.262	0.79 (0.59, 1.07)	0.122	1.02 (0.81, 1.27)	0.881	0.92 (0.70, 1.21)	0.539

Public bus stops within 500 m buffer, ≥1	1.09 (0.81, 1.48)	0.562	0.86 (0.51, 1.47)	0.587	1.03 (0.67, 1.56)	0.903	1.09 (0.64, 1.85)	0.761
Unhealthy food environment within 300 m buffer								
1 to 10	0.99 (0.77, 1.28)	0.952	1.09 (0.64, 1.84)	0.750	0.97 (0.67, 1.40)	0.868	0.69 (0.44, 1.09)	0.111
≥10	0.99 (0.78, 1.26)	0.937	1.24 (0.77, 2.01)	0.379	0.85 (0.61, 1.19)	0.344	0.93 (0.64, 1.36)	0.709

*Models adjusted for maternal education, pre-pregnancy BMI, age, smoking during pregnancy, history of asthma or atopy, area-level SES, and child's sex, ethnicity, and season of birth.

Table S6. Multiple-exposure associations of *in utero* urban environment exposures with lung function (n=4968).

	FEV_1 z-score change (95%CI)	p-value	**FVC** z-score change (95%CI)	p-value	**FEV_1/FVC** z-score change (95%CI)	p-value	**$FEF_{25-75\%}$** z-score change (95%CI)	p-value
NO_2	0.04 (-0.01, 0.08)	0.103	0.03 (-0.01, 0.07)	0.201	0.01 (-0.03, 0.05)	0.666	-0.05 (-0.10, 0.00)	0.052
$PM_{2.5}$	0.09 (0.04, 0.14)	<0.001	0.10 (0.05, 0.14)	<0.001	-0.03 (-0.07, 0.02)	0.244	-0.10 (-0.15, -0.05)	<0.001
Day evening and night noise, Lden<55 Ref								
55-59.9	0.02 (-0.06, 0.10)	0.641	0.03 (-0.04, 0.11)	0.389	-0.04 (-0.12, 0.04)	0.364	0.02 (-0.07, 0.11)	0.663
60-64.9	0.03 (-0.05, 0.11)	0.406	0.02 (-0.06, 0.09)	0.657	0.03 (-0.05, 0.11)	0.496	0.02 (-0.06, 0.11)	0.602
≥65	-0.05 (-0.14, 0.04)	0.298	-0.03 (-0.11, 0.06)	0.519	-0.03 (-0.12, 0.05)	0.447	-0.02 (-0.12, 0.08)	0.748
Night noise, Ln<50 Ref								
50-54.9	0.03 (-0.04, 0.11)	0.417	0.01 (-0.06, 0.08)	0.781	0.04 (-0.03, 0.12)	0.250	0.04 (-0.04, 0.13)	0.310
55-59.9	-0.05 (-0.14, 0.05)	0.320	-0.04 (-0.13, 0.05)	0.337	-0.01 (-0.10, 0.08)	0.814	-0.06 (-0.16, 0.04)	0.249
≥60	-0.02 (-0.16, 0.12)	0.754	-0.02 (-0.16, 0.12)	0.790	0.01 (-0.13, 0.15)	0.847	0.05 (-0.11, 0.20)	0.561
Inverse distance to nearest road	0.02 (-0.01, 0.05)	0.114	0.02 (-0.01, 0.05)	0.207	0.01 (-0.02, 0.04)	0.592	0.00 (-0.03, 0.04)	0.869
NDVI within 300 m buffer	0.03 (-0.02, 0.08)	0.293	0.03 (-0.02, 0.07)	0.297	0.00 (-0.05, 0.05)	0.880	-0.02 (-0.08, 0.03)	0.439

Size of nearest major blue space (>5000 m²)	0.02 (0.00, 0.04)	0.017	0.02 (0.00, 0.03)	0.059	0.01 (-0.01, 0.03)	0.239	0.02 (0.00, 0.04)	0.088
Building density within 300 m buffer	0.03 (-0.01, 0.08)	0.172	0.01 (-0.03, 0.06)	0.605	0.04 (0.00, 0.09)	0.072	0.02 (-0.04, 0.07)	0.542

* Air pollution models were adjusted for noise, traffic, green spaces, blue spaces and built environment. Noise models were adjusted for air pollution, traffic, green spaces, blue spaces and built environment. Traffic, green spaces, blue spaces and built environment models were adjusted for each other. All models were additionally adjusted for maternal education, pre-pregnancy BMI, age, smoking during pregnancy, history of asthma or atopy, area-level SES, and child's ethnicity and season of birth.

Table S7. Multiple-exposure associations of *in utero* urban environment exposures with wheezing patterns (n=4197) and asthma (n=4698).

	Early wheeze		**Late wheeze**		**Persistent wheeze**		**Current asthma**	
	OR (95%CI)	p-value	OR (95%CI)	p-value	OR (95%CI)	p-value	OR (95%CI)	p-value
NO_2	1.07 (0.95, 1.20)	0.264	1.04 (0.83, 1.30)	0.717	0.88 (0.74, 1.04)	0.138	0.99 (0.81, 1.22)	0.962
$PM_{2.5}$	0.97 (0.85, 1.10)	0.590	1.16 (0.91, 1.48)	0.245	0.82 (0.69, 0.99)	0.041	0.95 (0.76, 1.18)	0.575
Day evening and night noise, Lden<55 Ref								
55-59.9	0.91 (0.74, 1.13)	0.385	0.90 (0.59, 1.39)	0.634	1.18 (0.89, 1.57)	0.248	0.97 (0.66, 1.42)	0.830
60-64.9	0.99 (0.80, 1.22)	0.896	1.19 (0.80, 1.76)	0.387	0.95 (0.70, 1.30)	0.767	1.34 (0.95, 1.90)	0.102
≥65	1.16 (0.92, 1.46)	0.216	1.01 (0.63, 1.62)	0.954	1.03 (0.73, 1.44)	0.867	0.98 (0.65, 1.49)	0.899
Night noise, Ln<50 Ref								
50-54.9	1.00 (0.81, 1.23)	0.979	1.32 (0.91, 1.91)	0.138	1.00 (0.74, 1.34)	0.963	1.36 (0.97, 1.90)	0.076
55-59.9	1.26 (0.99, 1.59)	0.058	1.04 (0.64, 1.71)	0.868	0.97 (0.67, 1.40)	0.871	0.78 (0.48, 1.25)	0.272
≥60	1.05 (0.72, 1.52)	0.738	0.84 (0.38, 1.84)	0.660	1.35 (0.83, 2.20)	0.228	1.69 (0.98, 2.94)	0.055
Inverse distance to nearest road	1.00 (0.92, 1.08)	0.967	1.06 (0.91, 1.24)	0.457	0.95 (0.84, 1.07)	0.367	0.97 (0.84, 1.12)	0.678

NDVI within 300 m buffer	0.93 (0.80, 1.07)	0.280	1.02 (0.78, 1.34)	0.892	1.14 (0.93, 1.39)	0.207	1.05 (0.82, 1.34)	0.817
Size of nearest major blue space (>5000 m²)	0.99 (0.94, 1.05)	0.744	0.93 (0.83, 1.04)	0.184	1.10 (1.02, 1.17)	0.008	0.92 (0.85, 1.01)	0.091
Building density within 300 m buffer	0.99 (0.88, 1.13)	0.984	1.00 (0.78, 1.28)	0.987	1.19 (0.99, 1.42)	0.052	1.09 (0.88, 1.36)	0.397

* Air pollution models were adjusted for noise, traffic, green spaces, blue spaces and built environment. Noise models were adjusted for air pollution, traffic, green spaces, blue spaces and built environment. Traffic, green spaces, blue spaces and built environment models were adjusted for each other. All models were additionally adjusted for maternal education, pre-pregnancy BMI, age, smoking during pregnancy, history of asthma or atopy, area-level SES, and child's sex, ethnicity, and season of birth.

Table S8. Maternal and child characteristics in the clusters of urban exposures.

	Cluster 1 (n=1533)	Cluster 2 (n=2907)	Cluster 3 (n=1184)	p-value
Maternal characteristics				
Age (years)	31.09 (4.90)	30.87 (5.04)	31.20 (4.62)	0.104
Pre-pregnancy BMI (kg/m2)	23.53 (4.21)	23.54 (4.14)	23.17 (3.89)	0.023
Education, low	815 (53%)	1432 (49%)	526 (44%)	<0.001
Smoking, %				
Never	1163 (76%)	2234 (77%)	892 (75%)	0.670
Before pregnancy	132 (9%)	245 (8%)	115 (10%)	
During pregnancy	238 (16%)	428 (15%)	177 (15%)	
History of asthma or atopy, yes	558 (36%)	1054 (36%)	428 (36%)	0.991
Child characteristics				
Sex, female	771 (50%)	1457 (50%)	594 (50%)	0.994
Preterm birth, yes	69 (5%)	143 (5%)	49 (4%)	0.535
Low birth weight, %	62 (4%)	133 (5%)	44 (4%)	0.418
Season of birth				
Spring	347 (23%)	642 (22%)	344 (29%)	<0.001
Summer	383 (25%)	788 (27%)	345 (29%)	
Autumn	466 (30%)	829 (29%)	246 (21%)	

Winter	337 (22%)	648 (22%)	249 (21%)	
Ethnicity, %				
Caucasian	1238 (81%)	2246 (77%)	971 (82%)	<0.001
African/American	97 (6%)	249 (9%)	69 (6%)	
Asian	106 (7%)	173 (6%)	61 (5%)	
Other/Mixed	92 (6%)	239 (8%)	83 (7%)	
Age (years)	9.78 (0.34)	9.80 (0.36)	9.78 (0.34)	0.039
FEV_1 (z-score)	0.18 (0.97)	0.16 (0.99)	0.14 (0.97)	0.673
FVC (z-score)	0.21 (0.91)	0.20 (0.96)	0.19 (0.92)	0.825
FEV_1/FVC (z-score)	-0.10 (0.96)	-0.09 (0.97)	-0.13 (0.95)	0.551
$FEF_{25-75\%}$ (z-score)	0.46 (1.09)	0.46 (1.10)	0.40 (1.07)	0.305
Wheezing patterns				
Never	634 (59%)	1253 (58%)	535 (56%)	0.026
Early	258 (24%)	579 (27%)	272 (29%)	
Late	46 (4%)	111 (5%)	56 (6%)	
Persistent	138 (13%)	229 (11%)	86 (9%)	
Current asthma, %	69 (5%)	147 (6%)	52 (5%)	0.394

* Differences were calculated using Student's t test for continuous normally distributed variables, Mann-Whitney U test for continuous non-normally distributed variables, and chi-square test for categorical variables.

Table S9. Single-exposure associations between *in utero* urban environment exposures and lung function including only children with reproducible spirometries (n=4734).

	FEV_1 z-score change (95%CI)	p-value	**FVC** z-score change (95%CI)	p-value	**FEV_1/FVC** z-score change (95%CI)	p-value	**$FEF_{25-75\%}$** z-score change (95%CI)	p-value
Air pollution								
NO_2	0.01 (-0.03, 0.05)	0.672	0.00 (-0.04, 0.04)	0.917	0.01 (-0.03, 0.05)	0.691	-0.05 (-0.09, 0.00)	0.031
NO_x	0.00 (-0.04, 0.04)	0.960	0.00 (-0.04, 0.03)	0.823	0.00 (-0.03, 0.04)	0.803	-0.02 (-0.07, 0.02)	0.307
PM_{10}	0.05 (0.00, 0.09)	0.034	0.06 (0.02, 0.10)	0.008	-0.02 (-0.06, 0.02)	0.369	-0.10 (-0.15, -0.05)	<0.001
$PM_{2.5}$	0.06 (0.01, 0.11)	0.011	0.07 (0.02, 0.11)	0.003	-0.02 (-0.06, 0.03)	0.514	-0.11 (-0.16, -0.06)	<0.001
PM_{25abs}	0.01 (-0.03, 0.05)	0.652	0.01 (-0.02, 0.05)	0.434	-0.01 (-0.05, 0.02)	0.420	-0.05 (-0.09, -0.01)	0.019
PM_{coarse}	0.04 (0.00, 0.08)	0.056	0.04 (0.00, 0.07)	0.064	0.01 (-0.03, 0.04)	0.800	-0.05 (-0.09, 0.00)	0.031
$PM_{2.5cu}$	-0.01 (-0.03, 0.01)	0.473	-0.01 (-0.03, 0.01)	0.439	0.00 (-0.02, 0.02)	0.926	-0.02 (-0.04, 0.01)	0.139
$PM_{2.5fe}$	0.00 (-0.03, 0.02)	0.848	-0.01 (-0.04, 0.01)	0.391	0.01 (-0.01, 0.04)	0.277	-0.01 (-0.04, 0.02)	0.619
$PM_{2.5k}$	-0.02 (-0.05, 0.00)	0.080	-0.01 (-0.04, 0.01)	0.269	-0.02 (-0.04, 0.01)	0.163	-0.02 (-0.05, 0.01)	0.138
$PM_{2.5ni}$	-0.04 (-0.10, 0.02)	0.183	-0.04 (-0.10, 0.02)	0.188	0.00 (-0.06, 0.06)	0.926	0.05 (-0.02, 0.11)	0.166
$PM_{2.5s}$	0.03 (0.00, 0.06)	0.041	0.03 (0.00, 0.06)	0.077	0.01 (-0.02, 0.04)	0.518	-0.02 (-0.05, 0.02)	0.354
$PM_{2.5si}$	0.00 (-0.01, 0.01)	0.863	-0.01 (-0.01, 0.00)	0.140	0.01 (0.00, 0.02)	0.018	0.00 (-0.01, 0.01)	0.502
$PM_{2.5v}$	-0.04 (-0.10, 0.02)	0.190	-0.04 (-0.10, 0.02)	0.192	0.00 (-0.06, 0.06)	0.903	0.05 (-0.02, 0.11)	0.173
$PM_{2.5zn}$	-0.02 (-0.05, 0.01)	0.109	-0.01 (-0.04, 0.02)	0.377	-0.02 (-0.05, 0.01)	0.141	-0.03 (-0.06, 0.01)	0.124

Noise								
Lden	0.00 (-0.05, 0.05)	0.997	-0.01 (-0.06, 0.03)	0.606	0.02 (-0.03, 0.06)	0.418	0.00 (-0.05, 0.05)	0.895
Day evening and night noise, Lden (<55 Ref), dB								
55-59.9	0.01 (-0.07, 0.09)	0.812	0.03 (-0.05, 0.11)	0.421	-0.05 (-0.13, 0.03)	0.231	0.01 (-0.07, 0.10)	0.770
60-64.9	0.03 (-0.05, 0.11)	0.475	0.02 (-0.06, 0.09)	0.646	0.02 (-0.06, 0.10)	0.612	0.00 (-0.09, 0.09)	0.962
≥65	-0.05 (-0.13, 0.03)	0.247	-0.03 (-0.11, 0.05)	0.409	-0.03 (-0.11, 0.05)	0.485	-0.05 (-0.14, 0.04)	0.278
Night noise, Ln	-0.01 (-0.06, 0.04)	0.737	-0.02 (-0.06, 0.03)	0.508	0.01 (-0.04, 0.06)	0.656	0.00 (-0.06, 0.05)	0.956
Night noise, Ln (<50 Ref), dB								
50-54.9	0.02 (-0.06, 0.10)	0.631	0.00 (-0.07, 0.08)	0.929	0.04 (-0.04, 0.11)	0.345	0.03 (-0.06, 0.12)	0.487
55-59.9	-0.04 (-0.13, 0.05)	0.357	-0.04 (-0.13, 0.05)	0.348	-0.01 (-0.10, 0.08)	0.821	-0.06 (-0.17, 0.04)	0.215
≥60	-0.05 (-0.18, 0.09)	0.507	-0.03 (-0.16, 0.10)	0.666	-0.02 (-0.15, 0.12)	0.807	-0.04 (-0.19, 0.11)	0.603
Traffic								
Inverse distance to nearest road	0.03 (0.00, 0.06)	0.032	0.03 (0.00, 0.06)	0.075	0.01 (-0.02, 0.04)	0.540	0.01 (-0.02, 0.05)	0.484
Green spaces								
NDVI within 100 m buffer	0.03 (-0.01, 0.07)	0.169	0.03 (-0.01, 0.07)	0.192	0.00 (-0.04, 0.04)	0.936	0.01 (-0.04, 0.06)	0.668
NDVI within 300 m buffer	0.02 (-0.03, 0.07)	0.409	0.02 (-0.02, 0.07)	0.292	-0.01 (-0.06, 0.04)	0.693	-0.03 (-0.08, 0.02)	0.303
NDVI within 500 m buffer	0.01 (-0.04, 0.06)	0.662	0.01 (-0.04, 0.07)	0.561	-0.01 (-0.06, 0.05)	0.808	-0.02 (-0.08, 0.04)	0.556
Size of nearest major green space (>5000 m^2)	-0.01 (-0.03, 0.02)	0.528	-0.01 (-0.04, 0.01)	0.258	0.01 (-0.01, 0.03)	0.376	0.00 (-0.03, 0.02)	0.755

Major green space (>5000 m²) in a 300 m buffer, yes	0.01 (-0.06, 0.08)	0.799	-0.01 (-0.08, 0.06)	0.799	0.02 (-0.05, 0.09)	0.523	0.02 (-0.06, 0.10)	0.589
Distance nearest major green space (>5000 m²)	-0.03 (-0.07, 0.01)	0.174	-0.01 (-0.05, 0.03)	0.663	-0.03 (-0.06, 0.01)	0.182	-0.01 (-0.05, 0.03)	0.671
Blue spaces								
Size of nearest major blue space (>5000 m²)	0.01 (0.00, 0.03)	0.122	0.01 (0.00, 0.03)	0.160	0.00 (-0.01, 0.02)	0.616	0.02 (0.00, 0.04)	0.060
Major green space (>5000 m²) in a 300 m buffer, yes	-0.01 (-0.06, 0.05)	0.847	0.03 (-0.03, 0.08)	0.350	-0.07 (-0.12, -0.01)	0.017	0.00 (-0.06, 0.07)	0.917
Distance nearest major blue space (>5000 m²)	-0.01 (-0.05, 0.03)	0.653	-0.02 (-0.06, 0.02)	0.425	0.02 (-0.02, 0.07)	0.239	0.00 (-0.05, 0.04)	0.857
Built environment								
Population density	0.01 (0.00, 0.02)	0.181	0.00 (-0.01, 0.01)	0.773	0.01 (0.00, 0.02)	0.039	0.00 (-0.01, 0.01)	0.987
Building density within 100 m buffer	-0.02 (-0.06, 0.02)	0.306	-0.01 (-0.06, 0.03)	0.479	0.00 (-0.04, 0.04)	0.946	-0.01 (-0.06, 0.03)	0.559
Building density within 300 m buffer	0.02 (-0.03, 0.06)	0.425	0.00 (-0.04, 0.04)	0.905	0.03 (-0.01, 0.07)	0.136	0.01 (-0.04, 0.05)	0.771
Street connectivity within 300 m buffer	-0.01 (-0.05, 0.03)	0.617	-0.01 (-0.04, 0.03)	0.589	-0.01 (-0.04, 0.03)	0.746	0.01 (-0.03, 0.05)	0.620
Street connectivity within 100 m buffer	-0.03 (-0.08, 0.01)	0.155	-0.02 (-0.06, 0.02)	0.394	-0.03 (-0.07, 0.02)	0.218	0.00 (-0.05, 0.05)	0.871

Facilities density within 300 m buffer	0.01 (-0.04, 0.05)	0.779	0.00 (-0.04, 0.04)	0.999	0.01 (-0.03, 0.05)	0.497	-0.02 (-0.06, 0.03)	0.497
Facilities richness within 300 m buffer	0.00 (-0.04, 0.05)	0.905	0.00 (-0.05, 0.04)	0.927	0.01 (-0.03, 0.06)	0.552	-0.02 (-0.07, 0.03)	0.452
Land Use Mix (Shannon's Evenness Index) within 300 m buffer	0.02 (-0.02, 0.06)	0.424	0.03 (-0.01, 0.07)	0.097	-0.04 (-0.08, 0.00)	0.040	0.02 (-0.02, 0.07)	0.279
Walkability	0.00 (-0.04, 0.03)	0.826	0.00 (-0.03, 0.04)	0.858	-0.02 (-0.06, 0.02)	0.305	0.00 (-0.04, 0.04)	0.966
Public bus lines within 100 m buffer, ≥1	0.02 (-0.04, 0.08)	0.502	0.02 (-0.04, 0.08)	0.599	0.01 (-0.05, 0.07)	0.833	-0.03 (-0.10, 0.03)	0.328
Public bus lines within 300 m buffer, ≥1	-0.01 (-0.07, 0.04)	0.647	0.00 (-0.05, 0.06)	0.960	-0.03 (-0.08, 0.03)	0.392	-0.03 (-0.09, 0.04)	0.417
Public bus lines within 500 m buffer, ≥1	-0.02 (-0.10, 0.05)	0.541	-0.01 (-0.08, 0.06)	0.807	-0.03 (-0.10, 0.05)	0.484	-0.01 (-0.09, 0.07)	0.738
Public bus stops within 100 m buffer, ≥1	0.02 (-0.06, 0.10)	0.671	0.03 (-0.05, 0.11)	0.435	-0.03 (-0.11, 0.05)	0.427	0.01 (-0.08, 0.10)	0.806
Public bus stops within 300 m buffer, ≥1	-0.05 (-0.12, 0.01)	0.078	-0.04 (-0.09, 0.02)	0.220	-0.02 (-0.08, 0.03)	0.415	-0.04 (-0.10, 0.03)	0.295
Public bus stops within 500 m buffer, ≥1	-0.06 (-0.18, 0.05)	0.295	-0.04 (-0.16, 0.07)	0.432	-0.02 (-0.13, 0.10)	0.763	0.02 (-0.11, 0.14)	0.819

Unhealthy food environment within 300 m buffer								
1 to 10	0.03 (-0.07, 0.13)	0.591	0.05 (-0.05, 0.14)	0.325	-0.05 (-0.15, 0.05)	0.326	0.00 (-0.11, 0.11)	0.968
≥10	0.03 (-0.05, 0.12)	0.445	0.02 (-0.06, 0.10)	0.631	0.02 (-0.07, 0.11)	0.660	0.02 (-0.08, 0.12)	0.676

*Models adjusted for maternal education, pre-pregnancy BMI, age, smoking during pregnancy, history of asthma or atopy, area-level SES, and child's ethnicity and season of birth.

Table S10. Multiple-exposure associations of *in utero* urban environment exposures with lung function including only children with reproducible spirometries (n=4734).

	FEV_1 z-score change (95%CI)	p-value	**FVC** z-score change (95%CI)	p-value	**FEV_1/FVC** z-score change (95%CI)	p-value	**$FEF_{25-75\%}$** z-score change (95%CI)	p-value
NO_2	0.03 (-0.02, 0.07)	0.258	0.01 (-0.03, 0.06)	0.484	0.02 (-0.03, 0.06)	0.491	-0.05 (-0.10, 0.00)	0.051
$PM_{2.5}$	0.07 (0.02, 0.12)	0.004	0.08 (0.03, 0.12)	0.001	-0.01 (-0.06, 0.04)	0.666	-0.11 (-0.16, -0.05)	<0.001
Day evening and night noise, Lden<55 Ref								
55-59.9	0.01 (-0.07, 0.09)	0.799	0.03 (-0.04, 0.11)	0.409	-0.05 (-0.13, 0.03)	0.236	0.03 (-0.06, 0.11)	0.560
60-64.9	0.04 (-0.04, 0.12)	0.364	0.03 (-0.05, 0.10)	0.499	0.02 (-0.06, 0.10)	0.622	0.01 (-0.08, 0.10)	0.786
≥65	-0.05 (-0.14, 0.04)	0.284	-0.03 (-0.11, 0.06)	0.529	-0.04 (-0.13, 0.05)	0.389	-0.02 (-0.12, 0.08)	0.645
Night noise, Ln<50 Ref								
50-54.9	0.03 (-0.05, 0.10)	0.514	0.01 (-0.06, 0.09)	0.778	0.04 (-0.04, 0.11)	0.350	0.04 (-0.05, 0.13)	0.373
55-59.9	-0.04 (-0.13, 0.06)	0.445	-0.03 (-0.12, 0.06)	0.462	-0.01 (-0.11, 0.08)	0.788	-0.05 (-0.15, 0.06)	0.365
≥60	-0.05 (-0.20, 0.09)	0.477	-0.03 (-0.16, 0.11)	0.708	-0.03 (-0.17, 0.11)	0.704	0.01 (-0.15, 0.17)	0.880

Inverse distance to nearest road	0.03 (0.00, 0.06)	0.035	0.03 (0.00, 0.06)	0.075	0.01 (-0.02, 0.04)	0.605	0.01 (-0.02, 0.04)	0.543
NDVI within 300 m buffer	0.04 (-0.01, 0.09)	0.146	0.03 (-0.01, 0.08)	0.166	0.01 (-0.04, 0.06)	0.786	-0.02 (-0.08, 0.03)	0.427
Size of nearest major blue space (>5000 m^2)	0.02 (0.00, 0.03)	0.078	0.01 (0.00, 0.03)	0.129	0.01 (-0.01, 0.02)	0.457	0.02 (0.00, 0.04)	0.064
Building density within 300 m buffer	0.03 (-0.01, 0.08)	0.159	0.02 (-0.03, 0.06)	0.454	0.04 (-0.01, 0.08)	0.125	0.01 (-0.05, 0.06)	0.847

* Air pollution models were adjusted for noise, traffic, green spaces, blue spaces and built environment. Noise models were adjusted for air pollution, traffic, green spaces, blue spaces and built environment. Traffic, green spaces, blue spaces and built environment models were adjusted for each other. All models were additionally adjusted for maternal education, pre-pregnancy BMI, age, smoking during pregnancy, history of asthma or atopy, area-level SES, and child's ethnicity and season of birth.

Table S11. Associations of *in utero* urban exposure clusters with lung function including only children with reproducible spirometries [a].

	Cluster 2 n=2450		**Cluster 3 n=984**	
	Estimate (95%CI)	p-value	Estimate (95%CI)	p-value
FEV_1	-0.03 (-0.09, 0.04)	0.419	-0.06 (-0.14, 0.02)	0.136
FVC	-0.01 (-0.07, 0.05)	0.741	-0.04 (-0.12, 0.04)	0.300
FEV_1/FVC	-0.02 (-0.08, 0.05)	0.578	-0.04 (-0.12, 0.04)	0.356
$FEF_{25-75\%}$	-0.03 (-0.11, 0.04)	0.351	-0.09 (-0.18, 0.00)	0.060

[a] Associations are compared to the reference Cluster 1 (n=1300).
Models were adjusted for maternal education, pre-pregnancy BMI, age, smoking during pregnancy, history of asthma or atopy, and child's ethnicity and season of birth.

Table S12. Single-exposure associations of *in utero* urban environment exposures with lung function adjusted for postnatal urban environment exposures (n=4968).

	FEV_1 z-score change (95%CI)	p-value	**FVC** z-score change (95%CI)	p-value	**FEV_1/FVC** z-score change (95%CI)	p-value	**$FEF_{25-75\%}$** z-score change (95%CI)	p-value
NO_2	0.02 (-0.02, 0.06)	0.410	0.01 (-0.03, 0.05)	0.551	0.00 (-0.04, 0.04)	0.912	-0.04 (-0.09, 0.00)	0.053
$PM_{2.5}$	0.08 (0.03, 0.14)	0.002	0.08 (0.03, 0.13)	0.003	0.00 (-0.06, 0.05)	0.881	-0.09 (-0.15, -0.03)	0.003
Day evening and night noise, Lden<55 Ref								
55-59.9	0.02 (-0.06, 0.11)	0.588	0.03 (-0.05, 0.12)	0.484	-0.01 (-0.11, 0.08)	0.755	0.01 (-0.09, 0.11)	0.805
60-64.9	0.05 (-0.04, 0.13)	0.283	0.04 (-0.04, 0.12)	0.377	0.02 (-0.07, 0.11)	0.655	0.00 (-0.09, 0.09)	0.995
≥65	-0.05 (-0.13, 0.04)	0.297	-0.03 (-0.11, 0.05)	0.496	-0.03 (-0.12, 0.06)	0.515	-0.04 (-0.13, 0.06)	0.471
Night noise, Ln<50 Ref								
50-54.9	0.04 (-0.04, 0.13)	0.297	0.03 (-0.05, 0.11)	0.499	0.03 (-0.05, 0.12)	0.412	0.02 (-0.07, 0.12)	0.621
55-59.9	-0.05 (-0.15, 0.04)	0.283	-0.04 (-0.13, 0.05)	0.384	-0.03 (-0.12, 0.07)	0.602	-0.06 (-0.17, 0.05)	0.259
≥60	-0.01 (-0.15, 0.13)	0.925	-0.01 (-0.14, 0.13)	0.929	0.02 (-0.12, 0.16)	0.801	-0.02 (-0.18, 0.13)	0.796
Inverse distance to nearest road	0.02 (-0.01, 0.05)	0.281	0.02 (-0.01, 0.05)	0.217	0.00 (-0.04, 0.03)	0.775	0.01 (-0.03, 0.04)	0.668

NDVI within 300 m buffer	0.01 (-0.04, 0.06)	0.778	0.00 (-0.04, 0.05)	0.857	0.01 (-0.04, 0.05)	0.827	-0.04 (-0.09, 0.02)	0.156
Size of nearest major blue space (>5000 m²)	0.02 (0.00, 0.04)	0.089	0.02 (0.00, 0.03)	0.102	0.00 (-0.02, 0.02)	0.811	0.01 (-0.01, 0.03)	0.232
Building density within 300 m buffer	0.01 (-0.03, 0.06)	0.524	0.00 (-0.04, 0.04)	0.974	0.03 (-0.01, 0.08)	0.142	0.04 (-0.01, 0.09)	0.127

*Models adjusted for their corresponding postnatal exposure, maternal education, pre-pregnancy BMI, age, smoking during pregnancy, history of asthma or atopy, area-level SES, and child's ethnicity and season of birth.

Table S13. Single-exposure associations of *in utero* urban environment exposures with asthma adjusted for postnatal urban environment exposures (n=4698) [a].

	Current asthma	
	OR (95%CI)	p-value
NO_2	1.04 (0.87, 1.25)	0.682
$PM_{2.5}$	1.00 (0.77, 1.29)	0.994
Day evening and night noise, Lden<55 Ref		
55-59.9	1.00 (0.65, 1.52)	0.987
60-64.9	1.40 (0.96, 2.03)	0.079
≥65	0.93 (0.61, 1.41)	0.718
Night noise, Ln<50 Ref		
50-54.9	1.31 (0.90, 1.90)	0.161
55-59.9	0.74 (0.44, 1.22)	0.235
≥60	1.55 (0.89, 2.69)	0.122
Inverse distance to nearest road	1.01 (0.87, 1.17)	0.875
NDVI within 300 m buffer	1.03 (0.82, 1.29)	0.817
Size of nearest major blue space (>5000 m²)	0.90 (0.82, 0.99)	0.029
Building density within 300 m buffer	1.10 (0.89, 1.35)	0.388

[a]Models were not performed for wheezing patterns because the outcome was measured before the exposure.
Models adjusted for their corresponding postnatal exposure, maternal education, pre-pregnancy BMI, age, smoking during pregnancy, history of asthma or atopy, area-level SES, and child's sex, ethnicity, and season of birth.

Table S14. Associations of *in utero* urban exposure clusters with lung function, wheezing patterns, and asthma excluding preterm and low birth weight born children [a].

	Cluster 2 n=2709		**Cluster 3 n=1110**	
	Estimate (95%CI)	p-value	Estimate (95%CI)	p-value
Lung function (z-score change)				
FEV_1	-0.03 (-0.10, 0.04)	0.367	-0.05 (-0.13, 0.03)	0.228
FVC	-0.03 (-0.09, 0.04)	0.394	-0.04 (-0.12, 0.04)	0.351
FEV_1/FVC	0.01 (-0.06, 0.07)	0.851	-0.02 (-0.11, 0.06)	0.545
$FEF_{25-75\%}$	-0.03 (-0.11, 0.04)	0.378	-0.11 (-0.20, -0.02)	0.020
Wheezing patterns and asthma (OR)				
Early wheeze	1.14 (0.95, 1.37)	0.170	1.21 (0.98, 1.51)	0.080
Late wheeze	1.16 (0.81, 1.69)	0.415	1.42 (0.93, 2.16)	0.101
Persistent wheeze	0.83 (0.66, 1.06)	0.142	0.73 (0.53, 0.98)	0.040
Asthma	1.18 (0.87, 1.62)	0.288	0.99 (0.67, 1.47)	0.980

[a] Associations are compared to the reference Cluster 1 (n=1445).
Lung function models were adjusted for maternal education, pre-pregnancy BMI, age, smoking during pregnancy, history of asthma or atopy, and child's ethnicity and season of birth. Wheezing and asthma models were additionally adjusted for child's sex.

Table S15. Distribution of exposures and outcomes across area-level SES quintiles.

	Overall n=5605	**Quintile 1** n=593	**Quintile 2** n=646	**Quintile 3** n=663	**Quintile 4** n=616	**Quintile 5** n=3087	**p-value**
NO_2	38.48 (7.47)	36.29 (8.41)	40.05 (7.09)	36.24 (6.90)	40.54 (10.32)	38.80 (6.72)	< 0.001
$PM_{2.5}$	19.72 (3.83)	19.17 (3.77)	20.01 (3.91)	19.45 (3.39)	20.05 (3.85)	19.71 (3.84)	< 0.001
Day evening and night noise, Lden	54.00 (13.00)	53.00 (10.00)	52.00 (12.00)	52.00 (9.00)	58.00 (13.00)	55.00 (14.00)	< 0.001
Night noise, Ln	45.00 (13.00)	44.00 (10.00)	44.00 (11.00)	43.00 (8.00)	48.00 (12.00)	46.00 (14.00)	< 0.001
Inverse distance to nearest road	0.09 (0.05)	0.08 (0.05)	0.09 (0.06)	0.08 (0.05)	0.10 (0.05)	0.09 (0.06)	< 0.001
NDVI within 300 m buffer	0.39 (0.14)	0.48 (0.11)	0.42 (0.09)	0.49 (0.09)	0.36 (0.18)	0.35 (0.10)	< 0.001
Size of nearest major blue space (>5000 m^2)	$2.3 \cdot 10^{10}$ ($5.1 \cdot 10^{10}$)	$2.3 \cdot 10^{10}$ ($1.8 \cdot 10^{11}$)	$2.3 \cdot 10^{10}$ ($3.5 \cdot 10^{11}$)	$1.6 \cdot 10^{10}$ ($3.8 \cdot 10^{10}$)	$2.3 \cdot 10^{10}$ ($1.9 \cdot 10^{10}$)	$2.1 \cdot 10^{10}$ ($5 \cdot 10^{10}$)	< 0.001
Building density within 300 m buffer	$4.6 \cdot 10^5$ ($1.5 \cdot 10^5$)	$3.8 \cdot 10^5$ ($1.9 \cdot 10^5$)	$4.6 \cdot 10^5$ ($1.8 \cdot 10^5$)	$4 \cdot 10^5$ ($1.3 \cdot 10^5$)	4.6e x 10^5 ($1.3 \cdot 10^5$)	$4.9 \cdot 10^5$ ($1.4 \cdot 10^5$)	< 0.001
FEV_1 (z-score)	0.16 (0.98)	0.01 (0.94)	0.11 (0.96)	0.15 (0.95)	0.16 (0.93)	0.20 (1.00)	0.001
FVC (z-score)	0.20 (0.94)	0.09 (0.89)	0.17 (0.92)	0.22 (0.91)	0.22 (0.90)	0.22 (0.97)	0.034
FEV_1/FVC (z-score)	-0.10 (0.96)	-0.19 (0.94)	-0.15 (0.96)	-0.15 (0.91)	-0.15 (0.93)	-0.05 (0.98)	0.003
$FEF_{25-75\%}$ (z-score)	0.45 (1.09)	0.35 (1.09)	0.45 (1.07)	0.36 (1.10)	0.47 (1.09)	0.48 (1.10)	0.035
Wheezing patterns							

Never	302 (57%)	328 (55%)	330 (59%)	307 (62%)	1144 (57%)	2411 (58%)	0.005
Early	154 (29%)	185 (31%)	152 (27%)	116 (23%)	497 (25%)	1104 (26%)	
Late	24 (5%)	18 (3%)	31 (6%)	29 (6%)	111 (6%)	213 (5%)	
Persistent	49 (9%)	63 (11%)	50 (9%)	44 (9%)	246 (12%)	452 (11%)	
Current asthma	266 (6%)	24 (4%)	21 (4%)	32 (5%)	21 (4%)	168 (7%)	0.002

5.5. Paper V

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**Equal contribution*

Linkage of 719,858 parent and child electronic health records in a large longitudinal database in Spain: the Information System for Research in Primary Care (SIDIAP)

In preparation

Linkage of 719,858 parent and child electronic health records in a large longitudinal database in Spain: the Information System for Research in Primary Care (SIDIAP)

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Running title: Linkage of parent and child pairs in SIDIAP

Abbreviations: electronic health records, EHR; number of affiliation to the social security, NASS; personal identification code, PIC; International Classification of Diseases 10th revision, ICD-10; the Information System for Research in Primary Care, SIDIAP; Third Trusted party, TTP.

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ABSTRACT

Objective: The linkage of large electronic health records of parent and child pairs could provide a powerful resource for research. We aimed to create a new large birth cohort linking mother- and father-child pairs in the Information System for Research in Primary Care (SIDIAP) database in Catalonia (Spain).

Study Design and Setting: Children born from 2005-2018 were linked to their potential parent using the number of affiliation to the social security (NASS), the NASS status, the age of the potential parent, and the surname of parent and child. A sub-sample of 7,307 and 4,420 children with valid personal identification code (PIC) of mother or father, respectively, was used to validate parent-child linkage. Descriptive comparisons between linked and unlinked children were conducted.

Results: From 778,318 children in our study population, 719,858 (92.5%) were linked to at least one parent. 529,430 (68.0%) and 334,018 (42.9%) children were linked to a potential mother or father only, respectively. 143,590 (18.5%) children were linked to both parents. Linked parent-child pairs were representative of the overall population. In the validation sub-sample, 95.5% of mother-child pairs and 91.0% of father-child pairs were confirmed as true matches.

Conclusion: In this study, we successfully linked large longitudinal parent-child EHR data, providing a powerful birth cohort for future cohort studies in SIDIAP focused on the effects of parent's health and early life exposures in a wide range of child's outcomes.

Strengths and limitations of this study

- This study presents the linkage of a large sample size of longitudinal parent-child electronic health records in primary care.
- Children were linked to both mother and father.
- A validation study was performed and only approximately 5% of linkages were not confirmed.
- The proportion of unconfirmed linkages was higher in children from non-Spanish nationalities and living in most deprived areas.

INTRODUCTION

Parents health, as well as prenatal and early life events and exposures, are determinants of children's development and adult health[1,2]. Medication use during pregnancy is common; it has been estimated that up to 80% of women use at least one medication during pregnancy[3]. However, studies on the benefits and risks of drug exposures during pregnancy for both women and child are limited given that pregnant women are usually excluded from clinical trials for ethical and legal concerns[4]. The linkage of large health records of parent and child pairs could provide a powerful resource for investigating parent and children health associations, particularly for studies of pharmacovigilance, drug and vaccine safety during pregnancy, as well as the study of uncommon exposures or conditions from mother and father that could affect child's health.

Databases of routinely collected electronic health records (EHR) are becoming an increasingly valuable resource for epidemiology, as reflected by the number of recent studies using EHR data from primary care settings published in high impact journals[5–8]. These databases allow research on a wide range of topics and with greater statistical power than typically available in traditional cohort studies given the use of information from large and representative samples of the population[9–13]. The Information System for Research in Primary Care (SIDIAP) database includes extensive data on mother, father and child's health which are collected routinely in primary care centres in Catalonia, Spain[14]. However, the EHRs of parents and their children have still not been linked in SIDIAP.

Given the potential of linked parent and child EHR data for future research studies, we aimed to create a new parent-child birth cohort by linking EHR data of both mother- and father-child pairs in the SIDIAP database for children born from 2005 to 2018 in Catalonia. We also aimed to perform a validation of this linkage in a sub-sample of the population for which a direct linkage was available.

METHODS

Setting

This study was based on a large prospective population-based database from the SIDIAP (www.sidiap.org) platform in Catalonia, Spain. Since 2005, all visits in primary care centres of the Catalan Health Institute are recorded electronically. SIDIAP contains information recorded by health professionals in pseudo-anonymised patients' EHR for nearly six million people at primary care practices in Catalonia[14]. SIDIAP population is highly representative of the entire Catalan region in terms of geographic, age, and sex distributions[15]. It includes data collected during routine visits, including clinical diagnoses (International Classification of Diseases 10th revision [ICD-10]), laboratory test results, prescribed and dispensed drugs, vaccination, hospital referrals, anthropometric and clinical measurements performed during routine visits, demographic and lifestyle information. Additionally, it also includes specific paediatric and pregnancy follow-up data, and environmental exposures data (Supplementary Table 1). The high quality of these data has been previously documented[14–16], and SIDIAP has been successfully applied to epidemiological studies of key exposures and outcomes[17–20]. Therefore,

SIDIAP provides an excellent source of population-based data and reliably reproduces the actual conditions of clinical practice.

Study population

The Spanish health system is based on universal coverage with free access for all citizens. In primary care, children are visited by paediatricians and paediatric nurses until 15 years of age and they have a follow-up program for preventive activities and health promotion[21]. Children enter SIDIAP when they are assigned to a primary care centre, and are followed-up until the end of data collection, transferred out, or death. In Spain, legal partners, children or grandchildren up to 18 years of age are co-insured with a main insurance member without extra cost if they are not employed. Therefore, all subjects below the age of 18 should be registered in the national health system with the number of affiliation to the social security (NASS) of their parents, grandparents, or legal tutor. In this study, all subjects in SIDIAP born from January 1st 2005 to December 31st 2018 and with a valid NASS were included.

Parent and child linkage

Children were linked to their potential parent by a Third Trusted Party (TTP) when i) their NASS corresponded to the NASS of a female aged from 15 to 49 years (for a potential mother) or the NASS of a male aged 15 to 65 years (for a potential father) at the moment of the child's birth, ii) the NASS status of the child was "beneficiary" and the female/male candidate was the "holder", and iii) the first or second surname of the child corresponded to the surname of the potential parent (this last condition was only used for potential mothers aged 14 to 18 or 46 to 49

years in order to avoid linkage with possible siblings or grandparents). Additional information on date of birth obtained from the child's EHR and date of delivery obtained from the potential mother EHR was used in order to link mother and child when a NASS of the child was not linked to a female candidate. In this case, the first or second surname of the child had to correspond to the surname of the potential mother.

Linkage validation

The software used to register individuals' EHR data allows the registration of the personal identification code (PIC) of parents in the child's EHR. The PIC is a unique identification code for healthcare access of persons living in Catalonia. Although this information is not collected routinely, 7,307 and 4,420 children had a valid PIC of mother and father, respectively, registered in their EHR from 2005 to 2016. We used this information to validate the parent-child linkage.

Statistical analyses

Flowcharts were created to show the process of linkage including detailed population exclusions and the final number of linked pairs. Descriptive comparisons of those linked and unlinked children were conducted by child's sex (girls and boys), year of birth, nationality (Spanish and non-Spanish), and the ecological MEDEA index of deprivation[22] as a socioeconomic status indicator. The MEDEA index is calculated by census tract level in urban areas, categorised in quintiles, where the first and fifth quintiles are the least and most deprived areas, respectively. Rural areas were categorised separately since the index was not validated in these areas [23]. The proportion of confirmed linked pairs

was calculated for the sub-sample of the population with an available PIC of their parents in their EHR. All data analyses were performed using R studio version 2.13.0.

All linkage of data using personal identifying variables were performed by the Catalan Health Institute as a TTP. Pseudo-anonymised linked pairs were then included to the SIDIAP platform. This study was approved by the Clinical Research Ethics Committee of the IDIAPJGol in Barcelona (P16/179).

Patient and Public Involvement statement

Participants of this study were not involved in setting the research question or the outcome measures, nor were they involved in the design or implementation of the study. No patients were asked to advise on interpretation or writing of results.

RESULTS

Overall linkage

From 778,318 children born from 2005 to 2018 in SIDIAP, 719,858 (92.5%) were linked to at least one of both parents, 529,430 (68.0%) were linked to a potential mother, 334,018 (42.9%) were linked to a potential father, and 143,590 (18.4%) were linked to both parents (Figure 1). The proportion of children linked to their mothers was higher than those linked to their fathers during the full study period (Table 1). The percentage of linkage of parent-child pairs between 2005 and 2018 ranged between 86.5% (in 2018) and 95.2% (in 2013; data not shown).

We observed a small tendency of decrease in the number of parent-child pairs linked from 2015 onwards (Table 1).

Mother-child linkage

Overall, 428,855 mother-child pairs were linked through the NASS information and 100,788 through the dates of birth and delivery data (Figure 1). Following the algorithm criteria, the main reason for exclusion in the linkage through NASS was the age restriction of the potential mother at child's birth; 270,463 and 2,627 were excluded because the candidate mother was aged under 14 or above 49 years, respectively. There were 6,488 pairs, whose candidate mothers were aged 14 to 18 or 46 to 49, that were excluded because they did not meet the surname criteria, and 3,859 pairs excluded because children were linked to multiple women. Contrarily to the NASS linkage, the main reason for exclusion in the linkage through dates of birth and delivery data was the surname criteria (120,048 pairs excluded).

The proportion of linked children to their potential mothers was similar across sex but differed by year of birth, the MEDEA index and nationality (Table 1). The lowest percentages of linkage were observed among children born in 2005 (55.9%) and 2006 (57.9%), while the highest were observed in 2014 (72.8%), followed by 2013 (72.4%) and 2015 (72.1%). Regarding the MEDEA index, the highest proportion of linkages were observed among children in the 4th quintile (75.8%) and from rural areas (71.0%), and the lowest were observed among children from the 1st and 5th quintiles (least and most deprived area; 66.3% and 66.8%, respectively). Finally, the proportion of mother-child linkages

was lower among children with non-Spanish nationalities compared to Spanish children (51.0% vs. 70.7%, respectively).

Those linked to their potential mothers were representative of the overall population in terms of sex, year of birth, and the MEDEA index (Table 1). Except for a lower proportion of children of non-Spanish nationalities as compared to the overall population (10.1% vs. 13.5%, respectively). Children unlinked to a potential mother were also similar to the overall population in terms of sex, and the MEDEA index, but differed by year of birth and nationality. Unlinked children were more likely to be born at the first years of the study period and to be from non-Spanish nationalities compared to the overall population.

Father-child linkage

From 633,514 men and child pairs with matching NASS number, 335,970 (53.0%) were linked to a potential father (Figure 1). The main reason for the exclusion of linked pairs was the age restriction, with 289,343 and 668 pairs excluded because the candidate father was aged below 14 or above 65 years, respectively. Additionally, 4,620 pairs were excluded because they did not meet the surname criteria and 4,849 were excluded because they were matched to multiple potential fathers.

The proportion of linked children to their potential fathers was similar across sex but differed by year of birth, the MEDEA index and nationality (Table 1). The percentage of father-child linkage per year was 43.4% in 2005 and increased until 2009 (47%), but then started to decrease, with the lowest percentage of linkage observed among children born in 2018 (34.2%). Also, the proportion of father-child linkages

increased from 36.8% among children living in the least deprived areas (1st quintile of the MEDEA index) to 48.8% in children from most deprived areas (5th quintile of the MEDEA index). Contrarily to mother-child linkages, the proportion of father-child linkages was higher among children with non-Spanish nationalities compared to Spanish children (57.8% vs. 40.6%, respectively).

These children linked and unlinked to their potential fathers were representative of the overall population in terms of sex and year of birth, but differed by the MEDEA index and nationality. Higher proportions of children from most deprived areas and of non-Spanish nationalities were observed among the group of children linked to their fathers compared to all children (19.0% vs. 16.7% of children in the 5th quintile of the MEDEA index, and 18.1% vs. 13.5% of children from non-Spanish nationalities, respectively). On the other hand, unlinked children were more likely to be from least deprived areas and of Spanish nationality compared to the overall population.

Linkage validation

There were 7,307 and 4,420 children with available PIC of their mother and father registered in their EHR, respectively, which allowed us to validate the parent-child linkage. These subsamples of children with available PIC information of parents were representative of the paediatric SIDIAP population in terms of sex, rurality, and nationality (Supplementary Table 2). However, they differed in year of birth, with a higher proportion of younger cohorts, and in socioeconomic status since a

higher proportion of children from the most deprived areas (quintile 5th of the MEDEA index) was observed among children with PIC information of mother and father (20.6% and 18.7%, respectively) compared to the overall population (16.8%).

From the children with available PIC, 5,353 and 2,236 children were linked to a potential mother or father, respectively, using the algorithms described above (Table 2). Finally, 95.5% of mother-child pairs and 91.0% of father-child pairs were confirmed as true matches. The percentage of confirmed mother- and father-child pairs did not vary by child's sex, but differed by child's year of birth, the MEDEA index, and nationality. A higher percentage of mother- and father-child pairs were confirmed among children born in 2015 (97.5% and 94.9%, respectively) and 2016 (96.6% and 95.3%) compared to children born in 2005 (93.3% and 90.1%) and 2006 (94.8% and 85.8%). Also, children from the least deprived areas had a higher percentage of confirmed linkages with both mother and father (98.1% and 92.4%, respectively) compared to children from the most deprived areas (93.2% and. 88.4%), as well as children with Spanish nationality (95.7% and 92.2%) compared to children with non-Spanish nationalities (93.6% and 86.2%).

DISCUSSION

In this study, we were able to create a new birth cohort by linking the EHR of 719,858 (92.5%) children born from 2005 to 2018 to the EHR of at least one potential parent in SIDIAP; 68% of them were linked to a potential mother, 42.9% to a potential father, and 18.5% to both parents.

Linked children were representative of the overall paediatric population in SIDIAP in terms of sex, year of birth, and the MEDEA deprivation index, but children from non-Spanish nationalities might be underrepresented. In a subsample of the population for which the parent identification code was registered in the child's' EHR, 95.5% of mother-child pairs and 91.0% of father-child pairs linkages were confirmed. Unlinked children were more likely to be born in the first years of SIDIAP data collection, and to be from non-Spanish nationalities than the overall population.

Previous studies have linked maternal and child health data from a variety of sources including national medical prescription registries, congenital anomaly registries, national hospital discharge and birth registries, EHR and administrative claims databases[24–31]. The methods used were diverse and specific to the characteristics of each specific database. Some studies linked mothers and children through probabilistic approaches using maternal name, age, municipality[28,32], through a common birth record[29] or only diagnostic codes[30]. Similarly to the approach we followed in our study, a number of studies linked mothers and children through deterministic approaches using family identification numbers[26], national health service numbers[24,33], dates of birth and discharge[26,34], maternal and child characteristics[27] or algorithms combining the previous information[35]. In the present study, we were able to perform a direct linkage of parent-child EHR data in SIDIAP thanks to the access to personal data through a TTP, including surnames and co-insurance numbers. The combination of personal data to additional information such as age of potential parent, or date of birth and date of

delivery for potential mothers, helped avoiding possible misclassification of linkage to other female/male co-insurance (e.g. brother/sister or grandparents).

In our study we were able to link 92.5% of children to at least one of their parents, 68% to a potential mother and, 42.9% to a potential father. This is in line with the percentage of mother-child linkage reported in previous studies which ranged from 45 to 95%[36]. The comparability of the percentage of linked father and child pairs with other studies is difficult given that only few studies have previously linked large father-child health records, and often only the overall parent-child linkage rates are reported[31,37,38].

In a population subset, we were able to confirm the linkage in 95.5% and 91.0% of mother- and father-child pairs, respectively, which is comparable with the percentage of true matches (from 95% to 91%) reported in previous mother-child linkage studies[25,27]. In our study, unsuccessful linkages were more common during the first years of data collection, in children living in most deprived areas, and children from non-Spanish nationalities. These results suggest that co-insurance with a different family member (other than mother or father) and/or uninsurance status might be more frequent in these population subgroups.

Strengths and Limitations

The main strength of this study is the large sample size of linked parent-child pairs with available longitudinal data routinely collected by health professionals in EHR, which will allow future studies focused on the role

of parental exposures on child's health. Another strength is the collaboration with the main health provider in Catalonia which allowed us to perform a deterministic linkage, and the validation of the linkage of a pseudo-anonymised EHR database. It will also allow the continuous linkage of parent-child pairs of future newborns in SIDIAP, as well as the follow-up of the population linked in this study. Finally, several studies have shown the role of paternal exposures on child's health such as the association between paternal mental illness and risk of injuries in children and adolescents, or the correlation between increased paternal body mass index (BMI) and increased BMI in childhood[39–41]. In this study we were able to link the longitudinal EHR of 334,018 children to their potential fathers, providing a useful tool to investigate the impact of father's exposures and conditions on child's health and development. In addition, this newly created birth cohort contains detailed information on parental sociodemographic and lifestyle characteristics, pregnancy follow-ups, repeated health and development assessments through childhood, and environmental exposures. This provides a valuable source of data for future research on parental and child health in addition to early life determinants of children's health.

This study also has a number of limitations. Although we were able to use date of birth and date of delivery to link mother-child pairs for those children with a missing NASS of a potential mother, this was not possible for all children since SIDIAP does not include information from women using the private healthcare system for pregnancy follow-up and delivery. In the validation of our linkage algorithm, we found that approximately 5% of linkages were not confirmed. Finally, the

proportion of unconfirmed linkages was higher in children from non-Spanish nationalities and living in most deprived areas. Future studies are needed in order to better understand these unconfirmed linkages and to investigate whether parent-child linkage can be improved in these population subgroups.

Conclusion

In conclusion, we successfully linked and validated the linkage of large longitudinal health records of parents and children in a primary care Spanish population, providing a powerful resource for future studies focused on the effects of parent's health and early life exposures in a wide range of child's health and development outcomes.

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Potential competing interests

None to declare.

Data sharing statement

In accordance with current European and national law, the data used in this study is only available for the researchers participating in this study. Thus, we are not allowed to distribute or make publicly available the data to other parties. However, researchers from public institutions can request data from SIDIAP if they comply with certain requirements. Further information is available online (https://www.sidiap.org/index.php/component/content/article/11-

serveis/76-application-procedure) or by contacting Anna Moleras (amoleras@idiapjgol.org).

Author contributions

All authors contributed to the design of the study and interpretation of the results, and reviewed the manuscript. LMB applied the linkage algorithm using personal identified data. YD, MA and EH had full access to the pseudo-anonymised data used in the study, performed the statistical analysis, and acted as guarantors. TDS and AA wrote the first draft of the manuscript. All authors critically revised the manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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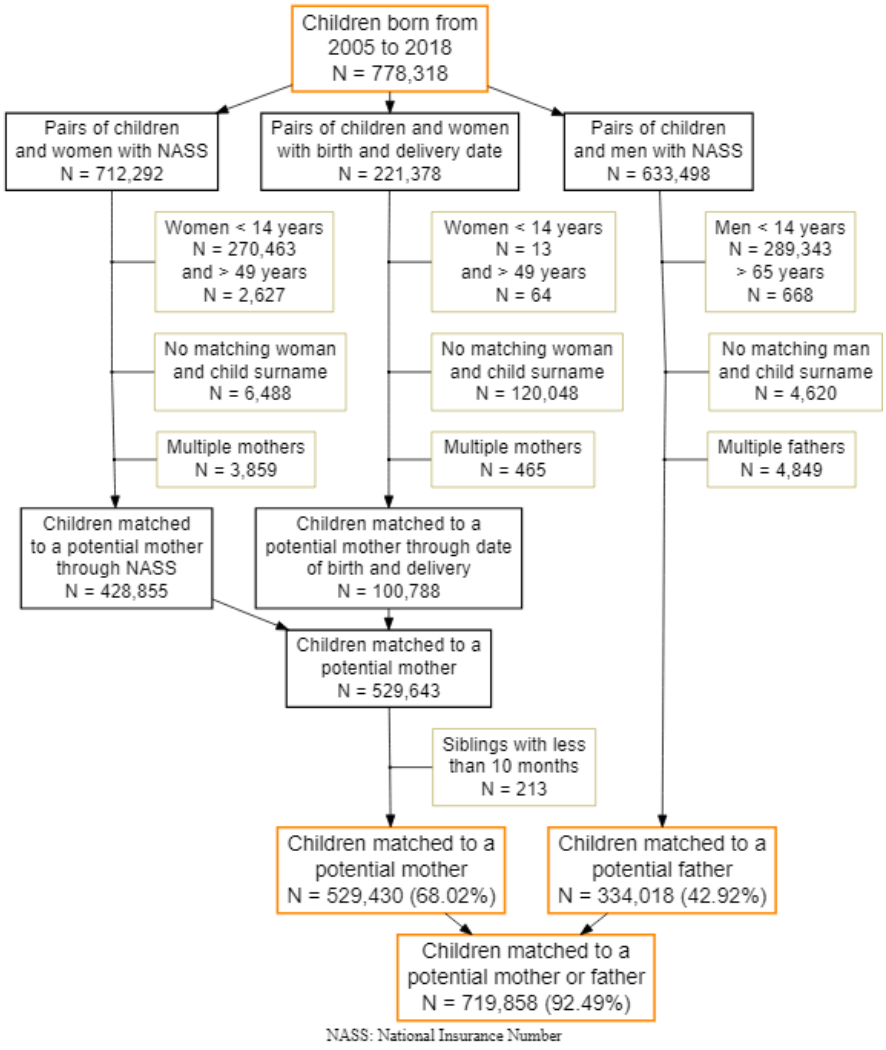
TABLES AND FIGURES

Figure 1. Flowchart of parent-child linkage from 2005 to 2018 in SIDIAP.



NASS: National Insurance Number

Table 1. Main characteristics of linked and unlinked children born between 2005 and 2018 in SIDIAP.

		Mother-child linkage			Father-child linkage		
N, %	**All children (N=778,318)**	**Linked pairs (N=529,430)**	**Unlinked pairs (N=248,888)**	**% of linked children**	**Linked pairs (N=334,018)**	**Unlinked pairs (N=444,300)**	**% of linked children**
Sex							
Girl	377,734 (48.5%)	257,387 (48.6%)	120,347 (48.4%)	68.1	161,919 (48.5%)	215,815 (48.6%)	42.9
Boy	400,584 (51.5%)	272,043 (51.4%)	128,541 (51.6%)	67.9	172,099 (51.5%)	228,485 (51.4%)	43.0
Year of birth							
2005	60,407 (7.76%)	33,757 (6.4%)	26,650 (10.7%)	55.9	26,240 (7.9%)	34,167 (7.7%)	43.4
2006	61,559 (7.91%)	35,646 (6.7%)	25,913 (10.4%)	57.9	26,933 (8.1%)	34,626 (7.8%)	43.8
2007	61,848 (7.95%)	39,844 (7.5%)	22,004 (8.8%)	64.4	28,733 (8.6%)	33,115 (7.5%)	46.5
2008	64,848 (8.33%)	45,408 (8.6%)	19,440 (7.8%)	70.0	30,629 (9.2%)	34,219 (7.7%)	47.2
2009	62,076 (7.98%)	43,367 (8.2%)	18,709 (7.5%)	69.9	29,299 (8.8%)	32,777 (7.4%)	47.2
2010	61,215 (7.87%)	42,387 (8.0%)	18,828 (7.6%)	69.2	28,786 (8.6%)	32,429 (7.3%)	47.0
2011	59,377 (7.63%)	41,752 (7.9%)	17,625 (7.1%)	70.3	26,172 (7.8%)	33,205 (7.5%)	44.1
2012	57,308 (7.36%)	41,056 (7.8%)	16,252 (6.5%)	71.6	24,473 (7.3%)	32,835 (7.4%)	42.7
2013	53,227 (6.84%)	38,550 (7.3%)	14,677 (5.9%)	72.4	22,592 (6.8%)	30,635 (6.9%)	42.4
2014	53,121 (6.83%)	38,655 (7.3%)	14,466 (5.8%)	72.8	22,220 (6.7%)	30,901 (7.0%)	41.8
2015	51,563 (6.62%)	37,172 (7.0%)	14,391 (5.8%)	72.1	20,626 (6.2%)	30,937 (7.0%)	40.0
2016	45,853 (5.89%)	31,999 (6.0%)	13,854 (5.6%)	69.8	16,806 (5.0%)	29,047 (6.5%)	36.7
2017	44,347 (5.70%)	30,848 (5.8%)	13,499 (5.4%)	69.6	16,282 (4.9%)	28,065 (6.3%)	36.7

2018	41,569 (5.34%)	28,989 (5.5%)	12,580 (5.1%)	69.7	14,227 (4.3%)	27,342 (6.2%)	34.2
MEDEA deprivation index							
Quintile 1 (least deprived)	108,065 (13.9%)	71,616 (13.5%)	36,449 (14.6%)	66.3	39,751 (11.9%)	68,314 (15.4%)	36.8
Quintile 2	112,387 (14.4%)	76,547 (14.5%)	35,840 (14.4%)	68.1	46,530 (13.9%)	65,857 (14.8%)	41.4
Quintile 3	113,623 (14.6%)	77,294 (14.6%)	36,329 (14.6%)	68.0	49,320 (14.8%)	64,303 (14.5%)	43.6
Quintile 4	117,784 (15.1%)	80,328 (15.2%)	37,456 (15.0%)	75.8	53,534 (16.0%)	64,250 (14.5%)	45.5
Quintile 5 (most deprived)	130,090 (16.7%)	86,913 (16.4%)	43,177 (17.3%)	66.8	63,506 (19.0%)	66,584 (15.0%)	48.8
Rural areas	147,058 (18.9%)	104,379 (19.7%)	42,679 (17.1%)	71.0	62,472 (18.7%)	84,586 (19.0%)	42.5
Missing	49,311 (6.3%)	32,353 (6.1%)	16,958 (6.8%)	65.6	18905 (5.7%)	30,406 (6.8%)	38.3
Nationality							
Spanish	673,375 (86.5%)	475,944 (89.9%)	197,431 (79.3%)	70.7	273,403 (81.9%)	399,972 (90.0%)	40.6
Non-Spanish	104,943 (13.5%)	53,486 (10.1%)	51,457 (20.7%)	51.0	60,615 (18.1%)	44,328 (10.0%)	57.8

Table 2. Main characteristics of linked parent-child pairs with PIC and confirmed parent-child pairs.

N, %	**Linked mother-child pairs with PIC (N = 5,353)**	**Confirmed mother-child linkage (N = 5,112)**	**Confirmed pairs (%)**	**Linked father-child pairs with PIC (N = 2,236)**	**Confirmed father-child linkage (N = 2,034)**	**Confirmed pairs (%)**
Overall		95.50%			91.00%	
Sex						
Girl	2,614 (48.83%)	2,486 (48.63%)	95.1	1,053 (47.09%)	955 (47.0%)	90.7
Boy	2,739 (51.17%)	2,626 (51.37%)	95.9	1,183 (52.90%)	1,079 (53.0%)	91.2
Year of birth						
2005	179 (3.34%)	167 (3.27%)	93.3	81 (3.62%)	73 (3.59%)	90.1
2006	212 (3.96%)	201 (3.93%)	94.8	120 (5.37%)	103 (5.06%)	85.8
2007	409 (7.64%)	387 (7.57%)	94.6	176 (7.87%)	153 (7.52%)	86.9
2008	426 (7.96%)	407 (7.96%)	95.5	165 (7.38%)	145 (7.13%)	87.9
2009	419 (7.83%)	394 (7.71%)	94.0	173 (7.74%)	158 (7.77%)	91.3
2010	390 (7.29%)	369 (7.22%)	94.6	186 (8.32%)	167 (8.21%)	89.8
2011	433 (8.09%)	414 (8.10%)	95.6	204 (9.12%)	184 (9.05%)	90.2
2012	456 (8.52%)	430 (8.41%)	93.7	221 (9.88%)	202 (9.93%)	91.4
2013	563 (10.5%)	542 (10.6%)	96.3	245 (11.0%)	224 (11.0%)	91.4
2014	613 (11.5%)	585 (11.4%)	95.4	216 (9.66%)	198 (9.73%)	91.7
2015	667 (12.5%)	650 (12.7%)	97.5	236 (10.6%)	224 (11.0%)	94.9
2016	586 (10.9%)	566 (11.1%)	96.6	213 (9.53%)	203 (9.98%)	95.3

MEDEA deprivation index						
Quintile 1 (least deprived)	413 (7.72%)	405 (7.92%)	98.1	184 (8.23%)	170 (8.36%)	92.4
Quintile 2	678 (12.7%)	647 (12.7%)	95.4	248 (11.1%)	237 (11.7%)	95.6
Quintile 3	629 (11.8%)	602 (11.8%)	95.7	254 (11.4%)	231 (11.4%)	90.9
Quintile 4	1,036 (19.4%)	998 (19.5%)	96.3	434 (19.4%)	391 (19.2%)	90.1
Quintile 5 (most deprived)	1,043 (19.5%)	972 (19.0%)	93.2	466 (20.8%)	412 (20.3%)	88.4
Rural areas	1,090 (20.4%)	1041 (20.4%)	95.5	504 (22.5%)	455 (22.4%)	90.3
Missing	464 (8.67%)	447 (8.74%)	96.3	146 (6.53%)	138 (6.78%)	94.5
Nationality						
Spanish	4,758 (88.9%)	4,555 (89.1%)	95.7	1,786 (79.9%)	1,646 (80.9%)	92.2
Non-Spanish	595 (11.1%)	557 (10.9%)	93.6	450 (20.1%)	388 (19.1%)	86.2

PIC, personal identification code.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Summary of information available on characteristics of linked parent-child pairs.

Parental and children's EHR	Diagnostic codes, specialist referrals, hospital discharges, laboratory measurements, anthropometric measurements, clinical measurements, sick leaves, vaccinations, COVID-19 diagnostics, hospitalizations, and vaccinations
Pharmacological data	Prescriptions and dispensations of medications
Sexual and reproductive health information	Pregnancy follow-up, pregnancy ultrasounds, risk of abortion, mode of delivery, gestational age
Children's health and development (0-14 years)	Birth outcomes, breastfeeding, repeated measurements on weight and height, puberty development, psychomotor development, oral health, ophthalmologic assessments
Sociodemographic and lifestyle characteristics	Age, nationality, MEDEA index of deprivation (at census tract level), individual income level, occupation status, alcohol consumption, smoking status
Environmental exposures (at census tract level)	Air pollution, noise, green spaces, built environment

EHR, Electronic Health Records.

Supplementary Table 2. Main characteristics of children born from 2005 to 2016 with a registered NASS and a personal identification code (PIC) of parents in SIDIAP.

	All children (N=692,402)	Children with mother's PIC (N=7,307)	Children with father's PIC (N=4,420)
Sex			
Girl	335,893 (48.5%)	3,552 (48.6%)	2,086 (47.2%)
Boy	356,509 (51.5%)	3,755 (51.4%)	2,334 (52.8%)
Year of birth			
2005	60,407 (8.7%)	315 (4.3%)	173 (3.9%)
2006	61,559 (8.9%)	378 (5.2%)	210 (4.8%)
2007	61,848 (8.9%)	566 (7.8%)	321 (7.3%)
2008	64,848 (9.4%)	556 (7.6%)	317 (7.2%)
2009	62,076 (9.0%)	544 (7.4%)	342 (7.7%)
2010	61,215 (8.8%)	552 (7.6%)	319 (7.2%)
2011	59,377 (8.6%)	584 (8.0%)	383 (8.7%)
2012	57,308 (8.3%)	587 (8.03%)	427 (9.7%)
2013	53,227 (7.7%)	757 (10.4%)	513 (11.6%)
2014	53,121 (7.7%)	801 (11.0%)	442 (10.0%)
2015	51,563 (7.5%)	869 (11.9%)	494 (11.2%)

2016	45,853 (6.6%)	798 (10.9%)	479 (10.8%)
MEDEA deprivation index			
Quintile 1 (least deprived)	97,355 (14.1%)	600 (8.2%)	358 (8.1%)
Quintile 2	101,567 (14.7%)	905 (12.4%)	540 (12.2%)
Quintile 3	102,259 (14.8%)	869 (11.9%)	524 (11.9%)
Quintile 4	105,739 (15.3%)	1,418 (19.4%)	838 (19.0%)
Quintile 5 (most deprived)	116,053 (16.8%)	1,505 (20.6%)	825 (18.7%)
Rural areas	132,114 (19.1%)	1,376 (18.8%)	1,009 (22.8%)
Missing	37,315 (5.4%)	634 (8.7%)	326 (7.4%)
Nationality			
Spanish	596,758 (86.2%)	6,249 (85.5%)	3,774 (85.4%)
Non-Spanish	95,644 (13.8%)	1,058 (14.5%)	646 (14.6%)

6. DISCUSSION

6.1. General Discussion

In this Thesis we evaluated some common environmental exposures during pregnancy and their effects on respiratory health during childhood. Given the importance of the early life period, we set up a new birth cohort based on EHR to set the basis for future studies on parental and child health from a real-world data perspective. The results of the different studies have been presented and discussed in Chapter 5 of this Thesis. This section aims to provide a general discussion of the methodological considerations and contributions to current knowledge. This section also reflects on the implications this Thesis has for public health and policy and provides suggestions for future research.

6.2. Methodological considerations

6.2.1. Study design and population

Birth cohorts

The use of prospective population-based birth cohorts in three of the studies included in this Thesis is a strength. Because of their prospective nature, birth cohort studies provide a valuable source of information to study causal relations of early life environmental exposures with childhood development and health. Birth cohorts allowed the collection of detailed information on the necessary exposures, covariates, and outcomes of interest in the consecutive follow-ups. The use of several birth cohorts from different European

regions in paper III is also a strength. This allowed us to capture the heterogeneity in the levels of exposures across regions, their background characteristics (e.g., different confounding structures) and susceptibilities across populations. In addition, in paper III we performed IPD meta-analyses. This approach provided a large sample size that enabled us to assess the small effects usually associated with exposure to environmental hazards such as bisphenols (222) and to assess sex as a potential effect modifier. Also, by performing prospective IPD meta-analyses, we increased the strength of evidence without relying on published data and thus limited potential publication bias and reviewer selection bias, which are common limitations of retrospective meta-analysis (223). However, birth cohorts also present some limitations. First, birth cohorts usually are limited in their sample size because they require a considerable amount of time and economic resources. Although we included a large number of participants in all the studies (from 1308 in paper II to 5624 in Study IV), sample size was limited to study some specific exposures such as BPS and BPF, with very few women with detectable levels during pregnancy, and outcomes such as wheezing patterns, with a low proportion of children classified as late-onset wheezers in papers III and IV. Second, as in any birth cohort, loss to follow-up was present and this may have led to bias if losses were related to the studied exposure, to the outcome, or to a relevant characteristic of the study population. In our studies, loss to follow up resulted in the selection of higher educated and more likely to be of European origin mothers. However, we assumed that losses happened at random or completely at random as they were not related

to the exposure or the outcome of interest. Third, information on some outcomes and covariates was collected through questionnaires. Although that is the most common approach in birth cohort studies to acquire such data, this implies the risk of recall bias and self-reporting bias in the information collected. Fourth, birth cohorts included in this Thesis were not initially designed to study the effects of bisphenols and hence the biosample collection strategy followed (i.e., collection of one or two spot urine samples) was not the most adequate to assess exposure to these compounds. Finally, birth cohorts provide information on past exposures (between 11 and 22 years for cohorts included in this Thesis) which may not reflect current populations exposure levels.

Real-world data

In paper V, we created a new parent-child cohort by linking their EHR. The main strengths of this novel cohort are its big sample size and the availability of data not only from mothers but also from fathers, which is information usually not available in most birth cohorts. This cohort was created from the SIDIAP population, which includes pseudo-anonymised information from a population sample that is representative of the overall Catalan population. Because EHR information is routinely collected, this cohort provides the source to conduct large studies with abundant data without the high costs that are usually needed in birth cohorts. Also, it allows researchers to conduct retrospective follow-ups, facilitating the creation of large longitudinal studies that are feasible in terms of time and costs. This is an open cohort that will be periodically updated. This means

that newborns entering the SIDIAP database every year will be automatically linked and included in the cohort, assuming a successful linkage process. However, this cohort also presents some limitations related to the nature of real-world data. First, the data available is routinely collected in clinical practice, and thus is not collected purely for research purposes. Second, despite the cohort contains a number of health data, it may lack information on relevant lifestyle or non-healthcare related characteristics that can be collected in other study designs like birth cohorts. However, this cohort includes socioeconomic data on deprivation index, nationality, type of residential area (urban, rural), individual income level (<18,000€, 18,000€ to 100,000€, >100.000€ per year) and type of occupation (active, retired). Third, there is the risk of under-reporting of some health factors. For example, there may be a portion of the population that despite having an ICS centre assigned, they may not make use of it (i.e., using private healthcare instead). In addition, this cohort includes the population assigned to a healthcare centre belonging to the ICS. Therefore, information of the population who attend healthcare facilities from other providers is not available. However, this might not result in selection bias because the ICS is the main provider, it covers around 80% of the Catalan population (205) and presents characteristics that are representative of the overall population (206). Linked parent-child pairs were also representative of the overall paediatric population of Catalonia in terms of sex, year of birth, and deprivation index. However, those from non-Spanish nationalities might be underrepresented because the linkage algorithm tended to be less successful among parent-child pairs from

a non-Spanish nationality. Thus, further studies are needed in order to better understand the reasons for unconfirmed linkages and to investigate whether parent-child linkage can be improved in these population subgroups.

6.2.2. Exposure assessment

OCs

In paper II, OCs were measured from maternal serum during pregnancy and/or cord blood samples at birth. The persistent nature of OCs, with half-lives of years, enables to estimate the overall exposure during pregnancy with a single measurement (224). Thus, in paper II, the risk of exposure misclassification is low. Since cord blood is considered the best proxy of prenatal OCs exposure (225), we estimated cord serum OCs in those participants that had only maternal serum measurements. We were able to create cohort-specific conversion factors from participants from INMA Gipuzkoa and INMA Valencia cohorts that had measurements in maternal serum and in cord blood. We performed sensitivity analyses using the maternal serum OCs measurements instead and results remained robust. Because OCs are lipophilic (i.e., accumulate in adipose tissue), we repeated the analyses using serum lipid-adjusted concentrations. Again, results remained robust. Levels of OCs in blood have been reported in previous studies to be generally highly correlated (76,226,227), thus making it difficult to ascertain the effects related to each specific compound. In our study population the correlations were generally low (e.g., r=0.14 for DDE and HCB) but

high between PCBs (e.g., r=0.88 for PCB-153 and PCB-180). In addition, we performed multipollutant models including all main OCs *(p,p'*-DDE, HCB, and ΣPCB) and results remained robust, which clarified the interpretation of the results obtained for each specific compound. Although we are aware of the existence of more advanced statistical approaches that allow the identification of the compound within a mixture more associated with the outcome, such as weighted quantile sum and Bayesian kernel machine regression, these methods were not fully developed at the beginning of this Thesis, and we did not apply them. Last, all the examined PCBs were non-dioxin-like. These PCBs have different biochemical and toxicological properties compared to dioxin-like PCBs, and therefore may have different effects on health (228). Thus, our results cannot be extrapolated to all PCBs.

Bisphenols

In paper III, we assessed BPA, BPF, and BPS from maternal urine samples collected during pregnancy. We adjusted bisphenols concentrations for creatinine to correct for urine dilution by dividing bisphenols concentrations by the concentration of urinary creatinine. This has been the most used standardisation approach to adjust for urine dilution in previous studies (229) although there are other methods such as specific gravity (230). In paper III we determined exposure to bisphenols from 1 or 2 spot urine samples. Because bisphenols are non-persistent organic pollutants with short half-lives (i.e., hours), the assessment from 1 or 2 spot urine samples might have led to exposure misclassification. However, misclassification is

suspected to be non-differential (i.e., not related to the outcome). Therefore, this is likely to have attenuated our results, biasing the estimates towards the null, without increasing the risk of bias towards false-positive results (231). Also, the high within-day and between-week variability (232) also limited the assessment of critical windows of exposure in our study (i.e., trimesters). Although we assessed BPF and BPS, measurements were only available in four of the eight cohorts included, and the number of samples with detectable levels was generally low. Thus, we were not able to consider the exposure as continuous and categorised them into detected and undetected. In our population, mothers among the detected group tended to smoke less and had a higher educational level compared to those among the undetected group. We suspect mothers with higher education and a healthier lifestyle tend to use more BPA-free products which in turn may contain BPA substitutes, as observed for parabens (233). Although we adjusted our models for lifestyle and socioeconomic factors, residual confounding cannot be ruled out, plus our results might reflect a selection of wealthier and healthier subsample than the associations with the exposures. A strength of paper III was the use of eight ongoing birth cohorts, but that implied that the exposures were measured at different laboratories. In order to ensure comparability of results between cohorts, we conducted an interlaboratory comparison. Although we observed poor correlations between Granada and Córdoba laboratories in relation to the other laboratories (Pearson $r<0.5$), after excluding INMA Gipuzkoa (Granada laboratory) or INMA Sabadell (Córdoba laboratory) cohorts from the analyses, results did not change.

Urban environment

A strength of paper IV is that we assessed a wide range of exposures related to the urban environment. We included 44 exposures that gave information on the maternal exposure levels of air pollution, noise, traffic, green and blue spaces, and built environment during pregnancy. This wide exposure approach might have helped to avoid publication bias and provided a better adjustment for confounding co-exposures, compared to single pollutant approaches. Exposure assessment of air pollutants was adjusted for temporal variability, thus limiting to some extent possible measurement error. Also, we included information on area-level SES, which provided an added value for the assessment of the associations of urban environment with childhood respiratory health. SES plays an important role in the associations of the urban environment with respiratory health in childhood. Disentangling the effects of SES on the associations of urban exposures with health outcomes is not straightforward and is likely to vary across urban settings (234,235). Thus, by considering area-level SES, in addition to individual level SES information, we could diminish the possible confounding of exposures due to SES.

Exposure assessment of the urban environment variables has some methodological considerations. First, exposures were assessed as the mean exposure levels during pregnancy estimated at the home address of the mothers. Although this is a common approach in population-based studies, especially those with big sample size, the estimation of exposures at the home address may not reflect the actual exposure of the mothers not considering their movements across the

city with various levels of urban exposures, other micro-environments such as the workplace, or time spent outdoors, for example. A more detailed and accurate exposure assessment such as personal monitoring could reduce exposure misclassification. However, personal monitoring is laborious and is highly time and economic costly. Second, measurement error bias might be of a different magnitude across exposures. Thus, the comparison of each exposure associations with respiratory health should be interpreted cautiously. However, as exposure assessment was independent of the health outcomes explored, we expect that any misclassification bias would be non-differential, and therefore might have attenuated the effect estimates without increasing the risk of false positives. Third, among non-time varying exposures or highly correlated exposures in time (e.g., green and blue spaces), we cannot exclude that observed effects might not purely reflect the exposure during pregnancy but the postnatal exposure. To ensure the associations reflected the exposures during pregnancy and were not confounded by postnatal exposures, we further adjusted our analyses by postnatal exposures measured the year prior to the outcomes assessment, and most results remained robust. Last, although we assessed a wide range of exposures, we did not include other exposures also related to the urban environment that might be of relevance to childhood respiratory health (e.g., ultrafine particles, volatile organic compounds, type and quality of green spaces, pollens, light exposure at night) and merit further investigation.

6.2.3. Outcome assessment

Lung function

In papers II, III and IV, lung function was measured from spirometry tests. Spirometry is a highly valuable tool to obtain an objective measure of lung function. In the three papers, spirometry was performed following the ATS/ERS guidelines (219). In all cases, lung function was measured by trained personnel and following common protocols established by the ATS/ERS guidelines, which reduced the risk of measurement error.

In paper II we assessed lung function in pre-school children (mean age 4 years). The validity of lung function testing by spirometry in pre-school children has been debated for many years. Performing spirometry tests in that age-range is challenging because of the poor cooperation of the participants (236). However, many studies have shown that it is possible to obtain reliable lung function measurements at these ages (132,237–240). In addition, the ATS/ERS stated that spirometry in children aged <6 years is possible (241). In paper II, to ensure the optimal reliability, spirometry tests were performed by pulmonologists or trained nurses that followed the ATS/ERS recommendations for that age and ensured a maximal collaboration of children. In addition, and for all spirometries performed within the INMA cohorts (used for papers II and III), we developed a protocol (Appendix G) to perform an additional cleaning process of spirometries in which a trained researcher thoroughly inspects each of the obtained spirometries and determines their

acceptability and reproducibility for their use in analyses. However, in the rest of the HELIX cohorts (BiB, EDEN, MoBa, and RHEA) (paper III), the additional cleaning process of spirometries was not done manually and the shape of each curve was not inspected. It was automatised following the ATS/ERS criteria. To ensure that this cleaning process did not introduce bias, 243 examinations from INMA were cleaned using both approaches (i.e., inspecting each curve manually vs the automatised cleaning process). The same manoeuvre was selected from both approaches in 79% of the cases. For the remaining 21%, the correlation between the FEV_1 of the different curves selected was very high (Pearson correlation r=0.96) (74). Measurement error of spirometry in all papers (papers II, III, and IV) was also examined by repeating the analyses among children who had reproducible spirometries. In all cases, results remained robust. Despite minimizing possible bias due to measurement error, lung function estimates need to be carefully interpreted since the spirometry measurement error might be greater than the estimates, when the estimates are expected to be small.

In papers II, III, and IV, the effects of several environmental exposures in relation to childhood lung function was examined in eight different birth cohort studies. To reduce possible bias due to between-cohort variation, all analyses were adjusted for cohort. In addition, we used the validated GLI age-, height-, sex-, and ethnicity-adjusted z-scores (220) to ensure comparability of lung function estimates across cohorts, especially in papers III and IV, where populations included were ethnically more diverse.

We based the lung function assessment on the FEV_1, FVC, and FEV_1/FVC parameters. Additionally, in papers III and IV we also explored $FEF_{25-75\%}$. FVC, FEV_1/FVC and $FEF_{25-75\%}$ are parameters more prone to measurement error, as they are highly dependent on a correct complete forced exhalation. This needs to be considered when interpreting results related to these parameters. However, we would like to highlight the added value of $FEF_{25-75\%}$ as an early marker of small airways impairment (242). Thus, it is worth putting efforts in obtaining reliable measurements in order to further explore this parameter in future studies. In our studies we did not consider neither lung function trajectories nor spirometric phenotypes (i.e., obstructive, abnormally low FEV_1/FVC, and restrictive, abnormally low FVC but normal FEV_1/FVC). Both approaches allow a more accurate identification of young children at higher risk of disease (9,243). Therefore, identifying early life environmental exposures in relation to lung function trajectories and spirometric phenotypes among children would be of relevance.

In our studies we did not perform the bronchodilator test for a better identification of an obstructive pattern because of the complications of performing that test in a birth cohort setting. Indeed, in the INMA Sabadell cohort we performed a pilot study when children were aged 11 years old to know if parents were in favor of administrating a bronchodilator to the child, and the majority of them did not accept.

Wheeze and asthma

Wheeze and asthma outcomes were explored in papers III and IV. These outcomes were assessed from parental reported questionnaires adapted from the ISAAC study, which is a validated tool and the most common approach to assess these outcomes in population-based birth cohort studies (23). Strengths on the outcome assessment in this Thesis include the assessment of wheezing patterns across childhood and the use of a validated asthma definition proposed by the MeDALL project (24) in paper III. In paper IV, a slightly more conservative definition was used. However, some limitations need to be discussed.

The reporting of outcomes from questionnaires might have led to recall bias and misclassification bias. For example, parents may not remember wheeze episodes suffered by their children if those are not particularly recurrent or do not progress to asthma. This is of concern in the wheezing patterns assessment in paper III. The data on wheezing episodes available across ages differed between cohorts. In Generation R and INMA cohorts, information on wheezing before the age of 4 years was available at two to four time points, whereas in BiB, EDEN, MoBa, and RHEA, data on wheezing before 4 years was only available at one time point. In these cohorts wheezing until 4 years was asked as "ever had wheezing until 4 years" and/or "had wheezing in the last year at the age of 4 years". We suspect that in these latest cohorts a considerable proportion of children that had wheezing episodes between 0 and 3 years were wrongly classified as never wheezers. However, when excluding these cohorts from the

models, estimates did not change. In paper IV, data on wheezing episodes was available for every year until the age of 5 years, but there was no information until 9 years of age. We suspect a proportion of children were wrongly classified as not having wheeze in the school-age period and might have affected late and persistent wheezing classification.

We considered doctor diagnose asthma, wheeze in the previous year, and asthma medication use in the previous year to define asthma. In paper III, asthma was defined as present if two of the three former conditions were met. In paper IV, a slightly stricter definition was used: doctor diagnose of asthma with either wheeze or asthma medication use in the previous year. However, assessing asthma in epidemiologic studies is challenging. When considering populations from different countries, variations in asthma may also reflect international differences in the asthma diagnosis practices or adherence to the guidelines (244). Another main challenge, and related to the previous, is the absence of a general consensus for its definition, which in part is consequence of its inherent heterogeneity (26). Asthma is an umbrella disease that covers many phenotypes. Until these phenotypes are better characterised and those are used in studies assessing environmental risks of asthma, the risk of outcome misclassification will be present, resulting in a dilution of risk estimates (26). Further, different asthma definitions across studies impedes the comparison of results and adds inconsistencies in reported estimates. An example of this was presented in a review (245) of 122 cohort studies assessing risk factors of childhood

asthma. They identified 60 different definitions of asthma were used. They estimated an overall agreement of 61% using the four most common definitions. This means that up to 39% of participants would be differently classified depending on the definition used (245).

Last, we did not assess other outcomes which might be of relevance such as respiratory tract infections, atopy, allergic sensitisation, biomarkers of inflammation, or the presence comorbidity such as the atopic triad (i.e., atopic dermatitis, allergic rhinitis, and asthma) (246), among others. Wheezing, especially at younger ages, might be a consequence of respiratory tract infections (247), and atopy is frequently present in children with asthma or presenting other respiratory or allergic symptoms that need to be considered under the asthma umbrella concept (248). This goes in line with the need to distinguish asthma phenotypes in children and use those as outcomes in studies instead.

6.3. Contribution to the current knowledge

This Thesis contributed to the understanding on the effects of common environmental exposures during pregnancy on children's respiratory health including chemical pollutants and exposures related to the urban environment, added new insights on the role of urban environments on children's respiratory health from a wide range of exposures perspective, and provided a new data source to develop studies on parental-child health, perinatal health, and early life exposures using Real-World Data. More specifically, and in concordance to the Thesis objectives, this Thesis contributed to the

understanding of: 1) the current knowledge on the effects of prenatal exposure to organic pollutants on children's lung function, 2) the effects of prenatal exposure to organochlorine compounds on children's lung function, 3) the effects of prenatal exposure to bisphenols on children's respiratory health, 4) the effects of maternal exposure to the urban environment during pregnancy on children's respiratory health, and 5) the creation of a new parent-child cohort linking EHR from the SIDIAP database in Catalonia.

6.3.1. Chemical pollutants

Current state of the evidence

In paper I, we provided a narrative review of the current evidence on the effects of prenatal exposure to organic pollutants on lung function in the offspring. We examined persistent organic pollutants (OCs and PFASs) and non-persistent organic pollutants (bisphenols, parabens, triclosan, benzophenone-3, phthalates, and currently used pesticides). We found insufficient evidence for all the assessed organic pollutants, with few studies available and inconsistent results across them.

Regarding OCs, only three studies have assessed prenatal exposure OCs in relation to children's lung function, including the study (paper II) developed as part of this Thesis. The three of them found evidence of lower lung function in relation to prenatal exposure to OCs, mainly *p,p'*-DDE. Evidence on prenatal exposure to bisphenols was focused on BPA. Of the five studies that explored the association with childhood lung function, only one reported decreased lung function

at 4y. The other studies reported null associations. There was no evidence on other bisphenols. In paper I we further discussed potential causes of inconsistencies and limitations of the studies conducted on the topic including exposure assessment, outcome assessment, statistical approaches, and their external validity. Based on the limitations, we provided recommendations for future research. Some of these recommendations were considered to conduct a meta-analysis of the association of prenatal bisphenol exposure with children's respiratory health in paper III.

Organochlorine Compounds

In paper II we highlighted the importance for the study of OCs because, although OCs were banned since the 1980s (65), we showed that population born two decades later were still exposed to them. We detected quantifiable levels of *p,p′*-DDE, HCB, PCB-138, PCB-153, and PCB-180 in more than 80% of the studied population. *p,p′*-DDE was the most prevalent compound and the one with the highest median concentrations. This showed that current populations, and consequently future generations, are and will be still exposed to these compounds.

In paper II, we examined the association of prenatal exposure to OCs with lung function at 4 years and 7 years of age in three INMA birth cohorts. We observed that prenatal *p,p′*-DDE was associated with reduced lung function during childhood, especially FEV_1 and at medium levels of exposure. We also observed reduced FVC at low exposure levels of HCB at 7 years and we did not find consistent

associations with any of the explored PCBs. In paper II we additionally included a repeated measurement analyses in a subsample of children that had lung function tested at 4 and 7 years of age. In these analyses we observed an overall association between prenatal exposure to *p,p'*-DDE and reduced FEV_1 across childhood. These results are in line with two previous studies. In a Danish birth cohort exposed they reported an association of very high levels of *p,p'*-DDE, HCB, and PCBs with increased risk of airway obstruction (FEV_1/ FVC < 75%) at 20 years of age (73). In a recent study assessing the totality of environmental exposures during pregnancy on lung function, *p,p'*-DDE tended to be associated with reduced FEV_1 at school-age, but results did not reach statistical significance (74). In paper II we observed reduced lung function in relation to *p,p'*-DDE even at levels much lower compared to those observed in the Danish birth cohort (73). Similar to previous studies on OCs and respiratory health, we observed associations at certain exposure concentrations, and not necessarily at the highest exposure levels. This contributed to the existing evidence on non-monotonic exposure-response that OCs may follow. This means that increasing concentrations will not necessarily have greater effects on the complete exposure range, and that lower exposures may be also of concern.

Bisphenols

In paper III, we assessed BPA and two emerging substitutes: BPF and BPS. This study was the first to assess prenatal exposure to BPF and BPS in relation to children's respiratory health. This is of importance

because these substitutes are becoming more present in daily life products as a result of production restriction and health concerns of BPA (83,249). In addition, BPF and BPS have similar biochemical structure and mechanisms of action than BPA and thus, may have similar deleterious health effects (86,250).

We detected concentrations of BPA among 90% of the samples across cohorts. This is of relevance because highlights the widespread exposure to BPA, which is of special concern in vulnerable populations such as pregnant women. Exposure to BPF and BPS was lower (detected in 40% and 70% of samples, respectively). However, it needs to be noted that such detection levels reflect exposures from 2002 to 2008, which not necessarily are the current levels. We would expect current exposure levels to BPA substitutes to be higher nowadays because of their progressive production and use in the market in the last decade due to the increasing concerns and stricter regulations of BPA. Therefore, further studies assessing the emerging BPA substitutes are needed.

Before this Thesis, the number of studies assessing prenatal BPA exposure in relation to childhood respiratory health was limited and results were inconsistent. In paper III, we observed that prenatal exposure to BPA increased the odds of asthma and wheeze at school-age among girls. This did not seem to be explained by lung function adaptations, but rather through immunomodulatory mechanisms. This could be explained by the ability of bisphenols to cross the placenta (251) and interfere with the developing respiratory and

immune systems by binding to a number of receptors related to inflammatory and oxidative stress pathways that have been observed in *in vitro*, animal, and human studies (91,252–254). Although animal studies have observed deleterious effects on the structural development of the lung after prenatal exposure to BPA (87,255), we could not confirm that in our study. In our study, BPA was positively associated with lung function at school age but only among boys, estimates were very small and generally towards the null, and associations disappeared in sensitivity analyses. Most previous birth cohort studies did not find associations between prenatal BPA and lung function at school age (74,93,94). Only one cohort study associated prenatal BPA exposure with a decrease in $\%FEV_1$ at 4 years but this association disappeared at 5 years of age (92). Our findings of an association between prenatal BPA and increased risk of asthma-related symptoms during childhood are in line with some (92,93,95–98) but not all (99–101) of previous studies.

Also, paper III adds evidence for potential sex-dependent effects of BPA exposure, only assessed in few studies that yielded contradicting results (96–98). The lack of consistency of sex-specific effects of previous studies might be partly explained by their small sample size. By combining eight cohorts and performing a meta-analysis with the largest sample size of studies on the topic to date, we were able to assess the sex-dependent effects with greater power than previous studies. Such sex-dependent effects could be explained by the hormonal activity of bisphenols. As an estrogenic chemical, BPA may exert different effects on male and female due to the complicated internal environment, such as different hormone level,

hormone type, and metabolic rate of BPA (256). Fluctuations of sex hormones and their consequences on immune functions can play a role in asthma pathogenesis, as observed in asthma incidence in hormonally changing periods such as puberty, pregnancy, and menopause (257).

6.3.2. Urban environment

In paper IV, we assessed a broad range of exposures related to the urban environment. This study was the first to assess the prenatal urban environment in relation to respiratory outcomes other than lung function. In this study we assessed multiple respiratory outcomes through childhood that provided a broader picture to understand the effects of urban exposures on respiratory health.

We observed that the median air pollution levels to which mothers were exposed during pregnancy (NO_2=38.48 (7.48) μg/m^3, PM_{10}= 31.36 (6.14) μg/m^3, $PM_{2.5}$= 19.72 (3.83) μg/m^3) were slightly below the current EU regulations (40 μg/m^3 for NO_2 and PM_{10}, and 25 μg/m^3 for $PM_{2.5}$), but above the threshold recommended by the WHO 40μg/m^3 for NO_2, 20 μg/m^3 for PM_{10}, 10 μg/m^3 for $PM_{2.5}$. Almost half of the mothers were exposed to daily noise levels above the EU regulations (Lden≥55 dB) and 37% were exposed to night noise levels above the EU regulations (Ln≥50 dB). Remarkably, exposure to natural spaces was high. Most mothers had access to major (>5,000 m^2) green (80%) and blue (54%) spaces.

In paper IV, we were able to explore the associations of the urban environment during pregnancy with childhood respiratory health by

combining three complementary approaches: 1) single-exposure analyses (ExWAS), 2) multiple exposure analyses (adjusting by co-exposures), and 3) cluster analysis of multiple exposures. Single-exposure analyses tested exposure-by-exposure associations with the outcomes. This approach displays a high sensitivity compared to other regression methods in the context where multiple exposures are examined (258). However, it presents two main limitations. First, this approach presents a high false discovery rate compared to other methods. To reduce false-positive results, we corrected for multiple hypothesis testing using an adapted version of the Bonferroni correction that takes into account the correlation structure of the data (259). Second, it does not control for between-exposure confounding. To overcome this limitation, we also performed two multiple-exposures analyses (approaches 2 and 3). In multiple-exposures analyses (approach 2), we adjusted by the models by co-exposures considering their causal structure. With this approach we reduced the rate of false-discoveries by adjusting for the most appropriate set of co-exposures. By taking into account the causal structure of exposures, we reduced the risk of over-adjustment of the models. Last, because each urban setting is unique, we performed a cluster analysis (approach 3) to evaluate specific urban exposure patterns of the study population in relation to children's respiratory health. However, this approach does not allow to capture the specific effect estimates of each exposure with the outcome.

An added value of paper IV is that we examined the associations taking into account the area-level SES in addition to individual level

SES characteristics. We adjusted single and multipollutant models by area-level SES and we standardised the exposures by area-level SES in cluster analyses. By standardising the exposures, we avoided the influence of the variance by SES, and minimised residual confounding by SES. The way how SES influences on studies of urban exposures and health is not clear. While in the US studies have shown an association of higher deprivation with more harmful environments, in the EU findings have been mixed (234). In our study population levels of exposure across SES quintiles was mixed. We did not observe an increasing pattern of air pollution with increasing deprivation, observing the highest median levels of NO_2 in quintiles 4 (highly deprived) and 2 (low deprived). Highest levels of noise were observed in the two most deprived quintiles. Quintiles 1 (least deprived) and 3 (medium deprived) presented the highest levels of green spaces, and quintiles 1, 2, and 4, the highest levels of blue spaces. Therefore, in our study population, some but not all harmful environments were related to higher deprivation.

In paper IV, results showed that in single and multiple exposure analyses, higher maternal exposure to NO_2 and $PM_{2.5}$ during pregnancy was associated with lower lung function only in mid-to-small airways in the child. Our results highlight the relevance of $FEF_{25-75\%}$ as an early marker of small airways impairment (242). Counterintuitively, higher levels of $PM_{2.5}$ increased FEV_1 and FVC. However, these associations were no longer observed when considering the urban environment as a whole in cluster analyses where we standardised exposures by area-level SES. Similar results

were observed in the first study that assessed the totality of the prenatal urban exposures in relation to childhood lung function (74). A positive association was observed between inverse distance to the nearest road (i.e., a proxy of traffic related air pollution) and lung function; the authors considered it a spurious finding (74). Air pollution exposure during pregnancy can affect the lung and immune system development because air pollutants inhaled by the mother during pregnancy can cross the placenta (117). Our results for $FEF_{25-75\%}$ are in line with a previous study where higher prenatal NO_x was not associated with FEV_1, FVC or FEV_1/FVC, but was with lower $FEF_{25-75\%}$ (21). Some single exposure studies (only assessing the effects air pollution) reported associations of higher exposure during pregnancy to $PM_{2.5}$ and PM_{10} with lower FEV_1, FVC or FEV_1/FVC (121,130,131,133) while others reported no associations (74,130,135,136). Our contradictory results of higher $PM_{2.5}$ with higher FEV_1 and FVC should be further explored before any strong conclusions could be made. Our study did not shed light on the current inconsistent evidence regarding prenatal exposure to air pollution and wheezing and asthma during childhood (121,129–136). We observed counterintuitive associations with wheeze and asthma outcomes because higher exposure to $PM_{2.5}$ was associated with decreased risk of persistent wheeze and no association was observed with asthma. In our population, air pollutants, mainly PMs, had narrow ranges, which IQR between 0.39 $\mu g/m^3$ in $PM_{2.5abs}$ and 6.14 $\mu g/m^3$ in PM_{10}. This might have biased the effect estimates by limiting adequate comparison groups, as lower exposed participants

were already exposed to high levels of air pollution that were very similar to those highly exposed.

This is the first study to show that high road traffic noise levels were associated with increased risk of early wheezing and asthma at 10 years of age. The two studies that have explored these associations reported null findings (74,171). This is also the first study to show an association of increasing size of the nearest major blue spaces with higher lung function and lower odds of asthma. Only one previous study assessed this and did not find any association (74). However, in that study they assessed blue spaces as their presence in a 300m buffer, to which we did not find any associations either. Given the potential health benefits of blue spaces and the scarcity of studies conducted, further research is guaranteed. We did not find any association with green spaces exposure. We observed narrow ranges of green spaces (IQR for NDVI was 0.14), which might also have underestimated the effect of green spaces on respiratory health by lack of adequate comparison groups. Also, we based our exposure assessment of green and blue spaces on availability and accessibility, but we did not have information on other characteristics about the quality, safety or use of such spaces that might be relevant for exposure-health assessments.

Our study was the first that applied a cluster analysis approach in relation to childhood respiratory health. Other studies have applied it in relation to other health effects such as obesity (260) and blood pressure (261). Although this approach has some limitations such as

that it cannot identify the urban exposure mostly associated with the outcome, it allows to distinguish children sharing similar urban exposure patterns. In our study, we observed that living during pregnancy in an area characterised by higher air pollution, noise, urbanisation, and lower levels of natural spaces was associated with lower $FEF_{25-75\%}$ and increased risk of early and late wheeze but lower risk of persistent wheeze. Finally, it is important to highlight that the study of the totality of the urban exposures in relation to health is an ongoing developing field and there is no consensus on which is the best strategy to follow to address their combined effect.

6.3.3. Linkage of parent-child EHRs

In paper V, we were able to successfully link the EHR of 719,858 children born from 2005 to 2018 to the EHR of at least one potential parent in SIDIAP, which represents 92.5% of the SIDIAP paediatric population born in that period. Two thirds of children were linked to their potential mother and 42.9% to their potential father. Additionally, 143,590 (18.5%) children could be linked to the EHR of both parents. We developed the linkage through a deterministic approach by combining information on the parents' and children's NASS, ages, surnames, and dates of delivery and birth. We further validated the linkage algorithm in a subgroup of 7,307 mother-child pairs and 4,420 father-child pairs with available information on their PIC, which is a unique identification code for healthcare access that allows a direct link of parents to their children. Overall, the validation confirmed 95.5% of mother-child pairs and 91.0% of father-child pairs.

With this linkage we did not aim to create a novel algorithm, but to apply a similar approach to those previously developed in other settings to the specific characteristics of the SIDIAP database to create a new parent-child cohort. Previous studies followed diverse approaches that were specific to the characteristics of each setting. Some opted for probabilistic approaches using for example maternal age, municipality, birth records or diagnostic codes (262–265). Others, similar to our approach, followed deterministic approaches through family identification numbers, national healthcare numbers or combined the previous with other characteristics such as dates of birth and discharge, and maternal and child characteristics (266–271). The choice of probabilistic or deterministic approaches is made depending on the information availability. Many national registries in Europe anonymise their EHR, which enhances the use of probabilistic approach using sociodemographic or clinical characteristics, while others make universal identification numbers available. Universal identifiers, such as the NASS, facilitate the use and reliability of deterministic linkages (272). In paper V we were able to perform a deterministic linkage of parent-child EHR data in SIDIAP thanks to the access to personal data through a Third Trusted Party, including surnames and co-insurance numbers. The combination of personal data to additional information such as age of potential parent, or date of birth and date of delivery for potential mothers, helped avoiding possible misclassification of linkage to other female/male co-insurance (e.g., brother/sister or grandparents).

This new cohort contains information with the potential to be used for different study designs (e.g., birth cohort, case-control, case-crossover). In addition, the EHR of each study participant has been linked to other databases including environmental exposures, socioeconomic, pharmacy, and hospital data. Thus, this new cohort also provides a valuable source of data for future research on parental and child health in addition to early life determinants of children's health. It includes detailed information on: 1) Parental and children's EHR including diagnostic codes, specialist referrals, hospital discharge, and laboratory and clinical measurements; 2) Prescriptions and dispensations of medications; 3) Sexual and reproductive health information, including also all pregnancy assessments (e.g., ultrasounds, mode of delivery, gestational age); 4) Children's health and development information from the "Childhood with Health" (*Infància amb Salut*) (273) program that periodically assesses multiple health and developmental outcomes in children attending the Catalan public healthcare services from birth to 14 years of age (e.g., birth outcomes, breastfeeding, weight, height, BMI, puberty development, psychomotor development, oral health, ophthalmologic assessments); 5) Sociodemographic characteristics (e.g., age, nationality, area-level SES, individual income level, occupation status); and 5) Environmental exposures estimated at the census tract level (e.g., air pollution, noise, green spaces, built environment).

6.4. Implications for Public Health and Policy

The promotion of safe and healthy environments, especially during vulnerable periods of life such as pregnancy and childhood, should be a major public health priority. Determinants of health and disease in these periods are of utmost importance as their effects may track into adult life. Therefore, by aiming at prevention strategies in early life, the beneficial effects may not be reduced to pregnant women and children's health, but to the overall population's health.

As shown earlier, the exposures studied in this Thesis are ubiquitous. This in itself should be a public health concern. Although the observed effects of chemical pollutants and urban environment on children's respiratory health may not be clinically relevant at an individual level, they are important from an etiological perspective and at a population level. As mentioned in paper I, an example of the relevance of small effects of environmental exposures at the populations health level is the case of lead exposure in children and the reduction of the intelligence quotient (IQ) by 6 points (274,275). This reduction at a population level is estimated to nearly double the number of people with an intellectual disability (i.e., IQ below 70) (276). Thus, even in the absence of consistent evidence about the detrimental health effects of a given exposure, the precautionary principle should prevail.

Overall, the results of this Thesis suggest that early life exposure to common environmental pollutants affect children's respiratory health. These exposures are some of the most common exposures.

They are present in food, daily life products, and compose the environments where most of the world's population live in. Because of that, this is not a matter of concern only to the Biomedical or Public Health field, but also, and most importantly, to international institutions with capacity to regulate the presence of those exposures. In this sense, it is important to consider this as a global issue that requires common strategies and regulations across countries. Individual measures to reduce harmful exposures can be taken, and it is important that populations are aware of them and of changes they can make to reduce them. However, the focus must be put on legislative bodies capable of making global and lasting changes. Only in this way we will achieve changes that revert into healthier and lasting effects on the population.

Specifically, the findings of this Thesis suggest that prenatal exposure to OCs negatively influences children's lung function. OCs were banned in the EU in 1983, and worldwide in 2004 when the Stockholm Convention entered into force (65). However, the use of *p,p'*-DDT is permitted for malaria vector control in endemic countries (277), which has generated a strong debate in the international community. This Thesis adds evidence on the detrimental effects of prenatal *p,p'*-DDE, a metabolite of *p,p'*-DDT, on children's lung function. Results of this Thesis provide further evidence for policy makers to include in the debate. This Thesis also adds evidence to add in the debate of currently used pesticides. The ban of persistent pesticides such as *p,p'*-DDT has led to the extensive use of non-persistent alternatives pesticides (e.g., organophosphate

pesticides or glyphosate) (278) that can also have detrimental health effects. The massive use of aggressive pesticides in agriculture is a consequence of the global agri-food market and is a threat to human health and the ecosystems. Based on the precautionary principle and from lessons learned from *p,p'*-DDT, pesticides suspected to have health effects should be reduced, if not eliminated. An alternative is to opt for a more locally focused production of food that does not rely on the extensive use of such pesticides.

This Thesis suggests that prenatal exposure to BPA negatively influences children's respiratory health. Our results provide a better understanding on the role of bisphenols in the causation of respiratory symptoms and asthma. This suggests important opportunities for asthma prevention since exposure to these chemicals is modifiable. When the Thesis started, policies to reduce exposure to BPA were in place in some countries such as France, but such measures were not widespread. For example, in Spain there is no national regulation on endocrine disrupting chemicals. In 2017, the European Chemical Agency considered BPA as a "substance of very high concern" (84,85). Consequently, in the last years, BPA production has been restricted in some countries, arising the emergence of substitutes such as BPF and BPS (86). From 2020, the EU has banned the use of BPA in thermal papers (Commission regulation 2016/2235) but has not included any regulation on its analogues. Hence, the results of this Thesis appear in a crucial moment in which regulation and exposure guidelines for endocrine disrupting chemicals are in the spotlight in Europe. We did not find conclusive evidence for BPF and BPS. We

were limited in their assessment by the small number of samples with detectable levels. However, they reflect levels from 2002-2008 which not necessarily are current levels, that we expect to be higher. Because they can have similar toxicity than BPA (250), similar regulations should be taken for the substitutes. Current EU chemical legislation is based on substance-by-substances approaches instead of chemical classes. This means that current regulation is focused on BPA, obviating analogues with suspected similar toxicity (279). Thus, regulation needs to move forward by limiting entire chemical classes instead of individual compounds.

This Thesis also suggests that the urban environment during pregnancy might shape children's respiratory health until school-age. Urban planning is of greatest importance in determining the health of the population. How a city is designed determines how people in that city live and what they are exposed to (280). Results from this Thesis suggest that living during pregnancy in an urban environment characterised by higher levels of air pollution, noise, urbanisation, and lower levels of natural spaces may negatively affect children's respiratory health. These results add evidence to the existing on how inadequate urban planning negatively affects the health of its inhabitants (194,281). It is important that policy makers shift priorities from private to active and public transportation modes (i.e., cycling, walking). In addition, urban planning needs to be inclusive in order to promote healthier environments in an equitable manner by reducing social inequalities that do not leave any collective or neighbourhood behind. Thus, improving urban planning in order to

minimise harmful and maximise beneficial urban exposures, along with reducing social inequalities related, is key to ensure a healthy respiratory development in childhood, that in turn, will result in a reduction of respiratory morbidity later in life.

Last, in this Thesis we provided a new birth cohort through the linkage of EHR of 719,858 parent-child pairs that can be of relevance for Public Health and policy decision making as it contains data reflecting current populations health status and trends. Also, it can help detect vulnerable populations in terms of disease or healthcare use or accessibility. Further, it provides a relevant monitoring and surveillance tool in a real-world context. In addition, it can be used to examine the status of healthcare delivery (e.g., practice patterns, quality of care) and safety and effectiveness of drugs and vaccines. Pharmacovigilance studies are of key importance, especially among pregnant women, who are usually excluded from clinical trials for obvious ethical concerns (282). A good and most current example of the potential of this cohort to support policy decision-making is its capacity to monitor COVID-19 infections in pregnant women and their related pregnancy and birth outcomes, and to perform pharmacovigilance and safety studies of vaccination among pregnant women and children.

6.5. Future research

6.5.1. Study designs and populations

Birth cohorts are usually limited in their sample size, which may hinder the assessment of some environmental exposures on health,

especially when effects are expected to be small. In this sense, collaborations across existing birth cohorts offer a powerful opportunity. These can increase the level of evidence to explore smaller effects and identify vulnerable groups across countries. An example of current collaborative research to expand and improve studies on early life determinants of health is the Horizon2020-funded LifeCycle Project, that joins 19 birth and childhood cohorts (283).

The linkage methodology used to create the EHR-based parent-child cohort tended to be less successful among subgroups of the population. Future studies are needed to explore the reasons for the unsuccessful linkages in order to improve the algorithm and link the maximum number of parents and children, regardless of their sociodemographic characteristics. This new cohort offers many opportunities for future research using real-world data because it contains detailed information on a number of maternal, paternal, environmental, clinical, and sociodemographic factors as well as extensive information on children's health and development. Future studies could broaden the scope of their research questions with longer follow-up time. Future studies could make use of this cohort for studies assessing urban environmental exposures on children's respiratory health, as it has been done with other health outcomes (284).

6.5.2. Environmental exposures

In paper I we showed that most of the current evidence regarding organic pollutants is based on the same few birth cohorts, often

limited in their sample size, which limits the generalisation of the results in other population settings with different use of chemicals and thus of exposure levels, and different sociodemographic characteristics. Also, in most studies on non-persistent organic pollutants such as bisphenols, the exposure was assessed from a single or few spot urine samples, which might have led to misclassification and attenuation of the results. Therefore, further studies, with larger sample size to study susceptible groups, conducted in different population settings, with a thoughtful sampling design, and including a number of repeated non-persistent pollutant measurements during several days of the week in the three trimesters of pregnancy are needed. This thoughtful sampling design has already been applied in some recently established birth cohorts demonstrating its feasibility in large population-based studies (285). Also, in order to improve current EU chemical legislation, further research is needed on the assessment of temporal variabilities of non-persistent pollutants exposure in relation to health outcomes.

Most previous studies on OCs and bisphenols have followed single-exposure approaches. However, populations are exposed to many pollutants simultaneously. Further studies are needed assessing multiple exposures and chemical mixtures as well as consider non-linear exposure-outcome relationships that are commonly observed in endocrine disrupting chemicals. Further, future studies should also include chemicals with increasing presence in the market such as BPF or BPS, of which very little is known.

The studies included in this Thesis, as the vast majority of epidemiological studies evaluating the health effects of chemical pollutants, rely on concentrations of chemicals determined in maternal biospecimens as a proxy of foetal exposure. However, these levels do not consider the modification of the mother's physiology due to pregnancy, the transfer of chemicals to the foetus, and the variations of chemicals exposure by the mother (286). The impact of pregnancy on the maternal levels of chemicals can be predicted by physiologically based pharmacokinetic (PBPK) models that employ a compartmental structure that incorporates anatomic and physiologic characteristics of the body and its tissues to map chemical movement. Future studies assessing the respiratory effects of chemical pollutants can consider the application of materno-foetal PBPK models to estimate the internal dose of the foetus.

Regarding urban environmental exposures, several improvements on the exposure assessment can be considered for future studies. For example, the use of personalised exposure assessments that account for the time, location, and activity patterns during the exposure assessment period, provides a more accurate assessment. However, the higher precision obtained is in expenses of very high costs and complexity in their implementation in birth cohorts. Also, better assessments on the type, the quality, and the use of urban and natural spaces could improve the understanding of how these features influence children's respiratory health. In relation to this, more studies are needed to understand the pathways by which prenatal urban exposures can influence children's respiratory health. In

addition, little is known about the complex relationship between urban exposures in relation to children's respiratory health. Further studies assessing possible interaction, mediation, or confounding between co-existing urban exposures is needed.

6.5.3. Respiratory outcomes in childhood

Most previous cohort studies, as the ones included in this Thesis, have used spirometry parameters at a specific time point as a measure of children's lung function. However, studying lung function trajectories and spirometric phenotypes in relation to early life environmental exposures would be of relevance for future research. These approaches may allow a better understanding on the influence of the early life predictors because they allow a more accurate identification of children at higher risk of later disease (9,243). Also, it is important that future studies examine whether the effects seen during childhood persist at later ages.

Further research needs to focus on improving asthma characterisation. As discussed in a previous chapter, asthma is an umbrella term that covers many heterogeneous phenotypes. Those phenotypes are not fully characterised, which implies that studies assessing environmental effects on a general definition of asthma are at risk of outcome misclassification (26). Thus, further research is needed to improve the characterisation of asthma phenotypes that would, in turn, help establishing a consensus on a better asthma assessment in observational studies and reduce its outcome misclassification. In this sense, large studies using real-world data such as the cohort presented in paper V offer a good opportunity. In

large cohorts using real-world data, disease phenotyping and characterisation is feasible, as demonstrated previously (287).

In line with the need to improve asthma phenotyping, assessing other often related outcomes such as respiratory tract infections, atopy, biomarkers of inflammation, or the presence of comorbidity such as the atopic triad (i.e., atopic dermatitis, allergic rhinitis, and asthma) (246,288) would be of relevance. This would not only improve the characterisation of the outcome assessed, but also provide some insights on the underlying mechanisms by which environmental exposures affect children's respiratory and immune health.

7. CONCLUSIONS

- In the last decade the number of studies assessing the effects of prenatal exposure to organic pollutants on childhood lung function have increased; however, the evidence is still limited and inconsistent. It is unclear whether inconsistencies might reflect true null effects or methodological limitations such as limited sample size or exposure misclassification in the case of non-persistent organic pollutants.

- Spanish populations born between 2004-2007 were widely exposed to OCs during prenatal life, with 80% of samples presenting detectable levels of *p,p'*-DDE, HCB, and PCBs. The most prevalent OC was *p,p'*-DDE. Prenatal exposure to *p,p'*-DDE was associated with lower lung function until 7 years of age, especially FEV_1, and at medium levels of exposure.

- In six EU birth cohorts established between 1999-2010, exposure to BPA was prevalent with 90% of maternal samples presenting detectable levels. BPF and BPS were only detected in 27% and 49% of samples, respectively. Prenatal exposure to BPA was associated with higher risk of asthma and wheeze among school-age girls.

- The urban environment during pregnancy, especially air pollution, road traffic noise, and availability of blue spaces, is of relevance to the offspring's respiratory health through childhood. Living during pregnancy in an urban environment

characterised by higher levels of air pollution, noise, urbanisation, and lower levels of natural spaces may contribute to poorer respiratory health during childhood.

- We created a new birth cohort by linking the EHR of 719,858 children born from 2005 to 2018 to the EHR of at least one potential parent in SIDIAP, which represents 93% of the SIDIAP paediatric population born in that period. This new cohort provides a valuable source of data for future research on parental and child health in addition to early life environmental determinants of children's health.

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APPENDIX

A) Other co-authored papers

Peralta GP, **Abellan A**, Montazeri P, Basterrechea M, Esplugues A, Gonzalez S, Roda C, Santa Marina L, Sunyer J, Vrijheid M, Casas M, Garcia-Aymerich J. Early childhood growth is associated with lung function at seven years: a prospective population-based study. Eur Respir J. 2020.

Sangüesa J, Sunyer J, Garcia-Esteban R, Bustamante M, **Abellan A**, Basterrechea M, Esplugues A, Garcia-Aymerich J, Guxens M, Julvez J, Irizar A, Rodriguez-Delhi AC, Vioque J, Tardon A, Torrent M, Vrijheid M, Casas M. Prenatal and child vitamin D levels and the development of allergy and asthma in children. *Under review*.

Stapleton AE, Casas M, Garcia-Aymerich J, Garcia-Esteban R, Sunyer J, Guerra S, **Abellan A**, Lavi I, Dobaño C, Vidal M, Gascon M. Associations between pre- and postnatal exposure to air pollution and lung health in children and assessment of CC16 as a potential mediator. *Under review*.

Wang G, Hallberg J, // **Abellan A** //, Maitland van der Zee AH, Melén E. Spirometric phenotypes from early childhood to young adulthood – A CADSET (Chronic Airway Disease Early Stratification) study. *Under review*.

Puche P, **Abellan A**, Lertxundi A, Zabaleta C, Iñiguez C, Lopez-Espinosa MJ, Fernandez MF, Vrijheid M, Sunyer J, Casas M. Prenatal exposure to Parabens, Benzophenone-3 during pregnancy and lung function, asthma and allergy in school-aged children. *In preparation*.

Rivas I, Guxens M, Iñiguez C, Alonso L, Estarlich M, Basterrechea M, **Abellan A**, Garcia-Aymerich J, Zabaleta C, Ferrero A, Basagaña X, Vrijheid M, Sunyer J, Casas M. Intrauterine and postnatal exposure to outdoor NO_2 and lung function at school age. *In preparation.*

Koch S, Peralta GP, Carsin AE, Cirugeda L, Sunyer J, Vrijheid M, Garcia-Esteban R, **Abellan A**, Guxens M, Torrent M, Ballester F, Ferrero A, Iñiguez C, Zabaleta C, Basterrechea M, Casas M, Garcia-Aymerich J. Lung function trajectories and their determinants in 4-18 year-old children from the INMA birth cohort. *In preparation*.

B) Children's health status report

Abellan A, Casas M, Sunyer J. Informe de salut infantil de Sabadell (Children's health report for Sabadell city council). 2018. Available from: https://www.sabadell.cat/ca/salut/estudi-sobre-habits-de-salut-en-infants/117219.

C) Participation at national and international conferences

International Society for Environmental Epidemiology – Early Career Researchers Conference, online, 2021. Oral presentation "Early life exposure to urban environment and lung function and asthma in school-age children".

European Respiratory Society International Congress, online, 2020. Attendance.

International Society for Environmental Epidemiology Conference, online, 2020. Attendance.

European Respiratory Society International Congress, Madrid, 2019. Oral presentation "Prenatal exposure to phenols and lung function, wheeze, and asthma in school-age children from 8 European birth cohorts".

International Society for Environmental Epidemiology Conference, Utrecht, 2019. Poster presentation "Prenatal exposure to phenols and lung function, wheeze, and asthma in school-age children from 8 European birth cohorts".

European Respiratory Society International Congress, Paris, 2018. Poster presentation by Maribel Casas "Prenatal exposure to organochlorine compounds and lung function until early adulthood".

American Thoracic Society International Conference, San Diego (CA), 2018. Attendance.

Prenatal Programming and Toxicity (PPTOX) VI Conference, Faroe Islands, 2018. Poster presentation by Maribel Casas "Prenatal exposure to organochlorine compounds and lung function until early adulthood".

International Society for Environmental Epidemiology – Early Career Researchers Conference, Munich, 2018. Oral presentation "Prenatal exposure to organochlorine compounds and lung function until early adulthood".

Barcelona-Boston Lung Conference, Barcelona, 2018. Oral presentation "Prenatal exposure to organochlorine compounds and lung function until early adulthood".

D) Grants and awards

International Society for Environmental Epidemiology – Early Career Researchers Conference award for 2nd best abstract of the conference. February 2021.

LifeCycle fellowship. Grant for a 6-months stay at the Erasmus Medical Center (The Netherlands), funded from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733206. January-July 2020.

ISGlobal PhD symposium award for best oral presentation. Grant to attend and cover expenses of courses or conferences of interest. November 2019.

Barcelona-Boston Lung Conference award for oral presentation. Grant to attend and cover expenses of the American Thoracic Society International Conference, San Diego (US). January 2018.

E) Additional academic training during the PhD

Real World Epidemiology, Oxford Summer School. NDORMS, University of Oxford. 2019.

Weighed Quantile Sum, ISEE Pre-conference workshop. International Society for Environmental Epidemiology. 2019.

Causal Diagrams: Draw Your Assumptions Before Your Conclusions. Harvard University through edX platform. 2018.

Multipollutant models, ISEE Young Pre-conference workshop. International Society for Environmental Epidemiology. 2018.

How to create a visually stunning scientific poster. PRBB intervals. 2018.

Introduction to R. ISGlobal. 2017.

The craft of the scientific research article. PRBB intervals. 2017.

F) Education and outreach activities

Supervision of a Master's Thesis "Exposure to parabens and benzophenone-3 during pregnancy and lung function in school-aged children" from the Master in Public Health. Universitat Pompeu Fabra.

Invited speaker for "Research Methodology" course of the Bachelor in Nursery. Escola Superior d'Infermeria del Mar, Universitat Pompeu Fabra.

Seminar on Endocrine Disrupting Chemicals. *Cicle formatiu de Grau Superior* of Nutrition (equivalent to Certificate of Higher Education). IES Guineueta.

Creation of a yearly newsletter for families participating in the INMA Sabadell population-based birth cohort (years 2017, 2019, and 2020).

Article "Endocrine disruptors: what are they and how they affect us?" for Diputació de Barcelona. Available from: https://www.diba.cat/web/salutpublica/butlletins/-/newsletter/35521038/92/287977102/els-disruptors-endocrins-que-son-i-com-ens-afecten-

Article "Endocrine disruptors: a threat for health, even at low doses" for DKV Salud. Available from: https://360.dkvseguros.com/sociedad/consumo/disruptores-endocrinos

Contribution to the article “Health risks of Bisphenol A” for Instituto Profesional de Estudios de la Salud (IP Salud).

G) INMA Spirometry cleaning protocol

INMA SPIROMETRY CLEANING PROTOCOL

October 2019

Document developed by Alicia Abellan, Judith GarciaAymerich, Anna Delgado, Raquel Garcia, and Maribel Casas from the INMA WG on Respiratory and Allergies and revised by Felip Burgos from the Hospital Clinic

So far spirometries in INMA have been cleaned following different protocols; there is a need to harmonize the cleaning process in order to compare the spirometry results within and between cohorts and periods. Hence, the objective of this document is to describe the spirometry cleaning procedure. This protocol has been performed based on the ATS/ERS guidelines [1, 2], and the MeDALL, HELIX, and Generation R spirometry cleaning protocols.
We recommend that the cleaning process detailed as follows is conducted by a trained fieldworker with previous experience.

Abbreviations:
BEV back extrapolated volume
FEF forced expiratory flow
FET forced expiratory time
FEV_1 forced expiratory volume in 1 second
FVC forced vital capacity
PEF peak expiratory flow
PEFT peak expiratory flow time

1. BEFORE CLEANING: SPIROMETRY TEST

Spirometry tests should be performed following the INMA Spirometry protocol (available in the 14-16 years follow-up document: "Examen clínico visita 14-16 años_080119"). During the examination, we recommend saving all the curves performed for a given child. Each child can perform a maximum of 8 manoeuvres. Ideally, from each child we should obtain 3 acceptable and reproducible curves. However, there are cases in which none or only 1 or 2 acceptable curves can be achieved.

2. SPIROMETRY CLEANING

The process of cleaning spirometry tests consists mainly on two parts: quality control and selection of parameters.

a. QUALITY CONTROL

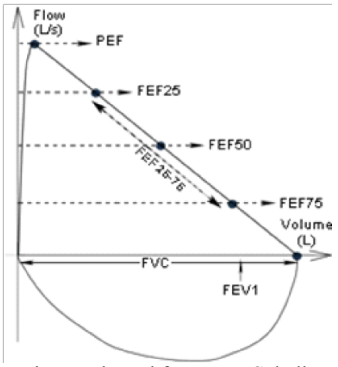
During the quality control we mainly determine which curves are acceptable and which ones are not. Note that we exclude technically non acceptable curves; we do not exclude curves that show respiratory problems such obstruction or restriction (see examples below). Acceptable curves must fulfil all the acceptability criteria (see step 2).

STEP 1. For each curve, generate the following variables (in bold) to evaluate the quality of the curves

- **BEV_ok** identifying a fast start. Takes values 1="yes" if **BEV**<0.1L and **PEFT**<0.10s; and 0="no" otherwise.
- **FET_ok** identifying a complete expiration. Takes values 1="yes" if 1s < **FET** < 10s; and 0="no" otherwise.
- **Good_shape_curve**. Takes value 1="yes" if the following three conditions are accomplished; and 0="no" otherwise.
  - *i.* Has a good start: sharp rise during the first second. No hesitation and no cough (check visually on the flow-volume curve; see Figure 1A).
  - *ii.* Has a good shape: no cough during the entire exhalation, no inhalation, no early termination or cut-off, the curve is constantly descending, no artefacts in the descending curve (check visually on the flow-volume curve; see Figure 1A)
  - iii. The volume-time curve has a plateau shape (check visually on the volume-time curve; see Figure 1B).
- Some curves with a bad shape can have an acceptable FEV_1 or FVC. For example, there are cases in which the curve has a good start, and therefore an acceptable FEV_1, but then there is an early termination (after the 1st second), meaning that FVC is not acceptable (please see examples of these curves at the end of the document). So, ONLY for those curves with Good_shape_curve="no", indicate whether the curve has an acceptable FEV_1 but not FVC (or viceversa) on the variables:
  - **FEV1_acceptable**: 1="yes"; 0="no"
  - **FVC_acceptable**: 1="yes"; 0="no"

Figure 1. Flow-volume and Volume-time curves

A. Flow-volume curve



B. Volume-time curve

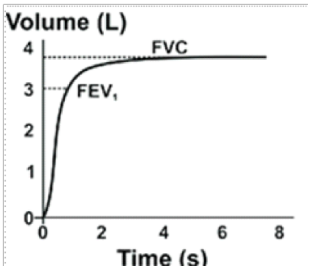


Figure adapted from Van Schalkwyk et al, 2004.

STEP 2. Select curves that are acceptable [a] ***OR those with acceptable FEV_1 or FVC*** [b]

Please note that in some INMA cohorts, the technician only records those acceptable; in this case, this step can be omitted. Please, check with your cohort if curves recorded have already been selected from those acceptable.

[a] A curve is acceptable if **BEV_ok**="yes" *AND* **FET_ok**="yes" *AND* ***Good_shape_curve***="yes"

Optional: A grade from each test is automatically given by the spirometer (from A to F). A and B indicate a good quality grad; C indicates that the test needs to be checked; D, E, and F indicate that the test is of poor quality. We do not usually use this criterion because a test could have a grade E but have some acceptable curves. Please, always check visually each curve to fulfill the rest of the acceptability criteria.

[b] **FEV1_acceptable**="yes" *OR* **FVC_acceptable**="yes" points out curves with acceptable FEV_1 or FVC

STEP 3. Determine if the spirometry test is reproducible

For each examination (identified by the variables "**RecNum"** and "**SerNr"** directly given by the spirometer) and considering only acceptable curves[a] (please note that curves without good shape but FEV_1 or FVC acceptables[b] are not used for the following calculations), generate the following variables (**in bold**):

- **N_accep** indicating the total number of acceptable curves.
- **FVC_reproducible_100** indicating whether the stringent (100 mL) reproducibility criterion is met. Takes the value 1="yes" when the difference between the largest FVC and the second largest FVC is < 100mL; 0="no" otherwise.
- **FVC_reproducible_150** indicating whether the loose (150 mL) reproducibility criterion is met. Takes the value 1="yes" when the difference between the largest FVC and the second largest FVC is < 150mL; 0="no" otherwise.
- **FEV1_reproducible_100**. Idem but for FEV_1
- **FEV1_reproducible_150**. Idem but for FEV_1
- **Accept_Repro_ATSERS** defining the acceptability and reproducibility according to ATS/ERS guidelines. Take the following values:

  0="Less than 2 acceptable curves"

  1="2 acceptable curves but not reproducible"

  2="At least 2 (≥2) curves acceptable and reproducible"

*Note: we have decided to consider that a child has acceptable and reproducible test if at least 2 curves are acceptable and reproducible (and not 3) because we have compared FEV_1 and FVC parameters in INMA children with 2 curves and those with equal or more than 3 curves and overall there are no big differences [see document in the INMA intranet: "INMA_spiro-curves_acceptable_Céline Roda"].

b. SELECTION OF PARAMETERS

The dataset may contain more than one value for each lung function parameter (FVC, FEV_1,...) since it may contain more than one curve per child (e.g. if a test has 3 acceptable curves, the dataset will contain 3 values of FVC).

To restrict to only one value for each of the lung function parameters, follow the steps below:

1. Select the highest FVC
2. Select the highest FEV_1

* Note that in addition to acceptable curves[a], those curves with bad shape but acceptable FVC or FEV_1[b] are also taken into account, respectively.

**Note: Highest FVC and FEV_1 can be from different curves

3. Select the remaining parameters (FEF_{25-75} etc) from the curve with the highest value of (FVC + FEV_1)

*** Note that only acceptable curves[a] are considered.

Send the final dataset to the INMA Data Manager containing the following variables:

cohort8 (5="Sabadell", 8="Sabadell2",...)
PatientID (child ID)
Date (date of the test)
FVC: Forced Vital Capacity (in L; the selected value)
FEV1: Forced Expiratory Volume in 1 sec (in L; the selected value)
FEF25: Forced Expiratory Flow 25% (in L/s; the selected value)
FEF50: Forced Expiratory Flow 50% (in L/s; the selected value)
FEF75: Forced Expiratory Flow 75% (in L/s; the selected value)
FEF2575: Forced Expiratory Flow 25-75% (in L/s; the selected value)
N_accep: Number of acceptable curves
FVC_reproducible_100 (yes/no)
FVC_reproducible_150 (yes/no)
FEV_1_reproducible_100 (yes/no)
FEV_1_reproducible_150 (yes/no)
Accept_Repro_ATSERS (0, 1, 2 – see above)

3. RECOMMENDATIONS FOR ANALYSES USING SPIROMETRY DATA

After some descriptive analyses and after contacting experts in the field (see ANNEX) we recommend:

1. To include all children with at least one acceptable curve in the main analyses and to perform two additional sensitivity analyses restricting first to children with at least two acceptable curves (reproducible or not reproducible), and second to children with at least two acceptable and reproducible curves.
2. To consider the following parameters: FVC, FEV_1, FEV_1/FVC, and FEF_{25-75}.
3. To construct z-scores of the lung function parameters applying the GLI-2012 equations and include such parameters in the analyses.

4. EXAMPLES OF SPIROMETRY CURVES

Visual examples of acceptable curves extracted from Miller et al, 2005:

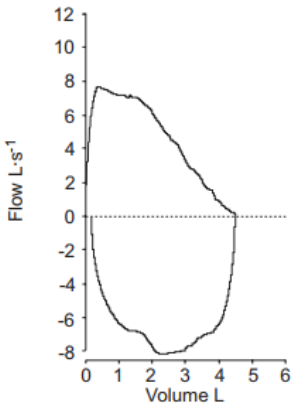


FIGURE 4. Flow–volume loop of a normal subject.

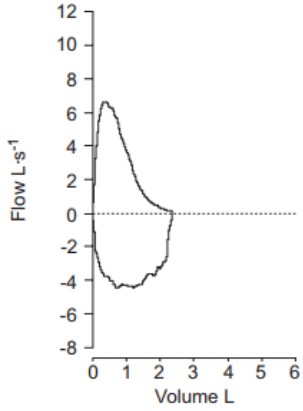


FIGURE 5. Flow–volume loop of a normal subject with end expiratory curvilinearity, which can be seen with ageing.

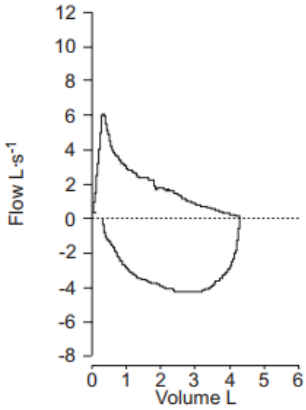


FIGURE 6. Moderate airflow limitation in a subject with asthma.

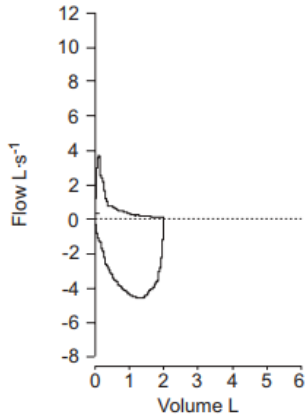


FIGURE 7. Severe airflow limitation in a subject with chronic obstructive pulmonary disease.

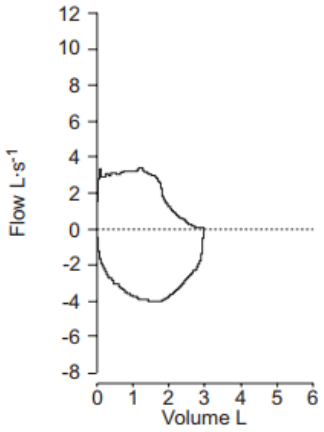


FIGURE 8. Variable intra-thoracic upper airway obstruction.

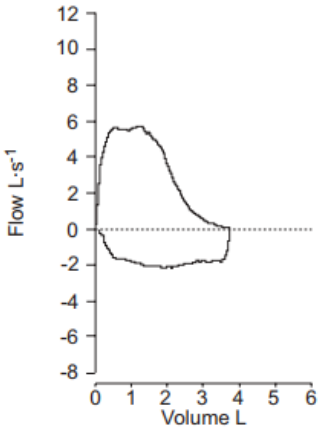
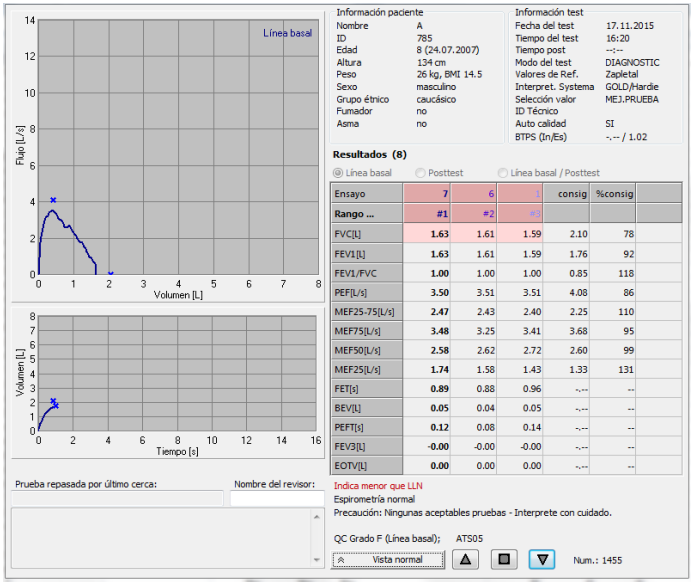


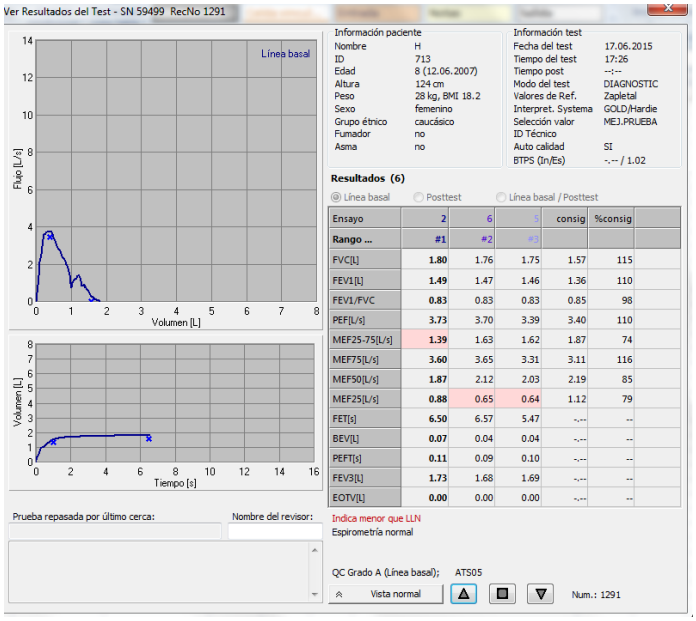
FIGURE 9. Variable extra-thoracic upper airway obstruction.

Visual examples of curves obtained from clinical practice with children using *EasyWare* software:

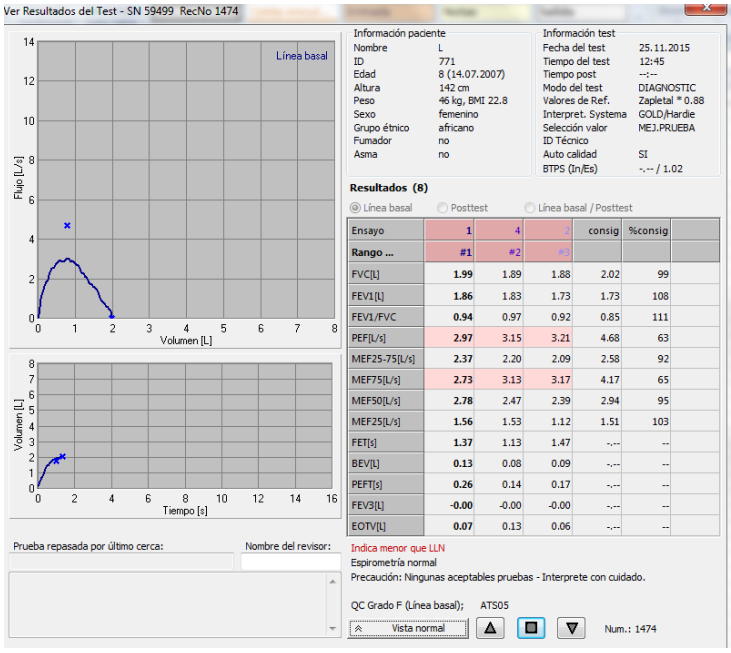
1) Early termination. FEV_1 is acceptable but FVC cannot be evaluated.



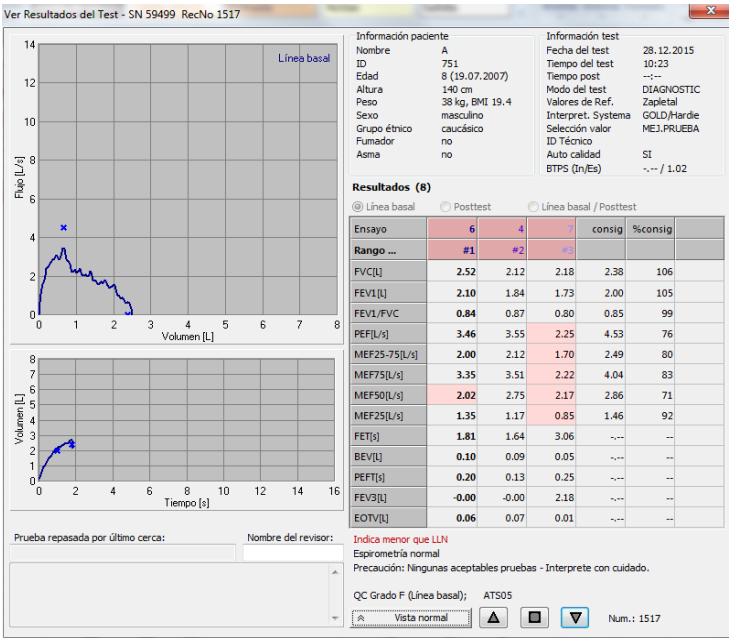
2) Artefact on the curve. Causes: cough, tongue obstructing the exhalation, etc. If FEV_1 is not affected by the artefact we can consider it acceptable.



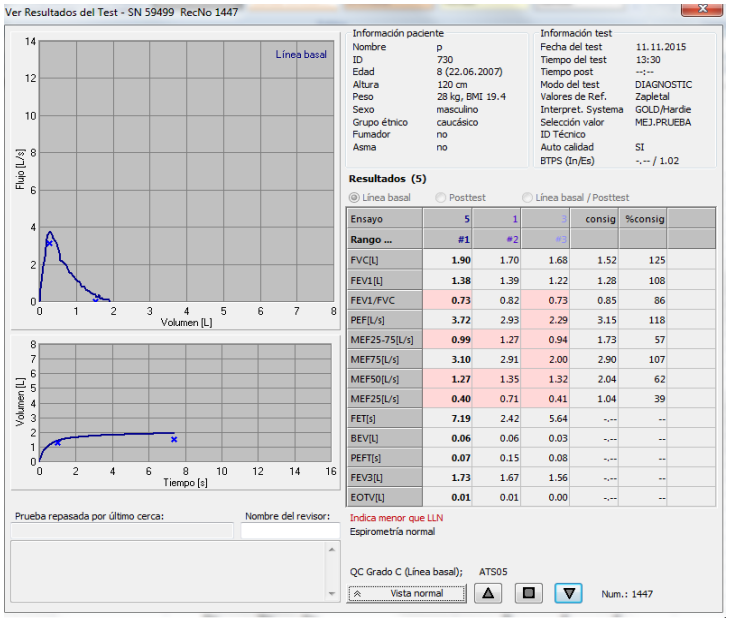
3) PEFT > 0.10s. Not acceptable.



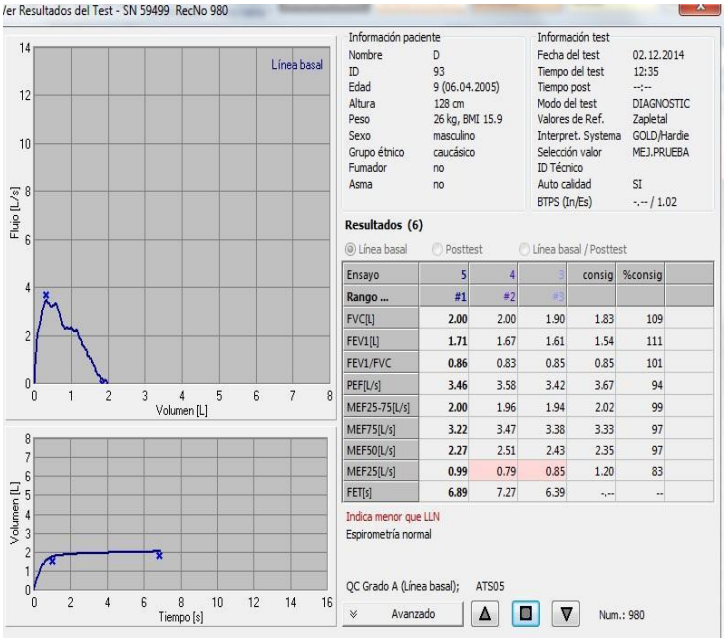
4) Artefacts and early termination. Not acceptable.



5) Acceptable curve. This is a typical obstructive pattern. It is common to see it in asthmatic children.



6) Acceptable curve. The “belly shape” of the descending curve is common in young people when the pulmonar pressure is not evenly distributed.


/er Resultados del Test - SN 59499 RecNo 980
Línea basal
Flujo [L/s]
Volumen [L]
Volumen [L]
Tiempo [s]
Información paciente
Nombre D
ID 93
Edad 9 (06.04.2005)
Altura 128 cm
Peso 26 kg, BMI 15.9
Sexo masculino
Grupo étnico caucásico
Fumador no
Asma no
Información test
Fecha del test 02.12.2014
Tiempo del test 12:35
Tiempo post --:--
Modo del test DIAGNOSTIC
Valores de Ref. Zapletal
Interpret. Systema GOLD/Hardie
Selección valor MEJ.PRUEBA
ID Técnico
Auto calidad SI
BTPS (In/Es) -.-- / 1.02
Resultados (6)
Línea basal
Posttest
Línea basal / Posttest
Ensayo 5 4 3 consig %consig
Rango ... #1 #2 #3
FVC[L] 2.00 2.00 1.90 1.83 109
FEV1[L] 1.71 1.67 1.61 1.54 111
FEV1/FVC 0.86 0.83 0.85 0.85 101
PEF[L/s] 3.46 3.58 3.42 3.67 94
MEF25-75[L/s] 2.00 1.96 1.94 2.02 99
MEF75[L/s] 3.22 3.47 3.38 3.33 97
MEF50[L/s] 2.27 2.51 2.43 2.35 97
MEF25[L/s] 0.99 0.79 0.85 1.20 83
FET[s] 6.89 7.27 6.39 -.-- --
Indica menor que LLN
Espirometría normal
QC Grado A (Línea basal); ATS05
Avanzado
Num.: 980

References:

1. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CPM, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J, ATS/ERS Task Force. Standardisation of spirometry. *Eur. Respir. J.* 2005; 26: 319–338. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16055882.
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3. van Schalkwyk EM, Schultz C, Joubert JR, White NW, South African Thoracic Society Standards of Spirometry Committee. Guideline for office spirometry in adults, 2004. *S. Afr. Med. J.* 2004; 94: 576–587. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15283308.

ANNEX

Advice from experts

We contacted three experts seeking for advice on the criterion to adopt when including spirometry tests in our studies. We asked how many acceptable curves (1, 2, >2) should have each child to be included, whether they had to be reproducible or not, and the most suitable lung function parameters to be included in the analyses.

1. **Dr Manuel Sánchez-Solís de Querol** (Paediatrics professor from Hospital Clínico Universitrio Virgen de la Arrixaca, Murcia. NELA Cohort in Murcia).

- He advices to include those children with at least 1 acceptable curve and perform a sensibility analysis restricting to at least 2 acceptable curves afterwards.
- He explains that in young children it is better to consider $FEV_{0.75}$ instead of FEV_1 because among the youngest, sometimes FEV_1 almost equals their FVC so you are measuring the same twice.

2. **Dr Liesbeth Duijts** (Paediatrician, Pulmonologist, Epidemiologist at the Erasmus MC-Sophia Children's Hospital, University Medical Center Rotterdam and Generation R cohort in the Netherlands).

- She also advices to include those children with at least 1 acceptable curve and perform a sensibility analysis restricting to at least 2 acceptable curves, and at least 2 acceptable and reproducible curves afterwards.
- She encourages the use of sex-, age-, height-, and ethnicity adjusted z-scores of the following lung function parameters: FVC, FEV_1, FEF_{25-75}, and FEF_{75}.

3. **Dr Enrico Lombardi** (Responsabile SOSA Broncopneumologia Pediatrica, Firenze - Italy).

   *Stefano Guerra recommended him for his experience in lung function in children from both research and clinical practice.

- He suggests making a comparison of spirometry parameters between those children in INMA with only one curve and those with 2 or more based on the z-scores. [Céline Roda did this comparison and overall the results are the same in children having only 1 curve and those having equal or more than 2 curves - see document in the INMA intranet: “INMA_spiro-curves_acceptable_Céline Roda”].

- He recommends using the following parameters: FVC, FEV_1, FEV_1/FVC and FEF_{25-75}. He does not recommend using $FEV_{0.75}$ since he thinks FEV_1 is sensitive enough.
- We need to check each curve manually – as a reviewer he would ask if the inspiration and expiration are good (plateau of at least 1 sec. etc).
- We can include those kids with only one acceptable curve and do sensitivity analysis excluding them to see whether the results change
- To test the short-term repeatability of the lung function tests, he recommended performing reversibility tests if possible. If not, what they do is to repeat the spirometry 15 minutes after the first spirometry in a subset of children. Potential sources of variability can be: different breathing patterns, different level of engagement and co-operation by the child, and any minute to minute fluctuations in airways calibre due to changes in airway tone. Reference paper:
    - Calogero C, Lombardi E. Respiratory impedance and bronchodilator response in healthy Italian preschool children. Pediatr Pulmonol. 2013 Nov;45(11):1086-94. doi: 10.1002/ppul.21292. PMID: 20672294