Modification by sex and atopy of the effect of chemical air pollutions and biological air pollutants including pollen and fungal spores on children respiratory health in the Western Cape, South Africa

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PREFACE

Plagiarism Declaration

I, **Yasmeen Nassr Mahmoud,** hereby declare that the work on which this dissertation is based is my original work (except where acknowledgement indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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I would like to express my immense appreciation to my supervisor, Professor Mohamed Aqiel Dalvie, and my co-supervisors, Professor Mohamed Jeebhay and Dr Toyib Olanian, for their guidance, encouragement, and support throughout the thesis process.

Thank you to my yet-to-be-born son for giving me the motivation and strength to continue to work hard to finish so that I can have the utmost priority for your arrival.

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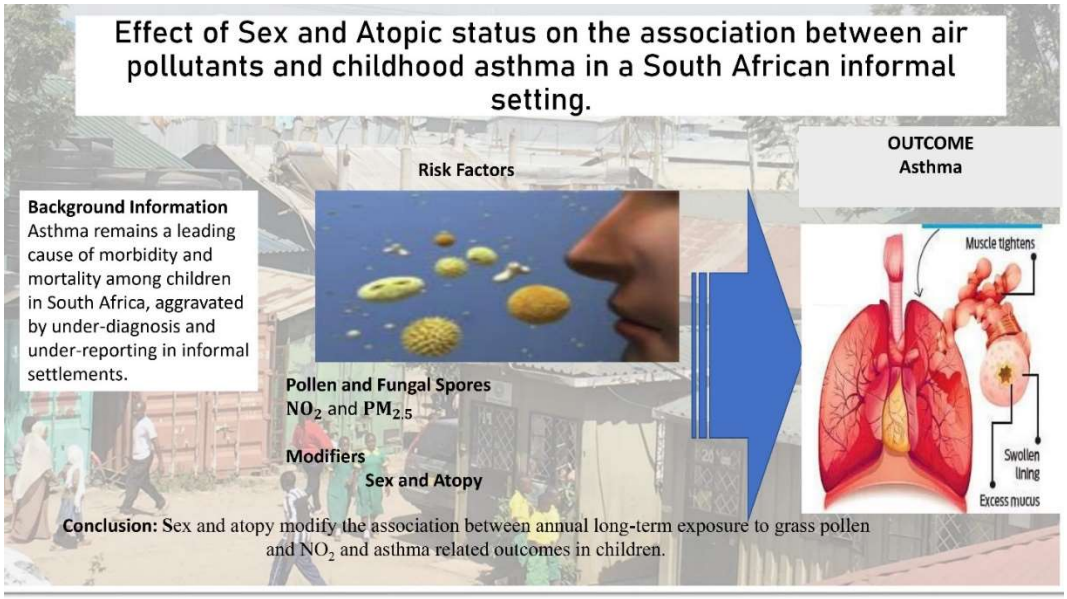
To my parents, Nassr and Mona, and my siblings, Hisham, Sarra, and Salma, thank you for being patient. I am beyond grateful. Thank you for your unconditional love, support, and encouragement throughout this academic journey.

Abstract

Asthma continues to be a leading cause of morbidity and mortality among children in Sub-Saharan Africa. Ongoing under-diagnosis and treatment in low- and middle-income countries, together with exposure to airborne pollutants and allergens, contribute to this disease burden. A cross-sectional study that recruited 590 school children residing in four informal settlements in the Western Cape province was conducted to assess the role of sex and atopic status on the association between air pollutants and childhood asthma. Exposure to NO_2 and $PM_{2.5}$ were estimated for individual homes using a land-use regression model, while pollen and fungal spores were measured using spore traps positioned in each study area during the follow-up study between May 2016 and September 2016. Asthma-related outcomes were obtained using the International Study of Allergy and Asthma in children (ISAAC) questionnaire, spirometry, and fractional exhaled nitric oxide (FeNO) testing. The study found that increased annual grass pollen levels and annual NO_2 levels were positively associated with airway obstruction (OR: 2.26, 95% CI: 1.02-5.03) for annual grass pollen and FeNO (OR: 2.43, 95% CI:1.19-4.98) for annual NO_2 and (OR: 3.43, 95% CI: 1.07-10.99) for grass pollen. Furthermore, there was evidence of effect modification by sex and atopy since associations were much stronger among boys and atopic children. Annual NO_2 levels were positively associated with FeNO>35ppb among boys (OR:2.77, 95% CI: 1.02-7.47) but not girls. A strong positive association was also observed between higher annual grass pollen levels and FEV_1< LLN in atopic children (OR: 4.46, 95% CI: 1.28-15.59). The study provides evidence that sex and atopy modify the association between annual long-term exposure to grass pollen and NO_2 and asthma-related outcomes in children.

Keywords: childhood asthma, chemical air pollution, biological air pollutants, effect modification, sex and atopy.



A graphical abstract on the effect of sex and atopic status on the association between air pollutants and childhood asthma in South African Informal Setting.

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PART A: RESEARCH PROTOCOL

1. Introduction

1.1 Background

Asthma is the most common respiratory disease in children, and its prevalence and morbidity are increasing (Esposito and Principi, 2001). Asthma is described as a chronic inflammatory disease of the airways, and it is characterized by reversible airway obstruction, shortness of breath, wheezing or cough (Esposito and Principi, 2001). The global burden of asthma is around 334 million people (Asher and Pearce, 2014). Globally, asthma is in the top 20 causes of Disability-adjusted Life Years (DALYs) in all ages and is the 10th cause of DALYs in schoolchildren (Asher and Pearce, 2014). Asthma is the most common chronic disease in children globally, affecting 14.2% of the paediatric population (Asher and Pearce, 2014). According to the International Study of Asthma in Children (ISAAC), the current prevalence of asthma in children aged 6-7 years is 11.5%, and in children aged 13-14 years is 14.2%. (Asher and Pearce, 2014). The common symptoms of paediatric asthma are wheezing, and cough triggered by allergy and other trigger factors such as air pollution and respiratory tract infection. However, these symptoms can develop in children without asthma (Bao *et al.*, 2017). In addition, the prevalence of asthma is increasing in LMIC and decreasing in many high-income countries, but these geographical differences did not suggest a direct causal association between poverty and asthma status (Beran *et al.*, 2015). Additionally, according to ISACC phase 111, 50% of children in LMIC have reported severe asthma symptoms, 30% have never been diagnosed, and more than 80% of global asthma-related mortality occurs in LMIC (Beran *et al.*, 2015).

In Africa, asthma ranges from 10% to 20% in central Africa. Furthermore, the prevalence of asthma is higher in urban areas than in rural areas in LMIC. Additionally, an analysis of (ISAAC) phase III indicated that although the prevalence of asthma is higher in developed countries, children in LMIC have reported more severe asthma symptoms than children in high-income countries. According to The Global Asthma Report 2018, South Africa is ranked 25th globally for asthma prevalence. Additionally, the rapid increase of urbanisation and population growth particularly in Kinshasa and Lago will ultimately expected to increase the incidence of non-communicable respiratory illness in the sub-Saharan region (Glenn *et al.*, 2022). Despite the substantial reduction in asthma mortality over the past decade, South Africa is ranked 5th for asthma mortality, and the prevalence of asthma is increasing in both urban and rural areas (Mosler *et al.*, 2020). Additionally, self-reported current wheeze was 20.3% in Cape Town and

18% in Polokwane in 2002 (ISAAC III) in children aged 13-14 years old, which exceeded the global estimate of 14.1% (Baard *et al.*, 2021).

The increasing burden of asthma in LMIC and in particular in South Africa is attributed to the higher rate of urbanization, urban lifestyle, the lack of appropriate diagnosis, poor access to medical services, poverty and malnutrition, environmental risk factors such as exposure to air pollution and tobacco smoke respiratory, infections genetic susceptibility to severe diseases, atopy stigma or combination of all these factors (Yusuf, Rathebe and Mbonane, 2022; Masekela *et al.*, 2018).

Childhood asthma is attributed to a list of risk factors such as genetic predisposition, exposure to tobacco smoke and environmental hazards (air pollutants, allergens, and pesticides), socio-economic status, childhood obesity and allergic rhinitis. Allergic rhinitis is associated with asthma and the severity of asthma in children and adults (Beasley, Semprini and Mitchell, 2015).

Environmental risk factors include exposure to air pollution (indoor and outdoor) such as environmental tobacco smoke, inhaled allergens including dust mites, moulds, cockroaches, pesticides, and outdoor allergens (Salam *et al.*, 2004). Air pollution is one of the major environmental risk factors for asthma (Chatkin, Correa and Santos, 2021). Air pollution is associated with premature death from cardiorespiratory disease, pulmonary diseases, lower respiratory disease, lung cancer and asthma (Tiotiu *et al.*, 2020). According to the World Health Organization (Who), air-pollution-related death was estimated to be 6.7 million deaths per year, where 3.2 million deaths are due to indoor air pollution and 3.5 are due to ambient air pollution with 91% of deaths occurring in lower and middle-income countries where air quality exceeded the recommended level of air quality by the World Health Organization (WHO, 2023). Population residing in low-and middle-income countries are disproportionately affected by adverse health effects caused by ambient air pollution (Katoto *et al.*, 2019a). However, the data obtained from LMICs outside of Africa is limited. Nevertheless, epidemiological studies have shown that higher concentrations of O_3, NO_2 and SO_2 are associated with an increased level of multiple respiratory diseases in vulnerable populations (Morakinyo, Mukhola and Mokgobu, 2020). Additionally, exposure to ambient air pollutants such as particulate matter, nitrogen dioxide, sulphur dioxides and Ozone have been shown to reduce lung function in children with a history of persistent asthma (Salvi, 2007). In addition to exposure to air pollution, early-life environmental risk factors for asthma and allergic reactions are associated

with outdoor biological pollutants, such as tree pollen, grass pollen, weed pollen and fungal spores (Simpson *et al.*, 2010).

1.2 Children's exposure to air pollution and biological air pollutants

Children in developing countries are particularly vulnerable population groups to exposure to air pollution. Children are exposed to outdoor air pollution in outdoor playgrounds. Children are exposed to chemicals in the outdoor environment resulting from the combustion of biomass from motor vehicles, industry and power plants (Shirinde, Wichmann and Voyi, 2014; Perera, 2018). In addition, children are exposed to indoor air pollution from the domestic combustion of biomass, candle incense burning and second-hand tobacco smoking (Shirinde, Wichmann and Voyi, 2014). Exposure to air pollutants starts in the early stages of development and occurs throughout life. Pregnant women exposed to air pollutants have the risk of delivering prematurely and have small, low-birth-weight children (Perera, 2018). In addition, air pollution can negatively impact the cognitive and neurodevelopment of children and can prompt asthma and childhood cancer (Lizana *et al.*, 2020). Infants and younger children have larger surface areas per unit of body weight; hence, the air passing will affect lung tissues (Peters, Treering and Shoham, 2009). Gisler *et al*. conducted a study on infant exposure to pollen and found that early life exposure was associated with an increased risk of respiratory symptoms (Harley *et al.*, 2000). Additionally, exposure to outdoor fungal spores, including Cladosporium and Alternaria, was associated with asthma exacerbation (Pongracic *et al.*, 2010) and asthma hospitalisation (Tham *et al.*, 2014) in children and adults.

During the early stages of development, younger children have a smaller lung surface area per kg closer to the ground, where the pollution concentration is higher (Salvi, 2007). Also, children are more at risk than adults as they perform a greater level of physical activity, hence breathing a higher air volume more rapidly and absorbing more pollutants during the lung's development (Peters, Treering and Shoham, 2009). Children exposed to biological and chemical agents as young as three months old have a higher risk of developing wheezing (Gilles *et al.*, 2020). The data from UNICEF indicated that 93% of children are exposed to higher than the recommended level of ambient fine particulate matter ($PM_{2.5}$). According to the (WHO), air pollution accounts for 1 in 10 deaths in children under the age of five years old, and Traffic-related air pollution is one of the significant sources of exposure (Matz *et al.*, 2019).

The observed health effects are attributed to a mixture of air pollutants such as Fine Particulate matter $PM_{2.5}$, Nitrogen dioxide and Ozone, which are associated with increasing the risk factors

for mortalities and morbidities from respiratory disease (Jiang, Mei and Feng, 2016). Although many epidemiological studies investigated the association between exposure to traffic-related air pollution TRAP and lung function in children, little is known about the strength of the association in lower- and middle-income countries, such as South Africa. Children in South Africa are exposed to indoor and outdoor air pollution. For instance, in a study conducted in Durban, school children residing in highly exposed and industrialized communities in the south presented a higher prevalence of asthma, asthma severity and marked airway hyperreactivity (R N Naidoo, MB ChB, PhD1, T G Robins, MD, MPH2, S Batterman, PhD2, G Mentz, PhD2, and C Jack, 2013). In a birth cohort study conducted in Paarl, Western Cape, South Africa, prenatal exposure to tobacco smoke was associated with the development and exacerbation of the upper respiratory tract during infancy (Vanker *et al.*, 2016).

2. Literature Review

This literature review was conducted to understand better the association between exposure to ambient air pollution and children's respiratory health, focusing on the modifying role of sex and atopy status. The electronic literature search was conducted using Google Scholar and PUBMED/MEDLINE. The keywords for the literature search were effect modification, children, asthma, sex, atopy, particulate matter, nitrogen oxides, and lung function. The data included all children exposed to ambient air pollution and the associated respiratory health effects. The search included data from different epidemiological studies and all countries.

2.1 Genetic susceptibility to air pollutants

Gene-environment interaction is important in developing allergies and asthma due to exposure to air pollution. Antioxidants such as vitamins E and C form the first line of defence in the airway epithelium and alveoli (Cross *et al.*, 1994). Glutathione S transferase (GST) enzyme provides a protective mechanism for oxidative stress for the epithelial cells in the airway and alveoli by reducing oxidative stress (Fletcher *et al.*, 2021). Additionally, (GST) neutralize reactive oxygen species ROS and detoxify electrophilic xenobiotics that are found in traffic-related air pollutants and second-hand tobacco smoke (SHS) (Hayes *et al.*, 2008). A common variation of the GST enzyme, such as the GST null phenotype, was observed in individuals with allergic reactions and severe asthma. Gilliand *et al.*, 2005, conducted a clinical trial on children exposed to SHS. The results showed that children with null *GSTM1* phenotype had an increase in allergic response to children with GSTM*1*(Gilliland *et al.*, 1993). The combined effect of Diesel Exhaust Particles (DEP) and allergen was associated with an increase in Histamine production and Th2 Cytokines release (Gilliland *et al.*, 2004). Additionally, DEP has been shown to enhance allergic response in young adults with null GSTM1 or GSTP1 Ile105 genotypes (Gilliland *et al.*, 2006) . Furthermore, exposure to Ozone was significantly associated with a deficit in mid-expiratory flow lung function FEF_{25-75} in Mexican children with GSTM1 null genotype (London, 2004). The study concluded that antioxidants supplements were more beneficial for children with GSTM1 null compared to GSTM positive children; this provides evidence of the role of air pollution and oxidative stress by GST enzymes in developing respiratory symptoms.

2.2 The role of sex in the association between childhood asthma and ambient air pollutants

Sex differences in air pollutants have been attributed to several factors, including biological factors such as growth rates, hormonal factors differences, respiratory mechanisms, and behavioural differences (Becklake and V, 1999).

Lung growth and maturation have been found to be more advanced and earlier in girls than in boys (Hayes *et al.*, 2008; Gilliland *et al.*, 1993; London, 2004). A study by McConnell *et al.* also reported a higher risk of asthma associated with outdoor activities among girls, attributed to a higher ventilation rate among girls compared to boys (Raherison and Filleul, 2002). The deposition of fine particulate matter (<1 um) in the lungs has also been found to be higher in females compared with males (Hu, 2021).

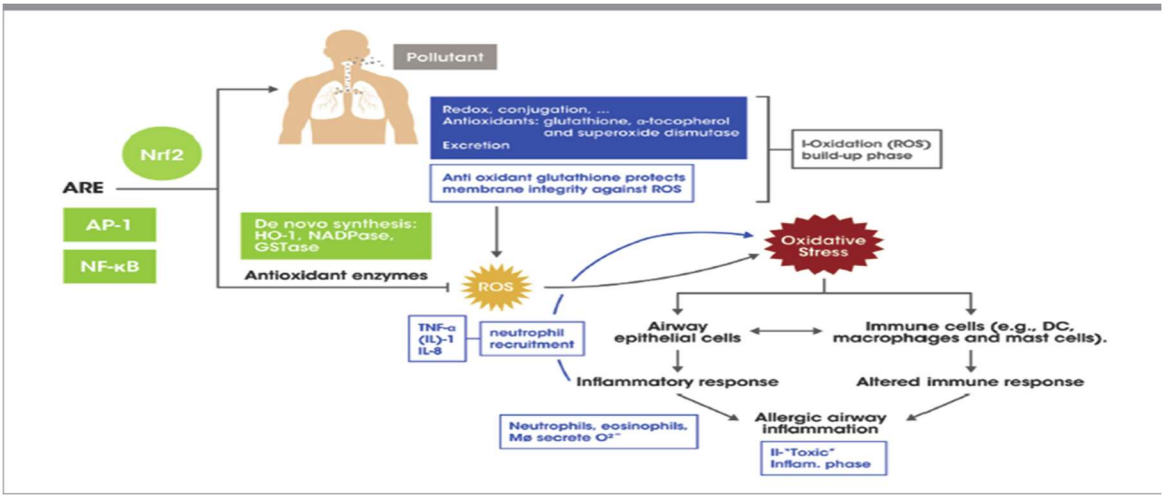
Sex differences in the effect of air pollutants on respiratory health due to hormonal levels are attributed to the effect of these hormones on bronchial smooth muscles function and airway inflammation (Card and Zeldin, 2009).

However, it is still not clear to what extent sex differences in the effect of air pollutants on respiratory health are due to biological and hormonal differences, differences in gender-related exposure activities or a combination of all.

2.3 The role of atopy in the association between childhood asthma and ambient air pollutants - Allergic inflammation

Atopy is defined as an exaggerated IgE immune response to harmless substances in the environment, also known as Type-I hypersensitivity or immediate reaction. Type-I hypersensitivity reaction occurs due to exposure to an antigen. The allergen binds to antigens in tissue mast cells, blood basophils and eosinophils, prompting the release of chemical mediators such as histamine and cytokines (Julge, Meriste and Bjo, 2002). The antigen response occurs in two stages: (A) the sensitization effect, which is the asymptomatic stage when the host is exposed for the first time to the antigen, and (B) the effect period, which occurs when the host is re-exposed to the antigen, and this leads to a type-I anaphylactic or atopic immune response (Carey *et al.*, 2008; (Townsend *et al.*, 2012). All atopic disorders are considered allergy disorders, but not all allergic disorders are atopic. Allergic reactions mostly affect the eyes, nose, lungs, and skin, causing allergic rhinitis and allergic asthma (Bousquet *et*

al., 2008). Studies on non-human primates (rhesus monkeys) showed that inhalation of ozone and dust mites escalate allergen sensitization (Card and Zeldin, 2009); (Julge, Meriste and Bjo, 2002). Inhalation of urban $PM_{2.5}$ may exacerbate allergic inflammation and lung eosinophilia in murine (He *et al.*, 2017). Additionally, exposure to aeroallergen from dust mites and pets in sensitized individuals initiates the activation of an allergen-specific Cluster of differentiation antigen 4 (CD4). Activation of Allergen-specific CD4 prompts the production of Th2-type cytokines (interleukin-4 and interleukin-13) to produce and initiate an asthmatic inflammatory responseClick or tap here to enter text..



Diagrammatic presentation of oxidative stress by pollutant: role in allergic airway inflammation. Adapted from ref (55).

2.4 Review of previous epidemiological studies that investigated effect modification due to sex of the association between air pollutants and respiratory health

Clougherty *et al.*, have conducted a review on the effect modification by gender on the association between air pollution and respiratory health in adults and children, assessing publications from 1989-2009 (Clougherty, 2010) (56). They identified 39 publications that examined the effect modification of gender or sex. Most publications were conducted in cohort and case-crossover study designs. Nevertheless, 22 studies reported gender and sex differences in respiratory health among children aged (0-24 years), of which ten studies reported stronger associations among girls, four studies reported stronger association among boys, and eight

studies reported null or mixed effect modification. The exposure measurements included ambient and outdoor exposure to NO_2, PM $_{2.5}$, PM_{10}, SO_2, O_2, O_3, CO, TRS, COH, EC, BS, truck traffic density and distance from busy and freeways. The outcome measurements included changes in lung function tests FVC, FEV_1, MMEF, PEF, FEF $_{25-75}$, respiratory hospitalization, the prevalence of wheeze and cough symptoms, asthma, and chest tightness. The review included studies conducted in 14 countries, mostly from Europe, the United States, and Canada, and only three developing countries: China, Poland, and Mexico. One limitation that the authors have stated in the reviewed studies is the different study designs, outcome measurements, exposure intensity and measurements, and reporting of results (odds ratio or risk ratios); thus, conducting a meta-analysis was not appropriate.

Additionally, only five studies were conducted in developing countries and none from Africa. It's important to have studies from Africa where the air pollutants composition and level, metrological conditions, age, and population health differ from those of other countries, resulting in different findings when conducting epidemiological studies. In addition, there is a lack of air quality measurement; only seven African countries have reliable air pollution monitors (Miller *et al.*, 2003). Furthermore, the reviewed studies did not measure pollen exposure and the fraction of exhaled nitric oxide FeNO. For instance, it is important to conduct studies during the pollen seasons, as the interaction between pollen particles and air pollutants might damage the cell wall of the Pollen, thus increasing the release of pollen allergen in the environment.

Furthermore, the FeNO test provides a non-invasive biomarker for eosinophilic inflammation. Additionally, atopy can be associated with a higher level of FeNO in both atopic asthmatic and atopic non-asthmatic individuals (Linhares *et al.*, 2011); (Scott *et al.*, 2010). In addition to age, gender was an important factor for a high level of FeNO in healthy children (Zhang *et al.*, 2013). Epidemiological studies showed mixed results on gender-related air pollution. Therefore, this current literature review will focus on studies published after the review by Clougherty *et al.* (i.e., 2010-2021).

PubMed was used to search using the keywords combined by Boolean operators such as 'AND/OR' commands. Keywords were identified using Population, Exposure and Outcome for the period between 2010-2021. The first stage of the search was broad, including the query: '(Children or Adolescent) AND (NO_2 OR particulate OR PM OR Ozone or Carbon black OR

Fungal spores OR tree pollens OR weeds OR grass pollen) AND (Spirometry OR airway Obstruction OR Decreased peak flow OR FEV_1)'. The broad search resulted in 155 papers in which the full text was screened. Only 18 articles examined air pollution effect modification by sex and/or atopy. A more specific search was also conducted, including the effect modifiers of interest; '(Children or Adolescent) AND (NO_2 OR particulate OR PM OR Ozone or Carbon black OR Fungal spores OR tree pollens OR weeds OR grass pollen) AND (Spirometry OR airway Obstruction OR Decreased peak flow OR FEV_1) AND (Effect modification OR Subgroup analysis OR Stratified analysis OR Stratification) AND (Sex OR Gender OR Atopy OR Sensitization OR Allergy sensitization)'. Only two publications examined the effect modification of sex and/or atopy. A sum of twenty articles was included in this literature review.

2.4.1 Evidence on effect modification by sex on the association between air pollution and children's respiratory health

Sixteen articles examined air pollution and the effect modification of sex in children. Studies had different study designs. The review included seven cross-sectional studies, five cohort studies, three panel studies and one population-based study.

In the studies examining sex as an effect modifier, six studies found a stronger association in girls compared to boys (Naclerio *et al.*, 2020); Linhares *et al.*, 2011; Wong, Coates and To, 2016; Yang *et al.*, 2020; Gao *et al.*, 2013), and fourteen studies found stronger associations in boys compared to girls (Clougherty, 2010; Scott *et al.*, 2010; Zhang *et al.*, 2013; Li *et al.*, 2020; (Chen *et al.*, 2018); (Wong, Coates and To, 2016); Yang *et al.*, 2020; Gao *et al.*, 2013) (YANG *et al.*, 2020; Xing *et al.*, 2020; Xu *et al.*, 2018; Chang *et al.*, 2012; B. Chen *et al.*, 2019; Xu *et al.*, 2020).

In studies with a stronger association among girls, one study found an association between PM_1 and FVC (Xing *et al.*, 2020), one study found an association between PM_1 and PEF (Yang *et al.*, 2020), one study found an association between $PM_{2.5}$ and FVC, FEV_1, PEFF (Li *et al.*, 2020; Chen *et al.*, 2018; Wong, Coates and To, 2016; Yang *et al.*, 2020). One study found an association between exposure to NO_2 and lower FVC and FEV_1 (Chen *et al.*, 2018), and one found an association between PM_{10}, FEV_1, FVC, and PEF (Chen *et al.*, 2018).

In the studies with the stronger association among boys, seven studies found an association between $PM_{2.5}$ and decline in FEV_1, FVC, FEV/FVC (Scott *et al.*, 2010; Zhang *et al.*, 2013;

Yang *et al.*, 2020; Xu *et al.*, 2018; Chang *et al.*, 2012; B. Chen *et al.*, 2019; Xu *et al.*, 2020), and One study found an association between exposure to traffic-related air pollutants CO, NO_x, NO and NO_2 (Li *et al.*, 2020). One study found that a distance of 25km from pollutant sources was associated with a more significant decrease in FEV_1 among boys compared to girls and a greater reduction in FVC among girls compared to boys (Gao *et al.*, 2013). The results indicate that the further away the children reside from the sources of pollutants, the better lung test results.

Fourteen studies found no significant differences between boys and girls as estimates overlap (Clougherty, 2010; Scott *et al.*, 2010; Li *et al.*, 2020; Chen *et al.*, 2018; Wong, Coates and To, 2016; Yang *et al.*, 2020; Gao *et al.*, 2013; Xing *et al.*, 2020; Xu *et al.*, 2018; Chang *et al.*, 2012; B. Y. Chen *et al.*, 2019; Xu *et al.*, 2020; Guo *et al.*, 2019). Only two studies with significant results among boys and girls found an association between $PM_{2.5}$ and more decrement in FEV_1 among boys (Zhang *et al.*, 2013), and one study showed an association between pm_{10} and more decrements in FEV_1 and FVC among girls (Linhares *et al.*, 2011).

The exposure measurements for PM_{10}, $PM_{2.5,}$ SO_2, NO_2, and CO were assessed by fixed monitoring stations, land regression models, timely adaptive modelling, satellite-based spatiotemporal model, national pollutant release inventory data and $PM_{2.5}$ measurement device (Agris, WP6930S). The outcome measurements were lung function tests FEV_1, FVC, FEV/FVC and a standardized asthma questionnaire.

2.4.2 Evidence on effect modification by atopy on the association between air pollution and children's respiratory health

Five articles examined the modifying role of atopy on the association between air pollution and respiratory health in children (Chang *et al.*, 2012; Xu *et al.*, 2020; Guo *et al.*, 2019; Lee *et al.*, 2011; Gehring *et al.*, 2013). The review included two cohort studies and three panel studies. The main exposure measurements were $PM_{2.5}$, SO_2, NO_2, CO, NOx, NO and metrological data. The outcome measurements were lung function tests FEV_1 and FVC, a standardized asthma questionnaire, a skin prick test to measure allergic sensitization, and nasal smear to measure IgE specific antibodies to certain allergies.

One article found no significant association between exposure to PM_{10} and declined lung function FEV_1 and FVC among asthmatic and healthy children (Xu *et al.*, 2020). One study found no association between exposure to $PM_{2.5}$ and a decline in PEF among asthmatic and healthy children (Xu *et al.*, 2020). One study found no significant association between

exposure to NO and NO_2 and decline in FEV_1 among asthmatic and non-asthmatic children (Lee *et al.*, 2011). One study found no significant association between exposure to NO and NO_2 and decline in FEV_1 among children with and without airway hyperresponsiveness (Lee *et al.*, 2011). Additionally, one study found no significant association between exposure to PM_{10} and NO_2 and decline in lung function FEV_1 among atopic children (Brown *et al.*, 2015) compared to healthy children. And one study found a marginal association between PM_{10} and NO_2 among and decline in PEF among sensitized children compared to healthy children (In, In and Paulo, 2013).

2.5 Areas for future research

The number of epidemiological studies investigating the effect-modification of sex or atopy on young children exposed to chemical air pollution is limited, and no studies on biological air pollutants could be found. Future studies should explore the relationship between exposure to ambient air pollution and lung function tests with the effect modification of sex and atopy status in children living in developing countries, particularly in Africa. Ambient air pollution is increasing in Africa, but most existing studies were conducted in high-income countries. In Africa, the sources and mixture of air pollution and spatial distribution are also different. In addition, studies in Africa are age-limited, mainly focused on adults. Studies investigating the environmental ambient chemical air pollutants and biological pollutants exposure and their role in asthma among schoolchildren residing in informal settlements is thus a research gap. Children are among the most vulnerable groups to the effect of air pollution as their lungs are growing; they are more active and breathe in more air and absorb more pollutants compared to adults. Children also spend more time in the ground where the concentration of some pollutants reaches its peak levels. Children residing in informal settlements are even more susceptible to the health effects of ambient air pollution due to lower socioeconomic status compared to children residing in urban settings. More studies should focus on children and children residing in industrial areas where the exposure to air pollution is more profound.

Although air pollution is a complex mixture of chemicals and biological pollutants, most epidemiological studies focus on single pollutant exposure. As humans are constantly exposed to a cocktail of air pollutants, there is an increasing need to shift the focus to a multi-pollutants approach. In addition, the previous studies on sex differences have been mixed and inconsistent. Identifying sex and atopy differences can help develop specific policies or

interventions to minimize the risks of health outcomes associated with ambient air pollution in children residing in informal settlements.

Additionally, there is a lack of studies focusing on objective measures of respiratory outcomes, particularly exhaled nitric oxide FeNO (a marker for airway inflammation) that provides simple and non-invasive measures for airway diseases such as asthma (Xu *et al.*, 2020; Guo *et al.*, 2019; Lee *et al.*, 2011).

Table 1 summary of the evidence from studies on the association between ambient air pollution, pollens, and respiratory health and its effect modification by sex and atopy in children

Study	Study aims and design	Sample size and population	Exposure Measurements	Outcome Measurements	Effect modifiers	Results	Conclusion
Acute effects of ambient PM2.5 on lung function among schoolchildren (Xu *et al.*, 2020)	Prospective panel study aimed to measure the short-term and lag effect of $PM_{2.5}$ among school-aged children using repeated measurements of lung function	848 schoolchildren in grades 3-5 with an average age of 9.74 in Zhejiang Province, China.	Daily air pollution data were derived from fixed-site monitoring stations. Air pollution indicators included $PM_{2.5}$, SO_2, NO_2, CO and daily 8-h maximum Ozone (O_3–8h).	Lung function tests (FVC, FEV1 and PEF).	Sex and asthma status	Association between $PM_{2.5}$, FVC, and FEV_1 were stronger for boys than girls but not significant in both. Association between $PM_{2.5}$ and PEF is stronger in asthmatic children compared to healthy children but not significant.	The results showed more deficit in lung function FEV_1(mL) and FVC (mL) among boys compared to girls and among asthmatic compared to non-asthmatic. However, the confidence intervals overlapped, and the groups' results were marginally different.
Acute Effects of Individual Exposure to fine Particulate Matter on Pulmonary Function in Schoolchildren (YANG *et al.*, 2020)	panel study aimed to determine the acute effects of exposure to fine particulate matter60 (PM2.5) on schoolchildren's pulmonary function	51 children aged 9-12 years, recruited from boarding schools in Begin, China.	The hourly concentration of $PM_{2.5}$ in classrooms, dormitories, canteens, and playgrounds for five consecutive days using $PM_{2.5}$ measurement devices (Agris, WP6930S).	Parent and schoolchild questionnaire, Physical examination and Lung function tests (VC, FEV_1, FVC, PEE, FEF)	sex	A 10μg/m^3 increase in $PM_{2.5}$ on the day of physical examination caused a decrease in FEV_1 and FEV_1/FVC in boys (62.26 mL and 25.85%), respectively. On lag 0–1 d caused a decrease in $FEF_{25\%–75\%}$ and FVC in boys of 99.92 mL/s and 82.11 mL, respectively. On lag 0–2 d and lag 0–3 d, it caused a decrease in FEV_1 in boys of 106.58 mL and 65.10 mL, respectively;	$PM_{2.5}$ is more associated with decrements in pulmonary function among boys compared to girls.

						and on lag 0–4 d, it caused a decrease in $FEF_{25\%–75\%}$ and FVC in boys of 66.92 mL/s and 16.50 mL, respectively. No significant changes in girls were observed on the same days.	
Chronic effects of ambient air pollution on lung function among Chinese children (Gao *et al.*, 2013)	A cross-sectional study design aims to examine long-term exposure to air pollutants and lung function among school children.	2060 schoolchildren aged 8-10 in Hong Kong	Two measures of short-term exposure of PM_{10}, NO_2, SO_2 and O_3; One hourly mean average between 7:00 to 14:00 during the 13 test days; and the other measurement for 24 h mean average of PM_{10}, NO_2, SO_2 and the 8 h maximal mean for O3 on one day before the test and metrological data.	Two self-administered questionnaires, lung function test FEV_1 FVC and metrological data.	sex	a 1 mg/m3 increase of annual PM10 was associated with a significant decrease in FEV_1 (L) [-0.008 (95%CI; -0.015, -0.002)] in boys, compared to FEV_1 (L) [-0.001(95%CI; -0.007, 0.005)] in girls.	The results showed more decline in FEV_1 among boys compared to girls, but the results between the groups are marginally significant as the confidence intervals overlapped.
Exposure to industrial air pollutant	A cross-sectional study to measure the long-term exposure and lung function test in participants residence within	2,833 children aged 6-18 years old.	The weighted sum of NO_X and $PM_{2.5}$ within 25 km of residence using national pollutant	In-home interviews and physical examinations, including lung function tests	sex	An industrial air emission within 25 km of the residence resulted in FVC of [103.21 (95%CI; 102.30 - 104.12)] in males compared to [102.93 (95%CI; 101.92 -	A distance of 25km was associated with better FVC in boys compared to girls. In contrast, better FEV_1 was observed in girls compared to boys. The confidence intervals are

emissions and lung function in children: Canadian Health Measures Survey, 2007 to 2011(63) (Wong, Coates and To, 2016)	25km from industrial emissions.		release inventory data.	FEV_1, FVC, FEV/FVC		103.93)] among females; and FEV_1 [98.63 (95%CI;97.70 to 99.56)] among males, compared to [99.09 (95%CI; 98.00 to 100.18)] among females.	overlapping; hence the decrements in lung function tests are not significant for both FVC and FEV_1 between males and females.
Interactions between ambient air pollution and obesity on lung function in children: The Seven North-eastern Chinese Cities (SNEC) Study (Li *et al.*, 2020)	A cross-sectional study aims to evaluate the joint effect of ambient air pollutant PM_1 and obesity on children's lung functions.	6740 children aged 7-14 years across seven north eastern Chinese studies from 2012-2013.	A spatiotemporal model uses daily particulate matter concentrations PM_1, $PM_{2.5}$, PM_{10} and NO_2.	Lung function measurement (FVC, FEV_1, PEF and MMEF).	sex	An IQR increase of 13.1 µg/m3 for PM_1 was associated with FVC (mL) [-55.0 (95%CI; -90.82 to -19.19)] among girls, compared to FVC (mL) [-15.80 (95%CI; -69.53 to 37.93)] in boys in the normal BMI category.	In the normal weight category, girls showed more decrement in FVC compared to boys. However, the Confidence intervals of the groups overlapped, and the results are marginally Significant.

Long-Term Exposure to Ambient Fine Particulate Matter (PM2:5) and Lung Function in Children, Adolescents, and Young Adults: A Longitudinal Cohort Study (Guo *et al.*, 2019)	Longitudinal cohort study aims to investigate the association between long-term exposure to ambient $PM_{2.5}$ and lung function in Taiwanese children.	24,544 participants aged 6-24 years.	Annual average concentration of $PM_{2.5}$ using satellite-based spatiotemporal model	Lung function tests FVC, FEV_1, using spirometry	sex	a 10 μg/m^3 increase in 2-year average of $PM_{2.5}$ was associated with FVC (mL) [-2.66 (95%CI -3.23 to -2.09] and FEV_1(mL) [-3.73(95%CI;(-4.37 to -3.08)] among males, compared to FVC (mL) [-1.72 (95%CI; -2.20, -1.23] and FEV_1(mL) [-1.86 (95%CI; -1.91, -1.82) among females.	Exposure to $PM_{2.5}$ was associated with more decrements in FVC and FEV_1 among boys compared to girls. Also, the results are significant between the two groups.
The short-term effects of air pollution on adolescent lung function in Taiwan (Chang *et al.*, 2012)	Cross-sectional study aims to assess the association between exposure to air pollutants and lung function tests.	2919 junior high school children aged 12-16 in Taipei City, Taiwan students	Hourly pollutants data for (SO_2, CO, O_3, PM_{10} and NO_2) from fixed monitoring stations and hourly metrological data	Lung function tests (FVC and FEV_1) using spirometer.	sex	An increase of 12ppb NO_2 was associated with FVC (mL) [-48 (95%CI; -55 to -41) and FEV_1 (mL) [-32 (95%CI; -39 to -26) in girls, compared to FVC (mL) [-45 (95% CI; -53 to -37) and FEV_1 (mL) [-26 (95% CI; -33 to -9) in boys.	Exposure to ambient air pollutants was negatively associated with a deficit in lung function tests in boys and girls, but the association was marginally stronger in girls for NO_2 exposure. However, the confidence intervals of FEV_1 and FVC among the two groups overlapped, and the results were insignificant.
Effect modifiers of lung function and daily air pollutant variability in a panel of schoolchildren	Panel study of school children to examine whether short-term air pollutants fluctuation was related to daily lung function among a panel of school	423 children, grades (3-6) with a mean age of 10.5 in Durban, South Africa	Monitoring stations were deployed in each school to allow for Continuous measurements of CO, SO_2, NO, NO_2 and Daily	Interviews, skin prick testing, lung function tests FEV_1 using a spirometer	Persistent Asthma or AHR.	On the 5 days average, an IQR increase of NO_2 (8.19ppb) was associated with more deficit in FEV_1 (mL) in asthmatic children [-0,034 (95%CI, -0.080,0.012)], compared to in FEV_1 (mL) [-0,031 (95%CI, -0.055, -0.008)] in	Although the results showed a decline in FEV_1 in the asthmatic and children with +AHR when exposed to NO and NO_2, the results are marginally significant as the confidence intervals overlapped.

(Mentz *et al.*, 2019)	children and whether these changes are modified by Airway hyperresponsiveness (AHR), location and Asthma severity.		measurements of $PM_{2.5}$			non-asthmatic children and more deficit in children with +AHR [-0,049(95%CI,-0.093,-0.004)], compared to [-0,033 (95%CI, -0.056, -0.009)] in children with -AHR. On the 5 days average, an increase of NO (29.7ppb) was associated with more deficit in FEV_1 (mL) in children with persistent asthma [-0,068 (95%CI, -0.123, -0.013)], compared to FEV_1 (mL) [-0,058 (-0.086, -0.030)] in non-Persistent Asthmatics. And more deficit in FEV_1 (mL) among children with +AHR [-0,050 (95%CI, -0.087, -0.013)], compared to FEV1(mL) [-0,064 (95%CI, -0.098, -0.03)] among children with -AHR.	
Acute effects of $PM_{2.5}$ on lung function parameters in schoolchildren in Nanjing, China: a panel study (Xu *et al.*, 2018)	Prospective cohort study aimed to assess the short-term effects of $PM_{2.5}$ on repeated measurements of lung function among school children.	86 students aged 9 years old were enrolled in two primary schools in China from grades three to five.	Daily air pollution data for $PM_{2.5}$ was obtained from fixed-site monitoring stations. Daily data of mean temperature and relative humidity from the	Standardized questionnaire and lung function measurement using electronic spirometer.	sex	An IQR increase of $PM_{2.5}$ (84.3µg/m3) was associated with decrease in FVC (mL) among girls [30.11 (95%CI, 14.10, 46.13)] compared to boys [17.75 (95%CI,4.98, 30.53)], FEV_1(mL) among girls; [24.02 (95%CI, 8.98, 39.07)] compared to Boys: [14.95 (95%CI, 2.46,	The association of $PM_{2.5}$ with lung function deficits was greater in girls compared to boys. However, the confidence intervals overlapped, and the results are marginally significant

			meteorological data network.			27.45)], PEF (mL/s) among girls [34.51(95%CI, −5.72, 74.73)], compared to Boys; [23.09 (95%CI) −20.16, 66.35)], And FEF25%; -75%(mL/s) among girls [30.64 (95%CI, 5.66, 55.62)] compared to Boys; [22.48 (−5.09, 50.05)].	
Changes in the relationship between ambient fine particle concentrations and childhood lung function over 5 years (Yang *et al.*, 2020)	Two Cross-sectional surveys aimed to study the temporal changes in the relationship between $PM_{2.5}$ and lung function among children.	Studies were conducted in Taiwan. 2011: 1358 schools from 22 elementary and middle schools with an average age of 11.9. 2016-2017: the same protocol was repeated (n=2557) with an average of 11.2. 2 elementary and one primary school did not participate.	Daily measurement of $PM_{2.5}$ and monthly calculated obtained from fixed monitoring stations. In the 2011 survey, $PM_{2.5}$ median (interquartile Range): 48.1 (33.8–48.1). In the 2016-2017 $PM_{2.5,}$ median (interquartile range): 30.9 (26.0–35.2)	Lung function tests (FVC and FEV) using Spirometry. Parents were asked to fill out the asthma questionnaire.	sex	In the 2011 survey: An IQR increase in PM $_{2.5}$ (40.6 µg/m3 in) was associated with FVC (mL) among boys [-3.3(95%CI, -6.4, -0.2)], FEV (mL) [-3.6 (95%CI, -7.0, -0.1)], compared to girls [-1.6 (95%CI, -4.8, 1.8) and FEV (mL) [-2.1 (95%CI, -5.2, 1.1)]. In 2016-2017 survey the PM $_{2.5}$ levels of 30.0 µg/m3 in was not significant for FVC (mL) among Boys [-0.6 (95%CI, -2.4, 1.1)] and FEV_1 (mL) [-0.2 (95%CI, -2.0, 1.6)], and among girls FVC (mL) [-0.7 (95%CI, -2.6, 1.3)], FEV_1 (mL) [-0.4 (95%CI, -1.9, 1.1)]	In the 2011 survey, the deficit in FEV1 and FVC was more among boys compared to girls, but the results were not significant as the confidence intervals overlapped. Also, the association was not significant in the 2016-2017 survey.

Regional PM2.5 and asthma morbidity in an agricultural community: A panel study (Loftus *et al.*, 2015)	A panel study to investigate the association between $PM_{2.5}$ and paediatric asthma exacerbation in an agriculture community in Washington state.	58 school-age children with an average age of 10.4.	24-h average $PM_{2.5}$ levels based on nephelometer measurements made from fixed monitoring stations. 24-h meteorological data.	Asthma symptoms survey and daily home lung function tests	Atopy	An IQR increase in PM $_{2.5}$ (7.9 $\mu g/m^3$) was associated with decrements in FEV $_1$(mL) among atopic children [-1.4 (95%CI, -2.7, -0.2)], compared to non-atopic [0.5 (95%CI, -0.7, 1.7)].	$PM_{2.5}$ is a risk factor for asthma morbidity among atopic children compared to non-atopic children living in an agricultural environment. The confidence intervals overlapped between the two groups, and the results were marginally significant.
Variation in Peak Respiratory Flow measurements associated to air pollution and allergic sensitization in children in Sao Paulo, Brazil (In, In and Paulo, 2013)	A prospective cohort study aims to investigate the effects of air pollution on Peak expiratory flow (PEF) and FEV_1 in children with and without allergic sensitization.	96 children aged 9-11 in Sao Paulo, Brazil.	The hourly concentration of (PM_{10}), nitrogen dioxide (NO_2), nitrogen oxide (NO), carbon monoxide (CO), sulphur dioxide (SO_2), and Ozone (O_3). Metrological data (temperature and humidity)	Spirometry Skin prick test Blood samples Nasal smear	Sensitization status	Children were categorized into three groups. Category 0: (no symptoms of allergy and the normal value of IgE, number of eosinophils in the blood, lower than 30% eosinophils in nasal smear and no positive reaction to skin brick test), Category 1 (children with any symptoms of allergy or one positive test), Category 2 (symptoms of allergy and all tests are positive) A 10 $\mu g/m^3$ increase in PM_{10}, NO_2 was associated with lower PEF in all categories. For the previous 24-h. A 10 µg/m3 PM_{10}; category 0 [-1.72 (95%CI, -3.12 to -	Results for those who are allergic were not significantly different from those who were not. $PM_{10.}$ It was only associated with a significant decrement in PEF in category 0, but the confidence intervals among the three categories overlapped, and the results are marginally significant. For no_2, the decrement was only significant for categories 0 and 1, but the confidence intervals of the three categories overlapped, and the results were marginally significant.

						0.33)], category 1[-0.52 (95%CI, -2.06 to 1.02), category 2 [−0.70 (95%CI, -2.95 to 1.55)]. For a 10 µg/m3 of NO_2, the declines of PEF in category 0 [-1.83 (95%CI, -3.01, -0.65)] and [−1.56 (95%CI, −3.02, -0.10)] and [-0.55 (95%CI, -3.02, 1.92)] in category 1 and 2, respectively.	
Effects of ambient air pollution on pulmonary function among school children (Lee *et al.*, 2011)	A population-based study aiming to maximize the variability and minimize the correlation in criteria outdoor pollutants based on historic routine air monitoring data.	4765 in grade Seven, age 12-13, in Taiwanese communities.	Hourly Outdoor pollutants data, including CO, NOx, NO, NO_2, O_3, SO_2, $PM_{2.5}$ and PM_{10}, were collected from a fixed monitoring station.	Parental questionnaires and pulmonary function tests.	sex	An IQR increase of NO2 (8.86 ppb) was associated with more decrements in FCV (mL) among boys [-120.7(95%CI, -216.8, -24.7)], compared to girls FVC [-58.7(95%CI, -127.8,10.5)]. An IQR increase of NO2 (8.86 ppb) is also associated with more decrement in FEV1 among Boys [-102.3 (95%CI, -180.8, -23.8)], compared to FEV1 in girls [-54.1 (95% CI, -119.1,10.9)].	Exposure to NO2 was associated with more decrements in FEV1 and FVC among boys compared to girls, but the association is not significant.
Is PM1 similar to PM2.5? A new insight into the association of PM1 and PM2.5 with	Cross-sectional to evaluate the associations between long-term exposure to PM_1 and $PM_{2.5}$ with children's lung function and	6740 children From primary school (6-12) years, and middle school (12-15) years in China.	Daily PM_1 and $PM_{2.5}$ and meteorological data and land use information based on satellite monitoring.	Lung function tests FEV1 FVC, MMEF, and PEF using an electronic Spirometer.	sex	An IQR increase of PM_1 (12.9 µg/m^3) was associated with more decrement in FEV_1 (mL) among boys [-119.4 (95%CI, -161.7 to -77.00], compared to girls [-116.4 (95%CI, −149.7, -83.1]. PM_1 had a more	PM_1 association among boys were marginally stronger than among girls for FEV_1. However, the associations were marginally stronger in girls for PEF and MMEF. PM_1 was associated with more decrements in FEV among boys compared to girls; the

children's lung function (58) (Yang *et al.*, 2020)	differentiate the associations of PM_1 from $PM_{2.5}$.					significant association in PEF (mL/s) among girls [-279.9 (95%CI, −359.5, −200.2], compared to boys [−175.6 (95%CI, −269.1, −82.1] and showed a decrement in MMEF among girls [-134.1 (95%CI, -194.5, -73.7] compared to boys [-38.6 (95%CI, -109.9, 32.7)].	association was more in PEF and MMEF among girls compared to boys. However, the association of all lung function tests between boys and girls was marginally significant as the confidence intervals overlapped.
Linkage between Particulate Matter Properties and Lung Function in Schoolchildren : A Panel Study in Southern China (Hu *et al.*, 2020)	Panel study to evaluate the changes of $PM_{2.5}$ concentration on lung function.	29 boys and 28 girls between the age of 8-13 from primary schools in Heshan, China.	Online measurements of air pollutants from fixed monitoring stations.	Lung function measurements (FEV, MMEF and PEF) using spirometer	sex	An IQR increase of $PM_{2.5}$ (19.1 g/m^3) was associated with more decrements in FEV_1 (ml), MMEF (ml/s) and PEF (ml/s) among boys compared to girls.	The association was stronger among boys compared to girls, but the confidence intervals of the associations overlapped, and none of the pollutant's interactions with gender were statistically significant.
Long-term exposure to PM2.5 and Children's lung function: a dose-based association analysis (Li *et al.*, 2020)	Cross-sectional study to estimate the long-term exposure to $PM_{2.5}$ and lung function in schoolchildren in China.	687 children aged 7-12 from	$PM_{2.5}$ measurements were collected using timely structure adaptive modelling	Lung function measurements using a spirometer	sex	In general, an IQR increase of $PM_{2.5}$1 µg/kg-d was associated with more decrements in FVC (mL) among girls [-0.86 (95%CI, -46.82, -14.89)] compared to boys [-2.78 (95%CI, -14.66, −9.11)], FEV_1 (mL)was more deficient among girls [-21.91	Girls reported a larger decrease in FEV_1 and FVC and PEF measurements with a unit increase in Average Daily Dose of $PM_{2.5}$ compared to boys. However, the confidence intervals of the association among boys and girls overlapped, and the results were marginally significant.

						(95%CI, −36.34, -7.48)], compared to boys [-2.78 (95%CI, −14.66, -9.11)]. PEF (mL/s) was deficient among girls [4.24 (95%CI, -39.29, 47.76)], compared to boys [-6.48 (95%CI, -37.80, 24.84)]	
Relationship between exposure to fine particulates and ozone and reduced lung function in children (Hwang *et al.*, 2015).	A prospective cohort study to assess the relationship between air pollutant exposure and lung function growth and to examine potential sex differences in the susceptibility of lung function growth to air pollution	2941 Taiwanese children aged 12 years old.	Hourly reported ambient air monitoring data for SO_2, NO_2, O_3, CO and $PM_{2.5}$ from fixed monitoring stations.	Questionnaires and Pulmonary function test to measure FVC and FEV_1 using two spirometers.	sex	Greater exposure to $PM_{2.5}$ (IQR, 17.92 μg/m^3) was associated with more decrements in FVC (mL) in boys [-76.89 (95%CI, -120.72, -33.06)], and FEV_1 (mL) [-85.14 (95%CI, -126.67, -43.62)], compared to FVC1(mL) [-63.97 (95%CI, -98.49, -29.46)] and FEV_1(mL) [-56.84 (95%CI, -93.12, -20.56)] among girls.	Long-term exposure to air pollutants was associated with more deficit in FVC (mL) and FEV_1(mL) among boys compared to girls and may have a detrimental effect on the development of lung growth in boys more than girls. However, the confidence intervals of the association overlapped, and the results were marginally significant.
Short-term effects of ambient air pollution exposure on lung function: A longitudinal study among healthy primary school	A stratified cohort study examines the association between short-term effects of ambient air pollutants and lung function among healthy primary school-aged children in China.	334 healthy children (7-11 years) from four cities in China.	Daily average concentrations of PM 2.5, PM_{10}, NO_2, and SO_2 were obtained from fixed-site monitoring stations around the schools.	Lung function measurements FVC, FEV_1 and PEF using Spirometry.	sex	Gender stratified analysis found that the adverse effects of air pollutants on lung function were stronger in girls compared to boys. An IQR level of PM_{10} (144 μg/m^3) was associated with more decrement in FVC (L) among girl [−5.76, (95%CI, -4.73, -3.18)], FEV_1 [-8.45(95%CI,-10.73,-6.12] and PEF [-6.69(95%CI,-	Short term exposure to PM_{10} was associated with more decrements in lung function among girls compared to boys. However, the confidence intervals of PEF overlapped, and the results were marginally significant. Only the results of FVC and FEV_1 were significant among boys and girls.

children in China (Chen *et al.*, 2018).						10.67,-2.52)], compared to FVC [0.66, (95%CI, (−1.93,3.32)], FEV_1 [-2.68(95%CI, -5.02,-0,27)] and PEF[-2.01(95%CI, -6.07,2.22)] among boys.	
Air Pollution Exposure and Lung Function in Children: The ESCAPE Project (Gehring *et al.*, 2013)	Analysis of birth cohort studies in European countries, aiming to study the association between residential exposure to air pollution and lung functions.	5,921 children 6-8 years of age.	Using a land-use regression model, routine annual air pollutant data from regional and urban areas measuring PM2.5, NOX, NO2 and black smoke.	Lung function measurements (FEV_1, FVC and PEF).	sex and asthma	An IQR increase of 10 µg/m³ for PM_{10} was associated with decreased in FEV_1 (L) in asthmatic children [-6.83 (95%CI, -19.8, 8.21)], compared to non-asthmatic [-0.90 (95%CI; -2.92, 1.16)]. an IQR of $PM_{2.5}$ (5 µg/m³) was associated with more decrements in FEV_1(L) among boys [-3.99 (95%CI; -8.05, 0.24)], compared to [-1.97 (95%CI; -4.38, 0.51)] among girls.	Stratified analysis showed no systematic difference between asthmatic and non-asthmatic children and boys and girls. Exposure to PM_{10} was associated with more decrements in FEV_1 among asthmatic children compared to non-asthmatic. However, the association is marginally significant as the confidence intervals overlapped. Also, exposure to $PM_{2.5}$ was associated with more decrements in FEV_1 among boys compared to girls, but the association is marginally significant.

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3. Aims and Objectives

3.1 Aims

This study aims to explore possible effect modification by sex and atopy of the association between ambient air pollutants $PM_{2.5}$ and NO_2 and biological air pollutants such as pollen and fungal spores with asthma outcomes among school children in four informal settlements in the Western Cape.

3.2 Objectives

To describe the demographic and host characteristics of schoolchildren residing in the four settlements.

- To investigate asthma-related outcomes, including lung function and airway inflammation, cross-sectionally
- To characterise the exposure of annual ambient air pollution and biological air pollution levels in the year prior to the outcome assessment.
- To assess the association between annual-term ambient air pollution exposure and biological air pollutants with asthma-associated outcomes, including lung function and airway inflammation, cross-sectionally among schoolchildren in informal settlements.
- To investigate the effect modification of sex and/or atopy of the association between ambient air pollution and biological pollutants on asthma-associated outcomes and lung function and airway inflammation among the children.

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4. Methods

4.1 Study Population and Design

This is a sub-study of the longitudinal study conducted to investigate the association of ambient air pollution on schoolchildren's respiratory health among schoolchildren residing in four informal settlements in the Western Cape in South Africa (Olaniyan *et al.*, 2017). The baseline study was conducted between February 2015 and August 2015, and the follow-up between May 2016 to September 2016. The current study will be a secondary cross-sectional analysis of the effect modification by sex and/or atopy on the association between asthma outcomes in 2016 and exposure to ambient air pollutants and biological air pollutants in the year prior to asthma outcomes measurement. A detailed description of the study population has been previously described by Olaniyan *et al.* In brief, at baseline, 590 primary school children were enrolled in the larger study from 7 schools. “The areas from which children were recruited include an industrialized urban area in Cape Town (Milnerton/Milnerton Ridge, including Marconi-Beam, Phoenix and Joe Slovo), a peri-urban area outside Cape Town with a large informal sector (Khayelitsha), and a rural area (Oudtshoorn)”. Additionally, a fourth area (Masiphumulele, Noordhoek) with less industrial development and a community of similar socio-economic status as the previously identified areas was included (Olaniyan *et al.*, 2017).

4.2 Sampling

The sampling frame was a list of schools in the four study areas from the Western Cape Department of Education. Six schools were selected based on accessibility and location to near air quality monitoring stations in the area, from which 590 grade-4 students were recruited. Grade four children were selected because the children are old enough to follow the instructions in the study and will not graduate and move to a different school before completing the full cohort study. The school's board granted the study permission, and a list of the students in grade 4 and their home addresses was obtained from the school principals. Informed consent was obtained from parents and guardians by trained staff after visiting the children's homes. Systematic sampling was used to select school children when children whose parents/guardians consented exceeded the targeted number of children for that school. Furthermore, all Grade-4 students were selected if they met the inclusion criteria. Spirometry was postponed if any of the children did not qualify for the inclusion criteria, for example, “if a child has a positive answer to the following: child vomiting or experiencing pain, on treatment for tuberculosis; having had flu, sinusitis, or lung infection in the last month” (Olaniyan *et al.*, 2017)

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Sample size

The sample size was calculated using a comparison of two samples proportions (exposed/nonexposed ratio=1, power = 90%confidence level=95), and using the result from a recent study by R N Naidoo *et al.* were the prevalence of doctor diagnosed asthma was higher in the schools in the north with high exposure (16.5%), compared to schools in the south with lesser exposure (13.0%). The required combined sample size calculated in R studio is N=264. The estimated sample of this study (n = 590) is therefore sufficient for this sub-study.

4.3 Exposure measurements

4.3.1 Ambient air pollution

A previous publication describes the land-use regression (LUR) models developed to estimate the annual average concentration of $PM_{2.5}$ and NO_2 for this study (Olaniyan, Jeebhay, Röösli, Rajen N Naidoo, *et al.*, 2020). Briefly, air pollution monitoring was performed during 2015-2016 in each study area in which $PM_{2.5}$ and NO_2 was measured weekly during winter and summer at 140 sites (40 sites each in three study areas, and 20 sites in Masiphumulele). Seasonal (winter/summer) and annual averages were obtained for each area by temporally adjustment using routinely monitored air quality measurements. "Data on household density, nearby traffic e.g major roads, bus stops, and train stations, waste burning sites, and land-use derived from geographic information system (GIS) collected on-site, were used to evaluate the spatial variation in the annual average concentrations" (Olaniyan, Jeebhay, Röösli, Rajen N Naidoo, *et al.*, 2020). To optimise the explained adjusted variance, regression models were developed, using a supervised stepwise approach, and the models were validated using leave-one-out-cross-validation (LOOCV). The annual average concentration of $PM_{2.5}$ and NO_2 was estimated at each participant's address (Olaniyan, Jeebhay, Röösli, Rajen N Naidoo, *et al.*, 2020).

4.3.2 Biological air pollutants

The Burkard 7-day recording volumetric Spore Trap was used for continuous spore trapping for tree pollen, weed pollen and mold spores. The pollens were measured in the four-study areas from July 2015 to March 2017. There are various concentrations of pollen and spores above the ground level. Consequently, the trap is placed above the ground at a minimum height of 1.5 mm to avoid dust contamination. The spore trap in each study area was located within a 10-km radius from each child's home and school address. Pollen and fungal spores were counted microscopically by trained

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personnel to estimate to estimate average daily concentrations for a one-calendar year and at each study area to include the seasonal peaks of these aeroallergens (Olaniyan *et al.*, 2017). The average annual concentrations of pollens are expressed as particles/m^3 (further details are provided elsewhere) (Brown *et al.*, 2015).

4.4 Outcome measurements

4.4.1 Asthma questionnaire

Respiratory health, asthma screening and allergy questionaries were based on the standardised and validated International Study of Asthma and Allergies in Childhood (ISAAC). The questionnaires were administered by a trained interviewer to the parents or legal guardians of the children during the baseline and follow-up studies and translated to both Xhosa and Afrikaans (Olaniyan *et al.*, 2017). The questionnaires included information about household exposure such as paraffine use, visible mould, dampness pets, passive smoking, birth history information such as birth weight and maternal smoking and respiratory outcomes information such as rhinitis, doctor-diagnosed asthma, ocular-nasal symptoms and wheezing (Appendix 2 and 3).

4.4.2 Asthma symptoms score

Asthma symptoms score was defined as the sum of positive answers to the following eight questions on asthma symptoms and bronchial hyperresponsiveness (Yarney *et al.*, 2016) :

1. How many episodes of wheezing or whistling has the child had in the past 12 months?
2. In the past 12 months, how many times has the child had wheezing that it hard for him or her to breathe or catch his or her breath
3. In the past 12 months, how many times has the child complained that his or her chest felt tight or heavy?
4. Does the child get short of breath walking with other children of his/her own age level ground?
5. In the past 12 months, how many times has the child coughed with exercise or running or playing hard?
6. In the past 12 months, how many times has the child's sleep been disturbed due to wheezing, coughing, chest tightness or shortness of breath?
7. Has a doctor or a nurse ever told you that the child has asthma?
8. Has the child ever taken any medication for asthma?

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4.4.3 Spirometry

A flow-volume spirometer was conducted to measure the lung function of interest FEV_1, FVC, FEV_1/FVC, FEF_{25-75}. The spirometer tests were performed according to the American Thoracic Society (ATS) and European Respiratory Society (ERS). The cut-off for normal lung function for children was conducted using the Global Lung Function initiative. The cut-off was pooled according to the child's age, sex, height, and ethnicity. The cut-off was measured by the Lower Limit of Normal (LLN).

FEV_1<LLN indicates large airway obstruction and FEF_{25-75}<LLN indicates small airway obstruction. Airway obstruction was characterised if FEV_1 was less than 80% and the ratio of FEV_1/FVC <0.8Click or tap here to enter text.. The spirometer was conducted during school hours at baseline and follow-up visits. The tests were conducted by a trained technologist who was not aware of the exposure status of the participants. Spirometry was calibrated at least twice a day using a 3-litre syringe; temperature and humidity were monitored daily. The technologist is blinded to the exposure status of the participants. The tests were conducted while children were upright with nose clips. A minimum of eight trials is to produce three acceptable curves. Reproducibility criteria are achieved with the variance of the two best values for FEV_1 and FVC values at no more than 150 ml or 5%. However, when the results don't meet reproducibility criteria, it does not exclude the results from the statistical analysis as they may indicate airway dysfunction. The best FEV_1 and FVC are used regardless of whether they belong to the exact tracing. Respiratory/lung volumes obtained by spirometer were adjusted for body temperature and pressure based on the temperature and atmospheric pressure measured throughout the day. Children were instructed not to take any anti-asthmatic inhalers for 12 hours or oral asthma medications for 48 hours before the Spirometry. Children with obstructive airways were given an inhaled bronchodilator and had the Spirometry repeated after a minimum of 10 minutes. Furthermore, emergency medical professionals, Oxygen and B2-adrenergic agents for nebulization were readily available and on-siteClick or tap here to enter text..

4.4.4 Airway inflammation

An experienced nurse conducted the non-invasive fractional exhaled nitric oxide (FeNO) to assess allergic airway inflammation during the baseline and follow-up studies. The FeNO test was conducted using a handheld portable nitric oxide sampling device (NIOX MINO® Airway Inflammation Monitor; Aerocrine AB, Solna, Sweden) (Brown *et al.*, 2015). Children are upright and advised to breathe normally for about five minutes and to inhale to total lung capacity (TLC). This is followed

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by immediate exhalation at a constant flow rate of 50 ml/s for four seconds. Following the American Thoracic Society and European Respiratory Society recommendations, two measurements were taken with at least a 30-second interval, and then the average value of the two measurements will be considered in the analysis. Children are advised to avoid strenuous exercise and eating or drinking at least one hour before the test. The last date of bronchodilator and History of passive smoking are recorded. FeNO is advised to take before Spirometry, as the latter may reduce exhaled NO levels. Measurements for children with upper and lower respiratory tract infections were postponed until recovery or had their measurements taken with infection state recorded. Children's body mass index (BMI) and ambient NO and temperature (Brown *et al.*, 2015) are recorded. FeNO levels are considered elevated if the concentration is more than 15ppb and high if the concentration is more than 35ppb (a marker of airway inflammation) (Olaniyan, Jeebhay, Röösli, Rajen N Naidoo, *et al.*, 2020).

4.5 Data Analysis

4.5.1 Variables

Outcome variables

The outcome variables developed include the following

- Asthma reported symptoms including Rhinitis, doctor-diagnosed asthma, ocular-nasal symptoms and wheezing will be analysed dichotomously (Yes/No).
- Asthma symptoms score will be defined as the sum of positive answers to the eight questions on asthma symptoms measured as a continuous variable and dichotomised at ≥ a score of 2.
- Airway obstruction measured from spirometer data. Airway obstruction will be analysed as a dichotomous variable characterised if FEV_1 was less than 80% and the ratio of FEV1/FVC <0.8.
- Airway inflammation will be analysed dichotomously based on elevated FeNO levels. Two dichotomous variables will be developed based on two cut-off levels ≥ 15ppb and ≥ 35ppb.

Exposure variables

The exposure variables will include:

- Annual ambient air pollution concentration of $PM_{2.5}$and NO_2 measured as a continuous variable.
- Annual biological air pollutants concentrations including tree pollen, weed pollen and fungal spores measured as a continuous variable.

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Effect modifiers

The effect modifiers include:

- Sex which will be analysed dichotomously
- Atopy which will be analysed dichotomously based on the atopy measurements.

Confounding variables

The confounding variables are described in the next section.

4.5.2 Statistical Analysis

Descriptive analysis will be conducted to examine the population demographic; distribution characterises of the exposure of interest, including annual and seasonal concentrations of ambient air pollution and biological air pollutants at 12 months of follow-up, distribution characteristics of asthma-related outcomes, including lung function and airway inflammation at 12 months follow-up.

Univariable analysis will be used to examine the potential association between respiratory outcomes of interest and different predictors variables, including age, sex, asthma medication use, weight, height, atopy status, birthweight of the child, maternal smoking, household dampness, mould, presence of pets, household smoking, paraffin cooking and study area. Confounders selected apriori include age, gender, BMI, race/ethnicity, atopic status, asthma medication use, history of respiratory allergy, and temperature.

A sensitivity analysis will compare the subjective lung function measurements obtained (from the questionnaire) to the objective measurements (lung function and airway inflammation).

Multivariant logistic regression will be used to assess the association between ambient air pollution and biological air pollutants on asthma-related outcomes, including lung function and airway inflammation at follow up. A stratified analysis will be conducted to examine the effect modification of sex or atopy on asthma-related outcomes, including lung function and airway inflammation in children exposed to ambient air pollution and biological air pollutants at the. Statistical data analysis will be conducted using R-studio software.

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5. Ethical approval and informed consent process

The main study proposal was approved by the University of Cape Town's Research Ethics Committee (HREC REF: 697/2014) and was conducted in accordance with the Declaration of Helsinki of the 25TH world medical Assembly (Appendix 4). Before recruitment, the study was granted permission to include school children from the Western Cape Department of Education. During recruitment, permission was obtained from the school principals of selected schools, school bodies and children's parents or caregivers to include the children in the study.

Trained field staff visited the houses of the participating children to explain and answer questions from the parents/guardian and obtain informed consent (Appendix 7). The consent forms explained the purpose of the study, described the testing and risks and discomfort of the tests, the expected benefits of the study and confidentiality of the data handling and storage and informed that the tests are free of charge from the participants. The informed consent was previously developed in the main study. In addition, participation in the study was voluntary, and parents and their participating children could decline at any stage not to continue participating.

This sub-study does not involve data collection, and strict COVID protocols will be observed during any other onsite interactions.

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PART B: MANUSCRIPT

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Preparation for submission

Journal: The thesis manuscript has been prepared for submission for publication in the journal “Environmental Pollution”. The journal’s instructions for authors in (Appendix 10) have been adhered to **apart from** the cover page with co-author details and the author credit statement which are not included in the dissertation paper.

Short running head: The association between biological and chemical air pollutants with children’s respiratory health.

Competing financial interest declaration: None

Student: Yasmeen Nassr Mahmoud

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Abstract

Asthma continues to be a leading cause of morbidity and mortality among children in Sub-Saharan Africa. Ongoing under-diagnosis and treatment in low-and middle-income countries, together exposure to airborne pollutants and allergens contribute to this disease burden. A cross-sectional study that recruited 590 school children residing in four informal settlements in the Western Cape province was conducted to assess the role of sex and atopic status on the association between air pollutants and childhood asthma. Exposure to NO_2 and $PM_{2.5}$ were estimated for individual homes using a land-use regression model, while pollen and fungal spores were measured using spore traps positioned in each study area during the follow-up study between May 2016 and September 2016. Asthma-related outcomes were obtained using the International Study of Allergy and Asthma in children (ISAAC) questionnaire, spirometry, and fractional exhaled nitric oxide (FeNO) testing. The study found that increased annual grass pollen levels and annual NO_2 levels were positively associated with airway obstruction (OR: 2.26, 95% CI: 1.02-5.03) for annual grass pollen and FeNO (OR: 2.43, 95% CI:1.19-4.98) for annual NO_2 and (OR: 3.43, 95% CI: 1.07-10.99) for grass pollen. Furthermore, there was evidence of effect modification by sex and atopy since associations were much stronger among boys and atopic children. Annual NO_2 levels were positively associated with FeNO>35ppb among boys (OR:2.77, 95% CI: 1.02-7.47) but not girls. A strong positive association was also observed between higher annual grass pollen levels and FEV_1< LLN in atopic children (OR: 4.46, 95% CI: 1.28-15.59). The study provides evidence that sex and atopy modify the association between annual long-term exposure to grass pollen and NO_2 and asthma-related outcomes in children.

Keywords: childhood asthma, chemical air pollution, biological air pollutants, effect modification, sex and atopy.

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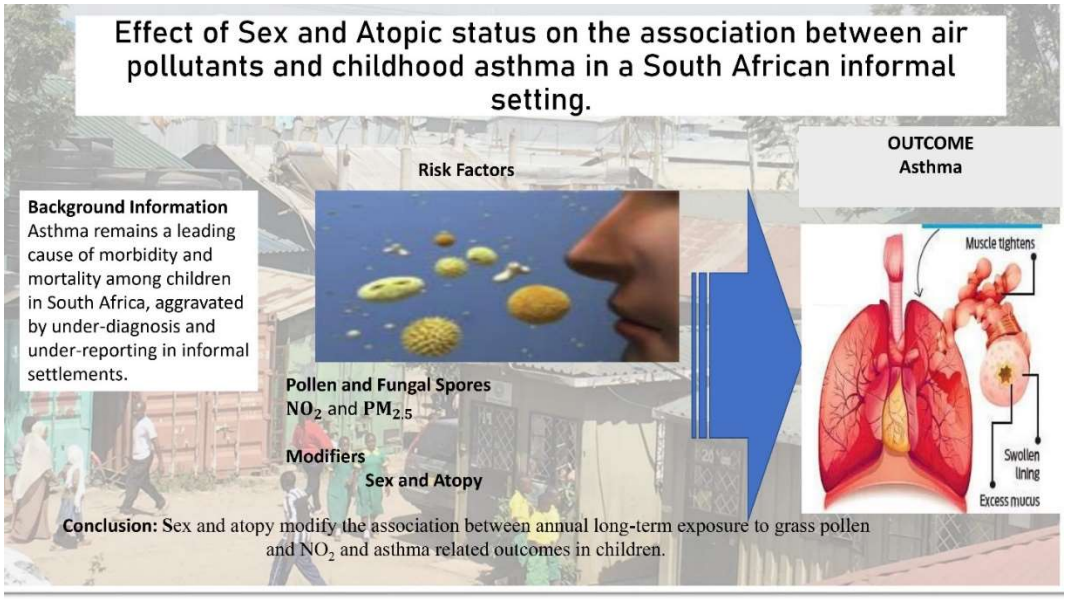

A graphical abstract on the effect of sex and atopic status on the association between air pollutants and childhood asthma in South African Informal Setting.

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Highlights

- Grass pollen was positively associated with child asthma outcomes.
- NO_2 was positively associated with child asthma outcomes.
- The association between grass pollen and asthma outcomes was stronger among boys.
- The association between NO_2 and asthma outcomes was significant only among boys.

Abbreviations

$PM_{2.5}$ – Fine Particulate Matter

NO_2- Nitrogen oxide

FeNO- Fractional exhaled nitric oxide

FEV_1- Forced expiratory volume in 1 second

FVC- Forced vital capacity

FEF- Forced expiratory flow

LLN- Lower Limit of Normal

Ppb- Parts per billion

BMI- Body mass index

LMIC- Low- or Middle-Income Country

GST- Glutathione S transfer

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1. Introduction

The World Health Organisation (WHO) has estimated that globally, around 300 million people are affected by the burden of asthma and asthma-related illness, and this trajectory is expected to reach up to 400 million in 2025 (Masoli *et al.*, 2004 ; Serebrisky and Wiznia, 2019). Asthma is a leading cause of morbidity and childhood mortality associated with chronic diseases with children in informal settlements bearing the most significant burden (Meme *et al.*, 2023). South Africa ranks among the countries with the highest asthma prevalence globally and is ranked as one of the leading countries with asthma-related child mortalities (Olaniyan *et al.*, 2017), with its urban and rural settings disproportionately affected (Olaniyan, Jeebhay, Röösli, Rajen N Naidoo, *et al.*, 2020).

Childhood asthma manifests as paroxysmal dyspnoea, wheezing or cough and has a significant adverse effect on the life of a child, including loss of school days (De Jong *et al.*, 2019; Martin, Townshend and Brodlie, 2022). Exposure to environmental factors, such as air pollution, plays a crucial role in asthma development in children (Tiotiu *et al.*, 2020; Chatkin, Correa and Santos, 2021; Sachdeva *et al.*, 2019). Air pollution has been identified as the leading environmental cause of asthma, but there is limited data in low- and middle-income countries (Landrigan *et al.*, 2018), including sub-Saharan countries in Africa (Katoto *et al.*, 2019; Landrigan *et al.*, 2018).

Chemical air pollutants, including nitrogen dioxide (NO_2), have been associated with adverse respiratory outcomes in children, such as a decline in lung function and asthma-related illness (Anenberg *et al.*, 2019; Tiotiu *et al.*, 2020). In addition to chemical pollutants, biological agents such as pollen and fungal spores contribute to the complex air quality landscape in South Africa (Gharbi *et al.*, 2023).

A study by Olaniyan et al. 2020 on ambient air pollution and respiratory morbidities, including childhood asthma in adolescents from the Western Cape Province, found that short-term exposure to biological pollutants was associated with reduced lung function. However, there is no data on the respiratory health effect of long-term exposure to biological contaminants in informal settlements. The respiratory effects of these biological pollutants may be further compounded in the presence of chemical pollutants NO_2, emphasizing the need for a comprehensive assessment that considers both categories of pollutants (Frank and Ernst, 2016; Kalisa *et al.*, 2019). As previously reported, the burden of asthma in South Africa is

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exacerbated by the high prevalence of under-diagnosed and untreated asthma among schoolchildren residing in informal settlements, which could be attributed to a weak healthcare system (Saucy *et al.*, 2018). Understanding the risk factors for asthma in children is vital to the prevention, treatment, and control of the chronic respiratory disease. The susceptibility of children to asthma is known to be modified by sex and atopy and may determine how children respond differently to exposure to air pollution (Demoulin-alexikova *et al.*, 2016).

Differences in childhood asthma stem from various factors, including variations in growth rates, hormonal factors, respiratory mechanisms, and behavioural differences, which differ in males and females (Raghavan and Jain, 2016). Before puberty, boys are more susceptible to asthma, but this phenomenon tends to reverse during adolescence, with women more likely to have severe and late onset of asthma (Fuseini and Newcomb, 2017a; Koper, Hufnagl and Ehmann, 2017; Fuseini and Newcomb, 2017b).

Atopy is characterized by an exaggerated immunoglobulin E (IgE) immune response to environmental substances, which can lead to allergic disorders such as asthma and allergic rhinitis (Naidoo RN, Robins TG, Batterman S, Mentz G, 2013). Despite the existing body of research on air pollution and respiratory health, there is a notable gap in our understanding of how sex and atopy modify the association of chemical $PM_{2.5}$ and NO_2 or biological pollutants pollen and fungal spores, with children's respiratory health. Epidemiological studies examining the modification of associations between chemical pollutants and asthma by sex among young children have found conflicting results. Stronger associations were reported among girls by Oftedal *et al.*, 2008; Rojas-Martinez *et al.*, 2007 and Rosenlund *et al.*, 2009, whereas studies by Peters et al. 1999; Gehring et al. 2002; Lindgren et al. 2009, found stronger associations among boys compared to girls.

Assessing the differential risk of asthma-related outcomes associated with exposure to long-term exposure to biological and chemical pollutants across sex and atopic status can help identify vulnerable sub-groups, tailor prevention and treatment strategies, and improve public health outcomes, particularly among this cohort of children which have previously been reported to have high underdiagnosis and underreporting of asthma; a scenario prevalent in resource-limited settings in informal settlements.

This study investigated the modification by sex and atopy of the associations between the chemical air pollutants, $PM_{2.5}$ and NO_2 or biological pollutants, fungal spores and pollen, and

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respiratory health among school children residing in four informal settlements in the Western Cape province of South Africa.

2. Methods and Materials

2.1 Study design, population, and sampling

This is a sub-study of the longitudinal study conducted to investigate the association between ambient air pollution and respiratory health among schoolchildren residing in four informal settlements in the Western Cape in South Africa (Olaniyan *et al.*, 2017). The baseline study was conducted between February 2015 and August 2015, and the follow-up between May 2016 and September 2016. The current study is a cross-sectional analysis of the effect modification by sex and/or atopy on the association between asthma-related outcomes measured during the follow-up study in 2016 and exposure to ambient air pollutants and biological air pollutants. A detailed description of the study population has been previously provided by Olaniyan *et al 2017.* In brief, at baseline, 590 primary school children were enrolled in the larger study from seven schools. The areas from which children were recruited include an industrialized urban area in Cape Town (Milnerton/Milnerton Ridge, including Marconi-Beam, Phoenix and Joe Slovo), a peri-urban area outside Cape Town with a large informal sector (Khayelitsha), and a rural area (Oudtshoorn). Additionally, a fourth area (Masiphumulele in Noordhoek) with less industrial development and a community of similar socio-economic status as the other three identified areas were included (Olaniyan *et al.*, 2017).

The sampling frame was a list of schools in the four study areas from the Western Cape Department of Education. Six schools were selected based on accessibility and location to near air quality monitoring stations in the area, from which 590 grade-4 students were recruited. Grade four children were chosen because the children are old enough to follow the instructions in the study and will not graduate and move to a different school before completing the full cohort study (Olaniyan *et al.*, 2017). The school's board granted the study permission, and a list of the students in grade 4 and their home addresses was obtained from the school principals. Informed consent was obtained from parents and guardians by trained staff after visiting the children's homes. Random sampling was used to select school children when children whose parents/guardians consented exceeded the targeted number of children for that school. Furthermore, all grade-4 students were selected if they met the inclusion criteria for the lung

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function test. Spirometry was postponed if any of the children did not qualify for the inclusion criteria, for example, if a child has a positive answer to the following: child vomiting or experiencing pain, on treatment for tuberculosis; having had flu, sinusitis, or lung infection in the last month (Olaniyan *et al.*, 2017).

2.2 Exposure measurements

2.2.1 Ambient air pollution measurements

A previous publication describes the land-use regression (LUR) models developed to estimate the annual average concentration of $PM_{2.5}$ and NO_2 for this study (Olaniyan *et al.*, 2019). Briefly, air pollution monitoring was performed during 2015-2016 in each study area in which $PM_{2.5}$ and NO_2 were measured weekly during winter and summer at 140 sites (40 sites each in three study areas and 20 sites in Masiphumulele). Seasonal (winter/summer) and annual averages were obtained for each area by temporal adjustment using routinely monitored air quality measurements. Data on household density, nearby traffic e.g major roads, bus stops, train stations, waste burning sites, and land-use derived from geographic information system (GIS) collected on-site, were used to evaluate the spatial variation in the annual average concentrations" (Olaniyan *et al.*, 2019). To optimise the explained adjusted variance, regression models were developed, using a supervised stepwise approach, and the models were validated using leave-one-out-cross-validation (LOOCV). The annual average concentration of $PM_{2.5}$ and NO_2 was estimated at each participant's address.

2.2.2 Biological air pollutants measurements

The Burkard 7-day recording volumetric Spore Trap was used for continuous spore trapping for tree pollen, weed pollen and mold spores. The pollens were measured in the four study areas from July 2015 to March 2017. There are various concentrations of pollen and spores above the ground level. Consequently, the trap is placed above the ground at a minimum height of 1.5 mm to avoid dust contamination. The spore trap in each study area was located within a 10-km radius of each child's home and school address. Pollen and fungal spores were counted microscopically by trained personnel to estimate to estimate average daily and annual concentrations in each study area to include the seasonal peaks of these aeroallergens (Olaniyan *et al.*, 2017). The average annual concentrations of pollens are expressed as particles/m^3, further details are provided elsewhere (Brown *et al.*, 2015).

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2.3 Outcome measurements

2.3.1 Questionnaire

Respiratory health, asthma screening and allergy questionaries were based on the standardised and validated International Study of Asthma and Allergies in Childhood (ISAAC). The questionnaires were administered by a trained interviewer to the parents or legal guardians of the children during the baseline and follow-up studies and backtranslated to both Xhosa and Afrikaans. The questionnaires included information about household exposure such as paraffine use, visible mould, dampness pets, passive smoking, birth history information such as birth weight and maternal smoking and respiratory outcomes information such as rhinitis, doctor-diagnosed asthma, ocular-nasal symptoms and wheezing (Appendices 2 and 3).

2.3.2 Asthma symptom score

Asthma symptom score was defined as the sum of positive answers (each positive answer = 1) to the following eight questions from the caregiver questionnaire on asthma symptoms and bronchial hyperresponsiveness (Pekkanen *et al.*, 2005) :

1. How many episodes of wheezing or whistling has the child had in the past 12 months?
2. In the past 12 months, how many times has the child had wheezing that it hard for him or her to breathe or catch his or her breath
3. In the past 12 months, how many times has the child complained that his or her chest felt tight or heavy?
4. Does the child get short of breath walking with other children of his/her own age level ground?
5. In the past 12 months, how many times has the child coughed with exercise or running or playing hard?
6. In the past 12 months, how many times has the child's sleep been disturbed due to wheezing, coughing, chest tightness or shortness of breath?
7. Has a doctor or a nurse ever told you that the child has asthma?
8. Has the child ever taken any medication for asthma?

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2.3.3 Spirometry

A flow-volume spirometer was conducted to measure the lung function of interest FEV_1, FVC, FEV_1/FVC, FEF_{25-75}. The spirometer tests were performed according to the American Thoracic Society (ATS) and European Respiratory Society (ERS). The cut-off for normal lung function for children was conducted using the Global Lung Function initiative (Cooper *et al.*, 2017). The cut-off was pooled according to the child's age, sex, height, and ethnicity. The cut-off was measured by the Lower Limit of Normal (LLN).

FEV_1<LLN indicates large airway obstruction and FEF_{25-75}<LLN indicates small airway obstruction. Airway obstruction was characterised if FEV_1 was less than the lower limit of normal LLN and the ratio of FEV_1/FVC <0.8. Spirometry was conducted during school hours at baseline and follow-up visits. The tests were conducted by a trained technologist who was not aware of the exposure status of the participants. Respiratory/lung volumes obtained by spirometer were adjusted for body temperature and pressure based on the temperature and atmospheric pressure measured throughout the day. Children were instructed not to take any anti-asthmatic inhalers for 12 hours or oral asthma medications for 48 hours before the Spirometry. Children with obstructive airways were given an inhaled bronchodilator and had the spirometry repeated after a minimum of 10 minutes. Furthermore, emergency medical professionals, oxygen and B2-adrenergic agents for nebulization were readily available and on-site (Olaniyan *et al.*, 2017).

2.3.4 Airway inflammation

An experienced nurse conducted the non-invasive fractional exhaled nitric oxide (FeNO) to assess allergic airway inflammation during the baseline and follow-up studies. The FeNO test was carried out using a handheld portable nitric oxide sampling device (NIOX MINO® Airway Inflammation Monitor; Aerocrine AB, Solna, Sweden). Children were upright and advised to breathe normally for about five minutes and to inhale to total lung capacity (TLC). This is followed by immediate exhalation at a constant flow rate of 50 ml/s for four seconds. Following the American Thoracic Society and European Respiratory Society recommendations, two measurements were taken with at least a 30-second interval, and then the average value of the two measurements will be considered in the analysis. Children are advised to avoid strenuous exercise and eating or drinking at least one hour before the test. The last date of bronchodilator and history of passive smoking were recorded. FeNO is advised to take before spirometry, as the latter may reduce exhaled NO levels. Measurements for children with upper and lower

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respiratory tract infections were postponed until recovery or had their measurements taken with infection state recorded. Children's body mass index (BMI) and ambient NO and temperature were recorded. FeNO levels are considered elevated if the concentration is more than 15ppb and high if the concentration is more than 35ppb (a marker of airway inflammation) (Olaniyan, Jeebhay, Röösli, Rajen N. Naidoo, *et al.*, 2020).

2.4 Statistical Analysis

The exposure variables of interest were chemical air pollution including $PM_{2.5}$ and NO and biological air pollutants including pollen (tree, weeds, and grass) and fungi (fungal spores, Ascospores, Alternaria and Cladosporium). Pollutants were analysed as continuous variables using means, medians, standard deviation, and interquartile range (IQR). The IQR was used to scale the chemical pollutants. For example, the IQR for annual $PM_{2.5}$ is 4.95 $\mu g/m^3$; and for the annual NO_2 is 14.22 $\mu g/m^3$, and the natural logarithm (In) was used for biological pollutant in the respective regression models.

The outcome variables of interest were reported asthma-related outcomes including rhinitis, doctor-diagnosed asthma, ocular-nasal symptoms, and wheezing, and airway obstruction (FEV_1, FVC, FEV_1/FVC, FEF_{25-75}) and air way inflammation. These were all analysed as dichotomous variables. Descriptive analysis was conducted to explore population demographics, the distribution of both the exposure and asthma-related outcomes across sex and atopic status. Bivariate analysis was used to identify potential associations between asthma-related outcomes and various predictor variables using simple logistic regression analysis. Confounders were controlled for in multivariable logistic regression models to assess the association between ambient air pollution and biological air pollutants and asthma-related outcomes at follow-up. The selection of confounders was *apriori* based on biological plausibility and on the strength of the association from the bivariate analysis.

The parsimonious (minimally adjusted) model that was used included age, gender, and BMI. The fully adjusted model included age, BMI, atopy, paraffin use, mold growth and smoker in the house. For analysis that included outcomes such as $FEV_1 < LLN$, and $FEF_{25-75} < LLN$, age and BMI were omitted as they were already accounted for in the calculation of the LLN. The fully adjusted model was used but the associations found using the two models were similar. Stratified analyses were conducted to examine effect modification by sex or atopy for the

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significant association between exposure and outcomes which included those between asthma-related outcomes and exposure to grass pollen and annual NO_2 The statistical analysis was carried out using R version 4.3.1.

2.5 Ethics

The main study proposal was approved by the University of Cape Town's Research Ethics Committee (HREC REF: 697/2014) as well as this sub-study (HREC REF:322/2022) and research was conducted in accordance with the Declaration of Helsinki of the 25th World Medical Assembly (Appendices 4 and 5). Before recruitment, permission to include school children was obtained from the Western Cape Department of Education. During recruitment, permission was obtained from the school principals of selected schools, school bodies and children's parents or caregivers to include the children in the study. Informed consent was obtained from parents or guardian (Appendix 8).

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3. Results

The study sample for this sub-study included the 522 children that participated in the follow-up study in 2016 who completed all the relevant testing.

3.1 Socio-demographic characteristics, asthma related outcomes and chemical and biological air pollutant exposures among participants

Table 1 summarises the socio-demographic characteristics and asthma-related outcomes among the study participants. The number of boys and girls participants were similar with the median age of 10-12 years for boys and 10-11 years for girls. Other characteristics such as BMI, weight (kg) and height (cm) were also similar between boys and girls. With regards to respiratory outcomes of interest, 31.4% reported ocular-nasal symptoms, of which 58.2% were non-atopic children. 2.1% of children reported doctor-diagnosed asthma, with girls accounting for 70% of them. In addition, asthma symptom score ≥2 was reported in only 17% with most of the children non-atopic. For the lung function indices, 22.8% of children reported FEV_1<LLN, with 53.8% non-atopic children. 23.4% of children reported FEV_1/FVC <0.8 and 24.5% reported FEF_{25-75} <LLN. For airway, the inflammatory marker, FeNO>35ppb was reported in 13% of the children, with 74.6% non-atopic.

Table S1 presents the annual concentration of biological and chemical air pollutants across the four informal settlements. Ascospores showed the highest median 1086.5 (IQR: 865.3-3143.8) spores/m^3 compared to the other fungi, with Milnerton having the highest median compared to the other study areas. Trees pollen showed the highest median, 16.9 (IQR: 16.8-73.2 grain/m^3), compared to grass and weed pollen, with Oudtshroon showing a concentration of 73.18 grain/m^3.

Regarding chemical pollutants, the overall median annual $PM_{2.5}$ concentration was 9.3 (7.3-12.2) μg/m^3, with Khayelitsha having the highest median concentration of 12.2 μg/m^3. For NO_2, the overall concentration was 14.0 (9.3 – 23.6) μg/m^3, with Khayelitsha having the highest median of 23.8 (22.9 – 24.9) μg/m^3.

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Table 1: Demographic characteristics and indoor exposures, reported across the four selected informal settlements of the Western Cape stratified by sex and atopic status

Variable	Total	n (%)	Sex		Atopy	
			Boys n (%)	Girls n (%)	Atopic n (%)	Non-atopic n (%)
Host characteristics						
Age (years): Median (IQR)	522	11 (10-11)	11 (10-12)	11 (10-11)	11 (10-11)	11 (10-11)
Gender:	522					
Girls		264 (50.6)	-	-	81 (30.7)	167 (63.3)
Boys		258 (49.4)	-	-	106 (41.1)	144 (55.8)
Atopy	498	187 (37.6)	106 (56.7)	81 (43.3)	-	-
Weight (kg): Median (IQR)	522	35.2 (31.0-42.5)	34.5 (30.5-40.4)	35.6 (31.4-43.3)	35.2 (31.3-42.8)	34.7 (30.6-41.4)
Height (cm): Mean (SD)	522	141.0 (7.46)	140.9 (7.5)	141.2 (7.4)	141.1 (7.3)	141.0 (7.5)
BMI (kg/m2): Median (IQR)	522	17.8 (16.2-20.1)	17.5 (16.1-19.4)	18.2 (16.3-20.8)	18.1 (16.4-20.0)	17.7 (16.1-19.9)
Birth history						
Low birth weight <2.5kg	487	24 (4.9)	14 (58.3)	10 (41.7)	13 (54.2)	11 (45.8)
Maternal smoking	487	60 (12.3)	28 (46.7)	32 (53.3)	24 (40.0)	34 (56.7)
Reported indoor exposure						
Visible mould growth	487	29 (6.0)	15 (51.7)	14 (48.3)	13 (44.8)	15 (51.7)
Dampness	487	33 (6.8)	19 (57.6)	14 (42.4)	15 (45.5)	17 (51.5)
Pet ownership	487	37 (7.6)	17 (45.9)	20 (54.1)	17 (45.9)	18 (48.6)
Paraffin-use	487	249 (51.1)	125 (50.2)	124 (49.8)	95 (38.2)	143 (57.4)
Smoker in the house	487	84 (17.2)	36 (42.9)	48 (57.1)	34 (40.5)	49 (58.3)
Respiratory Outcomes						

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Ocular-nasal symptoms	487	**153 (31.4)**	81 (52.9)	72 (47.1)	**60 (39.2)**	**89 (58.2)**
Rhinitis	487	18 (3.7)	10 (55.6)	8 (44.4)	5 (27.8)	12 (66.7)
Wheezing	487	22 (4.5)	10 (45.4)	12 (54.5)	11 (50.0)	9 (40.9)
Asthma symptom score ≥2	487	**83 (17.0)**	41 (49.4)	42 (50.6)	**34 (41.0)**	**49 (59.0)**
Doctor-diagnosed asthma	487	10 (2.1)	**3 (30.0)**	**7 (70.0)**	5 (50.0)	4 (40.0)
Asthma medication	487	8 (1.6)	2 (25.0)	6 (75.0)	4 (50.0)	3 (37.5)
Lung Function indices						
FEV_1 (litres): Mean (SD)	522	1.8 (0.4)	1.77 (0.4)	1.73 (0.3)	1.75 (0.4)	1.75 (0.3)
FVC (litres): Mean (SD)	522	2.1 (0.4)	2.1 (0.4)	2.1 (0.4)	2.10 (0.4)	2.08 (0.4)
FEF_{25-75}, litres/sec: Median (IQR)	522	2.1 (2.6-1.6)	2.1 (1.6-2.5)	2.1 (1.6-2.6)	2.1 (1.4-2.5)	2.1 (1.6-2.6)
FEV_1 < LLN	522	**119 (22.8)**	**66 (55.5)**	**53 (44.5)**	50 (42.0)	64 (53.8)
FVC <LLN	522	75 (14.4)	40 (53.3)	35 (46.7)	30 (40.0)	41 (54.7)
FEV_1/FVC < 0.8	522	**124 (23.4)**	63 (50.8)	61 (49.2)	54 (43.5)	65 (52.4)
FEF_{25-75} <LLN	522	**128 (24.5)**	**56 (43.8)**	**72 (56.3)**	52 (40.6)	69 (53.9)
Airway inflammatory marker						
FeNO >15 ppb	515	448 (87)	219 (48.9)	229 (51.1)	72 (16.1)	346 (77.2)
FeNO >35 ppb		**67 (13)**	35 (52.2)	32 (47.8)	**12 (17.9)**	**50 (74.6)**
Area						
Khayelitsha	487	122 (25.1)	56 (45.9)	66 (54.1)	46 (37.7)	72 (59.0)
Oudtshoorn		144 (29.6)	65 (45.1)	79 (54.9)	38 (26.4)	101 (70.1)
Milnerton		125 (25.7)	64 (51.2)	61 (48.8)	50 (40.0)	67 (53.6)
Noordhoek		96 (19.7)	56 (58.3)	40 (41.7)	37 (38.5)	53 (55.2)

Effect estimates for IQR – Inter-quartile range (25th – 75th percentiles). SD – standard deviation; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; FEF_{25-75}, forced mid-expiratory flow; LLN, lower limit of normal below the 5th percentile; FeNO, fractional exhaled nitric oxide; Asthma symptoms score computed from 8 asthma associated symptom questions. Continuous variable expressed as mean ± SD; Categorical variable expressed as n (%).

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3.2 Association between asthma related outcomes and host characteristics, birth outcomes and indoor exposures

Tables S2 and S3 describe associations between self-reported asthma related outcomes and host characteristics, birth outcomes and indoor exposures. The results of this analysis were previously described in (Olaniyan *et al.*, 2019).

3.3 Association between reported asthma-related outcomes, lung function decline and airway inflammation with biological and chemical pollutants

Table 2 presents the results of the analysis investigating the association between reported asthma-related outcomes and various biological and chemical pollutants using the fully adjusted multiple logistic regression analysis models.

The average weed levels were positively associated with ocular nasal symptoms (OR: 1.78, 95% CI: 1.17-2.72) and rhinitis (OR: 6.00, 95% CI: 1.29-27.80). There was a positive association between an IQR increase in annual NO_2 and ocular nasal symptoms (OR: 1.67, 95% CI: 1.06-2.61). There were no statistically significant associations between annual $PM_{2.5}$ levels and asthma related outcomes.

In Table 3, there were consistent strong positive associations between average grass pollen levels and airway obstruction and inflammation FEV_1< LLN (OR: 2.26, 95% C1: 0.02-5.03), $FEV_1/FVC < 0.8$ (OR: 2.38, 95% CI: 1.02-5.52) FEF_{25-75} < LLN (OR: 4.55, 95% CI: 2.05-10.1) and FeNO > 35 ppb (OR: 3.42, 95% CI: 1.07-10.91). There was also a statistically significant positive association between average levels of Alternaria and FeNO > 35 ppb (OR: 2.80, 95% CI: 1.30-6.03).

There were consistent positive associations between annual NO_2 levels ($\mu g/m^3$) and lung function indices and airway inflammation with the association between NO_2 levels and FEF_{25-75} < LLN (OR: 1.64, 95% CI: 1.02-2.63) and FeNO > 35 (OR: 2.43, 95% CI: 1.19-4.98). Associations between average annual $PM_{2.5}$ levels and lung function indices and airway inflammation were not statistically significant. On the other hand, there were negative associations between total fungal spores and ocular-nasal symptoms (OR: 0.48, 95% CI: 0.32-0.72). Cladosporium levels were negatively associated with ocular-nasal symptoms (OR: 0.44,

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95% CI: 0.32-0.61) and rhinitis (OR: 0.20, 95% CI: 0.06-0.68), trees pollen was negatively associated with ocular-nasal symptoms (OR: 0.50, 95% CI: 0.33-0.75) indicating a potential protective effect.

Other associations between asthma symptoms and fungal spores were not statistically significant. There were also negative associations between total fungal spores levels and levels of ascospores and FeNO > 35 ppb (OR: 0.37, 95% CI: 0.17-0.80) and (OR: 0.41, 95% CI: 0.21-0.81). Trees pollen were negatively associated with FeNO>35ppb (OR: 0.33, 95% CI; 0.16-0.68) and between weed pollen and FEF_{25-75}<LLN (OR: 058, 95% CI; 0.38-0.91). There were no other negative statistically significant associations between biological air pollutants and lung function indices and airway inflammation.

All associations between asthma-related outcomes and biological and chemical pollutants were similar when using the minimally adjusted model (Table S6 and S7). Sensitivity analysis, including area as an additional confounder in the models investigating the association between biological and chemical pollutants and asthma-related outcomes did not change the nature of the associations found.

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Table 2: Association between reported asthma-related outcomes with biological and chemical pollutants using adjusted multiple logistic regression models

Variables			Total (n)	Ocular-nasal symptoms (n=153) OR (95% CI)	Rhinitis (n=18) OR (95% CI)	Wheezing (n=22) OR (95% CI)	Asthma symptom score >2 (n=83) OR (95% CI)	Doctor-diagnosed asthma (n=10) OR (95% CI)
Biological exposures (Annual weekly Average count)	**Fungi** (spores/m^3)	Fungal spores	522	**0.56 (0.35-0.89)**	0.48 (0.14-1.68)	0.55 (0.16-1.82)	0.97 (0.54-1.73)	0.65 (0.12-3.36)
		Ascospores	522	0.78 (0.52-1.19)	0.80 (0.28-2.34)	0.68 (0.24-1.90)	0.99 (0.59-1.65)	0.65 (0.15-2.83)
		Alternaria	522	0.89 (0.55-1.44)	0.65 (0.21-2.06)	1.24 (0.40-3.82)	0.98 (0.54-1.77)	1.43 (0.27-7.54)
		Cladosporium	522	**0.44 (0.32-0.61)**	**0.20 (0.06-0.68)**	0.56 (0.24-1.34)	0.95 (0.64-1.41)	0.86 (0.28-2.61)
	Pollen(grain /m^3)	Trees	522	**0.50 (0.33-0.75)**	0.53 (0.16-1.70)	0.54 (0.17-1.69)	1.00 (0.60-1.66)	0.92 (0.21-3.93)
		Weed	522	**1.78 (1.17-2.72)**	**6.00 (1.29-27.80)**	1.26 (0.50-3.16)	1.10 (0.66-1.84)	1.31 (0.34-4.99)
		Grass	522	1.37 (0.64-2.92)	0.23 (0.03-1.67)	0.84 (0.17-4.29)	0.82 (0.32-2.09)	0.25 (0.02-3.38)
Chemical exposures		Annual $PM_{2.5}$ ($\mu g/m_3$)	484	1.21 (0.97-1.51)	1.21 (0.80-1.82)	1.10 (0.61-1.99)	0.99 (0.73-1.35)	0.55 (0.18-1.69)
		Annual NO_2 ($\mu g/m_3$)	483	**1.67 (1.06-2.61)**	1.44 (0.46-4.50)	2.29 (0.73-7.18)	1.20 (0.68-2.10)	2.72 (0.61-12.19)

Effect estimated as odds ratio (OR) at 95% confidence intervals (95% CI). Model adjusted for age, sex (ref: female), BMI, atopy (ref: yes atopy), paraffin use, visible mould, no smoker in the house.

Effects estimated per interquartile range increase in annual $PM_{2.5}$ of 4.95 $\mu g/m^3$; and annual NO_2 of 14.22 $\mu g/m^3$.

Scale used for biological pollutants is 1 unit log of the biological pollutants

Bold Estimates denote significance at 5% level.

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Table 3: Association between airway obstruction, lung function decline and airway inflammation with biological and chemical pollutants using adjusted multiple logistic regression models

Variables			Total (n)	FEV_1< LLN (n=119) OR (95% CI)	FVC < LLN (n=75) OR (95% CI)	FEV_1/FVC < 0.8 (n=124) OR (95% CI)	FEF_{25-75} < LLN (n=128) OR (95% CI)	FeNO > 35 ppb (n=67) OR (95% CI)
Biological exposures (Annual weekly Average count)	**Fungi** (spores/m^3)	Fungal spores	522	0.96 (0.58-1.58)	1.36 (0.73-2.53)	0.90 (0.54-1.50)	0.92 (0.56-1.50)	**0.37 (0.17-0.80)**
		Ascospores	522	0.96 (0.61-1.50)	1.24 (0.72-2.15)	0.89 (0.56-1.41)	0.92 (0.59-1.43)	**0.41 (0.21-0.81)**
		Alternaria	522	1.15 (0.68-1.94)	0.91 (0.48-1.71)	1.25 (0.74-2.10)	1.27 (0.76-2.14)	**2.80 (1.30-6.03)**
		Cladosporium	522	1.01 (0.73-1.42)	1.32 (0.87-2.01)	1.01 (0.71-1.43)	1.01 (0.72-1.41)	0.70 (0.42-1.16)
	Pollen (grain/m^3)	Trees	522	0.85 (0.55-1.30)	1.25 (0.74-2.10)	0.81 (0.52-1.25)	0.72 (0.47-1.11)	**0.33 (0.16-0.68)**
		Weed	522	0.74 (0.47-1.16)	0.76 (0.45-1.32)	0.72 (0.46-1.12)	**0.58 (0.38-0.91)**	0.58 (0.32-1.07)
		Grass	522	**2.26 (1.02-5.03)**	1.50 (0.56-4.00)	**2.38 (1.02-5.52)**	**4.55 (2.05-10.11)**	**3.43 (1.07-10.99)**
Chemical exposures		Annual $PM_{2.5}$ ($\mu g/m^3$)	484	1.01 (0.79-1.29)	0.91 (0.65-1.28)	0.99 (0.76-1.30)	1.11 (0.89-1.39)	1.21 (0.83-1.75)
		Annual NO_2 ($\mu g/m^3$)	483	1.29 (0.79-3.00)	0.87 (0.48-1.56)	1.44 (0.88-2.35)	**1.64 (1.02-2.63)**	**2.43 (1.19-4.98)**

Effect estimated as odds ratio (OR) at 95% confidence intervals (95% CI). Model adjusted for age, sex (ref: female), BMI, atopy, paraffin use, visible mould, smoker in the house.

* Age, sex (ref: female) and BMI have been accounted for already in FEV_1 < LLN, FEF_{25-75} < LLN and FVC < LLN, so they were **NOT** included.

Effects estimated per interquartile range increase in annual $PM_{2.5}$ of 4.95 $\mu g/m^3$; and annual NO_2 of 14.22 $\mu g/m^3$. Scale used for biological pollutants is 1 unit log of the biological pollutants

Bold Estimates denote significance at 5% level.

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3.4 Association between asthma-related outcomes and exposure to grass pollen and NO2 stratified by sex and atopy.

Tables 4 and 5 summarise the analysis for effect modification by sex and atopy for the associations between average grass pollen levels and annual NO_2 and objective asthma-related outcomes, and Tables S8 and S9 present these associations for reported asthma symptoms. Average grass pollen levels and annual NO_2 were the most strongly associated air pollutants associated with adverse asthma-related outcomes (Tables 2 and 3). Table 4 shows a strong positive association between average grass levels and $FEV_1/FVC < 0.8$ among boys (OR: 4.76, 95% CI: 1.44-15.61**)** but not for girls (OR: 1.90, 95% CI: 0.61-6.00).

Table 5 also shows a strong positive association between average grass levels and $FEF_{25-75} <$ LLN among both boys (OR: 6.89, 95% CI: 2.11-22.47) and girls (OR: 3.37, 95% CI: 1.13-10.4) but the association is stronger among boys. Annual NO_2 levels were also positively associated with FeNO > 35ppb among boys (OR: 2.77, 95% CI: 1.02-7.47) but not among girls (OR: 2.45, 95% CI: 0.81-7.43).

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Table 4: Association between airway obstruction and airway inflammation in relation to biological and chemical pollutants in adjusted multiple logistic regression models stratified by sex

Variables	Total (n)	FEV_1 < LLN (n = 119)* OR (95% CI)		FEV_1/FVC < 0.8 (n = 124) OR (95% CI)		FEF_{25-75} < LLN (n =128)* OR (95% CI)		FeNO > 35 ppb (n=67) OR (95% CI)	
		Boys (n=66)	**Girl (n=53)**	**Boys (n=63)**	**Girls (n=61)**	**Boys (n=56)**	**Girls (n=72)**	**Boys (n=35)**	**Girls (n=32)**
Grass Pollen (grain/m^3)	522	1.95 (0.64-5.93)	2.89 (0.89-9.35)	**4.76 (1.44-15.61)**	1.15 (0.34-3.93)	**6.89 (2.11-22.47)**	**3.37 (1.13-10.04)**	2.80 (0.57-13.65)	4.75 (0.81-27.83)
Annual NO_2 (µg/m^3)	483	1.11 (0.57-2.17)	1.64 (0.79-3.40)	1.24 (0.63-2.41)	1.76 (0.83-3.71)	1.62 (0.82-3.20)	1.66 (0.83-3.30)	**2.77 (1.02-7.47)**	2.45 (0.81-7.43)

Effect estimated as odds ratio (OR) for age, BMI, atopy (ref: yes atopy), paraffin use, visible mould, no smoker in the house.

* Age, and BMI have been accounted for already in FEV_1 < LLN, and FEF_{25-75} < LLN, so they were **NOT** included.

Bold estimates denote significance at 5% level. OR – odds ratio. 95% CI – 95% confidence interval.

Effects estimated per interquartile range increase in annual $PM_{2.5}$ of 4.95µg/m^3; and annual NO_2 of 14.22µg/m^3

Scale used for biological pollutant is 1 unit log of the biological pollutants.

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Table 5 shows a strong positive association between average grass levels and FEV_1< LLN among atopic (OR: 4.46, 95% CI: 1.28-15.6) but not for non-atopic children (OR: 1.32, 95% CI: 0.45-3.87), which demonstrates effect modification by atopy for this association. Strong positive association presented between average grass levels and $FEV_1/FVC < 0.8$ (OR: 3.39, 95% CI: 0.99-11.65) and (OR: 1.39, 95% CI: 0.42-4.60) and FEF_{25-75} < LLN (OR: 4.60, 95% CI: 1.35-15.74) and (OR:4.47, 95% CI: 1.56-12.80) among both atopic and non-atopic children with the associations of similar strengths in both groups. Annual NO_2 levels were also positively associated with FeNO > 35 ppm among atopic children (OR: 3.41, 95% CI: 1.51-7.68) but not for non-atopic children (OR: 0.45, 95% CI: 0.05-3.74). The results for the minimally adjusted model were also similar.

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Table 5: Association between airway obstruction and airway inflammation in relation to biological and chemical pollutants in adjusted multiple logistic regression models stratified by atopic status

Variables	Total (n)	FEV_1 < LLN (n = 114)* OR (95% CI)		FEV_1/FVC < 0.8 (n = 119) OR (95% CI)		FEF_{25-75} < LLN (n =121)* OR (95% CI)		FeNO > 35 ppb (n=62) OR (95% CI)	
		Atopic (n=50)	Non-Atopic (n=64)	Atopic (n=54)	Non-Atopic (n=65)	Atopic (n=52)	Non-Atopic (n=69)	Atopic (n=56)	Non-Atopic (n=6)
Grass Pollen (grain/m^3)	522	**4.46 (1.28-15.59)**	1.33 (0.45-3.87)	3.39 (0.99-11.65)	1.39 (0.42-4.60)	**4.60 (1.35-15.74)**	**4.47 (1.56-12.80)**	2.99 (0.85-10.48)	9.30 (0.34-255.81)
Annual NO_2 (µg/m^3)	483	1.45 (0.68-3.09)	1.21 (0.63-2.31)	1.59 (0.75-3.35)	1.29 (0.66-2.56)	2.08 (0.98-4.39)	1.41 (0.75-2.66)	**3.41 (1.51-7.68)**	0.45 (0.05-3.74)

Bold estimates denote significance at 5% level. OR – odds ratio. 95% CI – 95% confidence interval. Scale used for biological pollutant is 1 unit log of the biological pollutants.

Effect estimated as odds ratio (OR) for age, BMI, sex, paraffin use, visible mould, no smoker in the house. * Age, BMI, and sex have been accounted for already in FEV_1 < LLN, and FEF_{25-75} < LLN, so they were **NOT** included. Effects estimated per interquartile range increase in annual $PM_{2.5}$ of 4.95µg/m^3; and annual NO_2 of 14.22µg/m^3

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4. Discussion

This study found evidence of effect modification by sex and atopy of the association between asthma-related outcomes with long-term exposures to either biological or chemical pollutants among school children residing in four informal settlements of the Western Cape province of South Africa. These cohorts of children have previously been shown to have an increased risk of asthma, as shown by the underreporting and under-diagnosis of asthma-related outcomes (Olaniyan et al., 2018).

A previous analysis of the participants residing in Khayelitsha and Marcon-Beam found short-term exposure to Alternaria and Cladosporium fungal spores to be associated with reduced lung function (Olaniyan, Jeebhay, Röösli, Rajen N. Naidoo, *et al.*, 2020). Another previous analysis Olaniyan *et al* found long-term exposure to NO_2 to be associated with new asthma-related outcomes among the children residing in the four informal settlements. There was a significant association of new cases of airway inflammation at 12-month follow-up, defined by FeNO>35 ppb, with increased annual NO_2 exposure independent of co-exposure to $PM_{2.5}$. However, these studies on this cohort of children did not explore the association with long-term exposure to biological pollutants nor the potential effect modification by sex and atopy. The positive association between exposure to NO_2 and asthma outcomes were found at annual average levels slightly higher than the WHO air quality guidelines of 10µg/m3 for NO_2 (16.6 µg/m3), but lower than the South Africa National Air Quality Standards (SAAQS) ('Air Quality Standards and Objectives', 2014) of 40 µg/m3 for NO_2. It should also be noted that the annual average levels of $PM_{2.5}$ (10.0 µg/m3) is slightly higher than the WHO guideline of 5µg/m3 for $PM_{2.5}$ (OMS, 2021; Kan, 2022) but lower than the SAAQS limits of 20 µg/m3. There are no limits WHO and SAAQS limits for biological pollutants. It is important that exposure limits for biological pollutants are established.

Analysing the follow-up study data conducted 12 months after the baseline study, this current study found that increased exposure to grass pollen levels in the four informal settlements was associated with increased odds of airway obstruction (FEV_1< LLN, $FEV_1/FVC < 0.8$, FEF_{25-75}< LLN) and airway inflammation (FeNO > 35 ppb) and that these associations were modified by sex and atopy since the associations were stronger among boys and atopic children. The positive associations between pollen exposure and asthmatic outcomes are consistent with the results of four recent studies by Shrestha *et al.*, 2018; Lee *et al.*, 2019; De Roos *et al.*, 2020 and Gleason, Bielory and Fagliano, 2014 included in a recent meta-analysis that investigated the association between grass pollen

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exposure and severe asthma exacerbation in individuals under 18 years of age (Annesi-Maesano *et al.*, 2023). However, these studies were large studies, including three case-crossover studies and one retrospective cohort study assessing the association between pollen counts and asthma emergency hospitalisations in Australia, Korea and USA, as compared to the current study that investigated the association between pollen counts and subclinical outcomes in children residing in informal housing settings in South Africa. Another study conducted in Melbourne among 320 children found that doubling of grass pollen counts in the first seven days of life was associated with a decreased odds of FEV_1 (OR: −15.5 mL; 95% CI: −27.6, −3.3) and FVC (OR: −20.8 mL; −35.4, −6.1) at 12 years of age, and that lifetime 3-months grass pollen exposure was associated with decreased FVC at 12- and 18 years of age.

The present study found that an association between grass pollen and $FEV_1/FVC < 0.8$ was stronger in males (OR: 4.76, 95% CI: 1.44-15.61) compared to females (OR: 1.15, 95% CI: 0.34-3.93). Similarly, the association between grass pollen and FEF25-75 < LLN was stronger in males (OR: 6.89, 95% CI: 2.11-22.47) than females (OR: 3.37, 95% CI: 1.13-10.04), suggesting effect modification by sex. Consistent with the current study findings, the study by Shrestha et al. (2018) also found significantly higher odds of asthma hospitalizations with increased grass pollen levels among boys (OR: 1.04) than among girls aged between 2-18 (Shrestha et al., 2018). To the best of our knowledge, no other studies have investigated effect modification by sex in a formal manner as in the current study. One of the explanations for a higher prevalence of asthma among pubertal boys could be due to delayed airway growth (Fuseini & Newcomb, 2017) and possibly higher allergen sensitisation and atopy prevalence compared to girls (Chowdhury *et al.*, 2021; Kauffmann and Demenais, 2012). The higher prevalence of asthma among post-pubertal females is generally considered to be due to the effect of sex hormones, in that higher testosterone levels among males is associated with decreased asthma prevalence (Clougherty, 2010).

This study found evidence for effect modification by atopic status for the association between exposure to grass pollen and airway inflammation. The association was stronger between exposure to grass pollen and FEV_1 <LLN among atopic children (OR:4.46, 95% CI: 1.28-15.95) but not among non-atopic children (OR: 1.33, 95% CI; 0.45-3.87). The strong association between grass pollen and asthma outcomes among atopic individuals is to be expected, considering that atopy, with a strong genetic predisposition, has been consistently reported to be associated with asthma in children (Simpson *et al.*, 2010). The heightened response to allergens in atopic individuals is associated with specific genes involved in allergic reactions (Julge *et al.*, 2002). Cytokines such as IL-4 and IL-13

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have been linked to atopic predisposition (Son *et al.*, 2022). A study in Melbourne that recruited children aged 2-17 years that were admitted for asthma during the grass pollen seasons concluded that atopic status (sensitization) may lead to allergy priming, which then increases the risk of asthma exacerbations when pollen/spore levels are very high (Batra *et al.*, 2022).

In this study, there was evidence of effect modification by sex for the association between long-term NO_2 exposure and airway inflammation. The association between annual average NO_2 levels and airway inflammation (FeNO >35 ppb) was stronger in males (OR: 2.77, 95% CI; 1.02-7.47) compared to females (OR: 2.45, 95% CI; 0.81-7.43). This is consistent with the results of Gehring et al. (2002), which found that outdoor residential exposure to NO_2 was positively associated with asthma related outcomes in boys, including cough without infection (OR: 1.52, 95% CI; 1.16–2.00) and dry cough at night (OR 1.45, 95% CI; 1.07–1.98) but not in girls, among 1,756 German infants (Gehring et al., 2002).

However, it should be noted that three studies on children by Oftedal *et al.*, 2008; Rojas-Martinez *et al.*, 2007 and Rosenlund *et al.*, 2009 found stronger positive associations between NO_2, and lung function among girls compared to boys.

Moreover, this current study found an increased odds of airway inflammation FeNO>35 ppb from long-term NO_2 exposure among atopic children (OR: 3.41, 95% CI; 1.51-7.68) but not in non-atopic children (OR: 0.45, 95% CI; 0.05-3.74). This aligns with the review findings of Nanda *et al.*, 2022, which reported that air pollutants could worsen inflammation in atopic individuals by encouraging T helper 2 (Th2) cell activation. Additionally, exposure to $PM_{2.5}$ was not associated with decline in lung function indices and airway inflammation. This is in contrast with findings from a meta-analysis study that found

The higher levels of $PM_{2.5}$ in Khayelitsha compared to the other areas could be explained by the higher use of wood for cooking and heating in this area. It is important that communities in Khayetlitsha are made aware of the risks of using unclean fuels.

Although in our study there were no statistically significant association between annual $PM_{2.5}$ and asthma related outcomes and lung function indices, previous studies in other settings by Wei *et al.*, 2023 and Nordeide Kuiper *et al.*, 2021, did find a statistically significant positive associations between $PM_{2.5}$ exposure and asthma related outcomes.

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The negative associations found between fungal spores and ocular nasal symptoms, cladosporium and ocular nasal symptoms and rhinitis and tree pollen and ocular nasal symptoms are unexplained, and no evidence could be found in the literature supporting these associations"

There are some limitations in the current study that is worth noting. First, this study used a cross-sectional design conducted after a one-year period of exposure measurement model (Land-use Regression), which made it challenging to infer temporality of the associations observed between exposures to biological and chemical pollutants and asthma-related outcomes. Second, the sample size was relatively small (n=590) and may result in imprecise effect estimates for some asthma-related outcomes with a low number of cases, thus reduced the ability to detect significant associations for these outcomes. Third, relying on self-reported asthma symptoms may have led to outcome misclassification due to recall bias, which could have led to under or over-estimation of reported asthma symptoms. Additionally, the ISAAC that was used to report atopic status is found to be inaccurate due cultural differences and language untranslatability. This is unlikely with more objective measures of lung function and airway inflammation were observed. In addition, to study design, sample size, and outcome misclassification, using a single monitoring location for the biological pollutants may have led to potential exposure misclassification and thus impacted the observed associations for some of the other exposure metrics used in this study.

In conclusion, this study provided evidence that long-term exposure to grass pollen and NO_2 is associated with increased asthma-related outcomes among children and that these associations are modified by sex or atopy. This is the first time that evidence on these associations in an African setting have been reported. Targeted interventions such as public health policies aimed at controlling the emission of NO_2 and other chemical pollutants that influence the risk of asthma in children are recommended. Individual prevention measures could include public messaging of providing airborne chemical pollutant and pollen counts, and encouraging children to stay indoors when levels are high. Further longitudinal studies that track changes over time, at an individual and group level, should also investigate the synergistic or additive effects when there is concomitant exposure to both chemical and biological pollutants, to examine the interplay between several pollutant components and their impact on respiratory health.

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Declaration of competing interest

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

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PART C: APPENDICES

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Appendix 1: Supplementary Tables

Table S1: Annual Concentrations of biological and chemical air pollutants across the four informal settlements of the Western Cape

Variable		Total (n)	median (IQR)	Area			
				Khayelitsha Median (IQR)	Oudtshoorn Median (IQR)	Milnerton Median (IQR)	Noordhoek median (IQR)
Fungi (spores/m^3)	Fungal spores	522	2077 (1519-5688)	1518.62	5687.88	2076.98	3420.72
	Ascospores	522	1086.5 (865.3-3143.8)	865.30	3143.76	1086.45	2498.31
	Alternaria	522	140.0 (66.1-153.7)	140.03	66.06	158.28	56.30
	Cladosporium	522	629.6 (263.7-2090.5)	263.66	2090.55	629.63	385.47
Pollen (grain/m^3)	Grass	522	14.7 (11.7-22.5)	11.66	14.73	24.48	16.40
	Trees	522	16.9 (16.8-73.2)	16.89	73.18	16.88	32.14
	Weed	522	11.2 (7.8-19.8)	19.79	11.19	6.66	23.26
Chemical pollutants	Annual $PM_{2.5}$ (µg/m3): median (IQR)	484	9.3 (7.3 – 12.2)	12.2 (10.6 – 13.7)	6.9 (5.7 – 8.6)	10.7 (8.8 – 13.1)	9.1 (8.0 -10.4)
	Annual NO_2 (µg/m3): median (IQR)	483	14.0 (9.3 – 23.6)	23.8 (22.9 – 24.9)	7.9 (6.3 – 8.9)	22.9 (21.7 – 24.5)	12.5 (12.5 – 12.9)

IQR – Inter-quartile range (25th – 75th percentiles)

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Table S2: Association between reported asthma-related outcomes and host characteristics using simple logistic regression

Variable	Total (n)	Ocular-nasal symptoms (n=153) OR (95% CI)	Rhinitis (n=18) OR (95% CI)	Wheezing (n=22) OR (95% CI)	Asthma symptom score ≥2 (n=83) OR (95% CI)	Doctor-diagnosed asthma (n=10) OR (95% CI)
Host characteristics						
Age (years)	522	1.12 (0.91 – 1.37)	0.88 (0.52 – 1.49)	0.96 (0.52 – 1.49)	0.94 (0.73 – 1.22)	0.96 (0.48 – 1.90)
Gender: Female	498	1.22 (0.83 – 1.79)	0.78 (0.30 – 2.00)	1.18 (0.50 – 2.80)	1.00 (0.63 – 1.61)	2.32 (0.59 – 9.09)
Atopy	522	1.23 (0.83 – 1.85)	0.71 (0.24 – 2.03)	0.71 (0.24 – 2.03)	1.10 (0.67 – 1.81)	2.18 (0.57 – 8.22)
Weight (kg)	522	1.00 (0.98 – 1.02)	1.00 (0.96 – 1.05)	1.00 (0.96 – 1.05)	1.02 (0.99 – 1.04)	1.00 (0.93 – 1.06)
Height (cm)	522	1.00 (0.98 – 1.03)	0.96 (0.90 – 1.03)	0.98 (0.92 – 1.03)	0.99 (0.96 – 1.03)	0.94 (0.86 – 1.02)
BMI (kg/m2)	522	1.01 (0.96 – 1.06)	1.05 (0.94 – 1.17)	1.03 (0.92 – 1.14)	1.06 (0.99 – 1.12)	1.04 (0.87 – 1.19)
Birth history						
Low birth weight (<2.5kg)	487	0.89 (0.36 – 2.20)	1.14 (0.14 – 8.95)	N.C	0.20 (0.03 – 1.52)	N.C
Maternal smoking	487	**0.30 (0.14 – 0.64)**	0.41 (0.05 – 3.13)	N.C	1.11 (0.55 – 2.23)	N.C
Reported indoor exposure						
Visible mould growth	487	1.36 (0.63 – 2.95)	0.93 (0.12 – 7.22)	1.62 (0.36 – 7.30)	**2.34 (1.02-5.33)**	1.78 (0.22 – 14.56)
Dampness	487	1.01 (0.43 – 2.73)	**0.33 (0.13 – 0.88)**	0.57 (0.22 – 1.53)	1.09 (0.43 – 2.72)	0.89 (0.18 – 4.28)
Pet ownership	487	1.05 (0.51 – 2.15)	0.39 (1.08 – 1.42)	1.76 (0.23 – 13.48)	1.15 (0.49– 2.71)	0.32 (0.06 – 1.55)
Paraffin-use	487	**0.56 (0.38 – 0.83)**	0.40 (0.14 – 1.11)	**0.38 (0.15 – 0.98)**	0.64 (0.40 – 1.04)	0.69 (0.19 – 2.48)
Smoker in the house	487	0.97 (0.58 – 1.62)	1.40 (0.46 – 4.33)	1.86 (0.71 – 4.90)	**2.33 (1.35 – 4.03)**	0.52 (0.07 – 4.22)

N.C: not computable due to lack of model convergence;

Bold text denotes significance at 5% level.

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Table S3: Association between airway obstruction and airway inflammation in relation to characteristics using simple logistic regression

Variable	Total (n)	FEV_1< LLN (n=119) OR (95% CI)	FVC < LLN (n=75) OR (95% CI)	FEV_1/FVC < 0.8 (n=124) OR (95% CI)	FEF_{25-75} < LLN (n=128) OR (95% CI)	FeNO > 35 ppb (n=67) OR (95% CI)
Host characteristics						
Age (years)	522	**1.33 (1.07 – 1.66)**	**1.32 (1.02 – 1.70)**	**1.50 (1.21 – 1.87)**	**1.44 (1.63– 1.79)**	1.28 (0.98 – 1.68)
Gender: Female	522	1.37 (0.91 – 2.06)	1.20 (0.74 – 1.96)	1.08 (0.72 – 1.61)	1.74 (0.49 – 1.01)	1.14 (0.68 – 1.91)
Atopy	498	1.41 (0.92 – 2.15)	1.26 (0.76 – 2.10)	**1.54 (1.01 – 2.33)**	1.35 (0.89 – 2.05)	**22.89 (9.62 – 54.47)**
Weight (kg)	522	0.97 (0.95 – 1.00)	**0.96 (0.93 – 0.99)**	1.01 (0.99 – 1.03)	0.99 (0.97 – 1.01)	1.01 (0.99 – 1.04)
Height (cm)	522	0.98 (0.95 – 1.01)	0.98 (0.95 – 1.01)	1.00 (0.97 – 1.03)	1.00 (0.97 – 1.03)	0.99 (0.96 – 1.03)
BMI (kg/m2)	522	**0.93 (0.87 – 0.99)**	**0.89 (0.81 – 0.96)**	1.02 (0.97 – 1.08)	0.97 (0.92 – 1.03)	1.04 (0.98 – 1.11)
Birth history						
Low birth weight (<2.5kg)	487	1.11 (0.43 – 2.86)	1.22 (0.41 – 3.70)	1.10 (0.42 – 2.83)	1.03 (0.40 – 2.66)	1.47 (0.48 – 4.46)
Maternal smoking	487	0.72 (0.36 – 1.43)	0.92 (0.42 – 2.04)	0.80 (0.41 – 1.56)	1.04 (0.55 – 1.93)	1.04 (0.47 – 2.31)
Reported indoor exposure						
Visible mould growth	487	1.53 (0.68 – 3.47)	2.03 (0.83 – 4.96)	1.51 (0.67 – 3.42)	1.98 (0.91 – 4.32)	2.29 (0.94 – 5.61)
Dampness	487	1.06 (0.47 – 2.43)	1.09 (0.41 – 2.92)	1.97 (0.94 – 4.14)	**0.99 (0.43 – 2.26)**	0.66 (0.19 – 2.22)
Pet ownership	487	0.91 (0.40 – 2.04)	0.72 (0.25 – 2.09)	0.75 (0.32 – 1.75)	0.99 (0.45 – 2.17)	0.82 (0.28 – 2.35)
Paraffin-use	487	1.33 (0.87 – 2.04)	0.89(0.53 – 1.48)	0.76 (0.50 – 1.17)	0.75 (0.50 – 1.15)	**0.58 (0.33 – 1.00)**
Smoker in the house	487	1.04 (0.60 – 1.81)	0.92 (0.42 – 2.04)	0.80 (0.41 – 1.56)	1.21 (0.71 – 2.05)	1.15 (0.58 – 2.27)
Asthma medication	487	1.11 (0.22 – 5.55)	2.05 (0.41 – 10.37)	1.99 (0.47 – 8.45)	1.88 (0.44 – 7.98)	4.20 (0.97 – 18.03)

Bold estimates denote statistical significance at 5% level.OR – odds ratio. 95% CI – 95% confidence interval

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Table S4 Association between reported asthma-related outcomes in relation to biological and chemical pollutants using simple logistic regression analysis

Variables			Total (n)	Ocular-nasal symptoms (n=153) OR (95% CI)	Rhinitis (n=18) OR (95% CI)	Wheezing (n=22) OR (95% CI)	Asthma symptom score ≥2 (n=83) OR (95% CI)	Doctor-diagnosed asthma (n=10) OR (95% CI)
Biological Exposures	**Fungi (spores/m^3)**	Fungal spores	522	**0.51 (0.35 – 0.75)**	**0.29 (0.09 – 0.90)**	**0.27 (0.10 – 0.69)**	0.69 (0.43– 1.09)	0.40 (0.10 – 1.60)
		Ascospores	522	**0.67 (0.47 – 0.95)**	0.47 (0.19 – 1.18)	**0.35 (0.14 – 0.77)**	0.73 (0.47 – 1.13)	0.43 (0.13 – 1.48)
		Alternaria	522	1.08 (0.70 – 1.66)	1.16 (0.40 – 3.39)	2.57 (0.09 – 7.29)	1.30 (0.76– 2.23)	**2.30 (5.08 – 10.41)**
		Cladosporium	522	**0.47 (0.35 – 0.61)**	**0.15 (0.05 – 0.52)**	**0.39 (0.19 – 0.82)**	0.77 (0.58 – 1.04)	0.61 (0.25 – 1.48)
	Pollen (grain/m^3)	Average Trees	522	**0.50 (0.36 – 0.69)**	**0.37(0.14 – 0.98)**	**0.30 (0.11– 0.79)**	0.75 (0.51 – 1.10)	0.56 (0.18 – 1.74)
		Average Weed	522	**2.02 (1.34 – 3.05)**	**8.42 (1.96 – 36.06)**	1.45 (0.58– 3.61)	1.25 (0.76 – 2.06)	1.58 (0.41 – 6.09)
		Average Grass	522	1.44 (0.71 – 2.91)	0.16 (0.02 – 1.26)	0.96 (0.19 – 4.68)	0.77 (0.32 – 1.87)	0.22 (0.02 – 3.07)
Chemical Exposures		Annual $PM_{2.5}$ (µg/m3)	484	**1.02 (1.01 – 1.02)**	2.20 (0.85 – 5.68)	1.01 (0.99 – 1.03)	1.01 (0.99 – 1.01)	1.00 (0.97 – 1.02)
		Annual NO_2(µg/m3)	483	**1.90 (1.31 – 2.78)**	2.20 (0.85 – 5.68)	**3.41 (1.31– 8.84)**	**1.62 (1.02 – 2.58)**	3.54 (0.96 – 13.04)

Bold estimates denote statistical significance at 5% level.
OR – odds ratio. 95% CI – 95% confidence interval
Effects estimated per IQR increase for annual $PM_{2.5}$ of 4.95 µg/m^3; and annual NO_2 of 14.22 µg/m^3
Scale used for biological pollutants is 1 unit log of the biological pollutants.

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Table S5: Unadjusted associations between airway obstruction and airway inflammation in relation to biological and chemical pollutants using simple logistic regression analysis

Variable			Total (n)	FEV_1< LLN (n=119) OR (95% CI)	FVC < LLN (n=75) OR (95% CI)	FEV_1/FVC < 0.8 (n=124) OR (95% CI)	FEF_{25-75} < LLN (n=128) OR (95% CI)	FeNO > 35 ppb (n=67) OR (95% CI)
Biological Exposures	**Fungi (spores/m^3)**	Fungal spores	522	0.86 (0.58 – 1.28)	1.14 (0.72 – 1.81)	0.80 (0.54 – 1.18)	0.78 (0.53 – 1.15)	**0.42 (0.24 – 0.72)**
		Ascospores	522	0.88 (0.61 – 1.27)	1.09 (0.70 – 1.70)	0.81 (0.57 – 1.18)	0.80 (0.56 – 1.14)	**0.47 (0.29 – 0.78)**
		Alternaria	522	1.22 (0.77 – 1.94)	1.02 (0.59 – 1.77)	1.38 (0.87 – 2.18)	1.44 (0.91 – 2.26)	**2.41 (1.31 – 4.46)**
		Cladosporium	522	0.92 (0.72 – 1.19)	1.14 (0.85 – 1.54)	0.90 (0.70 – 1.16)	0.89 (0.70 – 1.14)	**0.59 (0.41 – 0.84)**
	Pollen (grain/m^3)	Average Trees	522	0.80 (0.58 – 1.11)	1.05 (0.72 – 1.54)	0.73 (0.52 – 1.01)	**0.70 (0.51 – 0.98)**	**0.37 (0.22 – 0.62)**
		Average Weed	522	0.77 (0.50 – 1.17)	0.70 (0.42 – 1.16)	0.68 (0.45 – 1.04)	**0.64 (0.43 – 0.98)**	0.72 (0.42 – 1.22)
		Average Grass	522	**2.43 (1.16 – 5.10)**	1.91 (0.79 – 4.61)	**3.23 (1.55 – 6.71)**	**3.76 (1.82 – 7.77)**	**4.26 (1.70 – 10.70)**
Chemical Exposures		Annual $PM_{2.5}$ (µg/m3)	484	1.01 (0.99 – 1.01)	0.99 (0.99 – 1.01)	1.00 (0.99 – 1.01)	1.00 (0.99 – 1.01)	**1.01 (1.00 – 1.02)**
		Annual NO_2(µg/m3)	483	1.31 (0.88 – 1.97)	0.95 (0.60 – 1.53)	**1.58 (1.06 – 2.35)**	**1.73 (1.17– 2.58)**	**2.43 (1.43 – 4.11)**

Bold estimates denote significance at 5% level.

OR – odds ratio. 95% CI – 95% confidence interval;

Effects estimated per IQR increase for annual $PM_{2.5}$ of 4.95 µg/m^3; and annual NO_2 of 14.22 µg/m^3

Scale used for biological pollutants is 1 unit log of the biological pollutants.

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Table S6: Associations between reported asthma-related outcomes in relation to biological and chemical pollutants in minimally adjusted multiple logistic regression models

			Ocular-nasal symptoms (n=153) OR (95% CI)	**Rhinitis (n=18) OR (95% CI)**	**Wheezing (n=22) OR (95% CI)**	**Asthma symptom score>=2 (n=83) OR (95% CI)**	**Doctor diagnosed asthma (n=10) OR (95% CI)**
Biological exposures	**Fungi (spores/m^3)**	Fungal spores	**0.48 (0.32-0.72)**	0.35 (0.12-1.05)	**0.35 (0.12-0.98)**	0.78 (0.48-1.27)	0.56 (0.35-2.33)
		Ascospores	**0.64 (0.44-0.93)**	0.56 (0.22-1.42)	0.44 (0.18-1.11)	0.83 (0.53-1.30)	0.57 (0.55-2.11)
		Alternaria	1.00 (1.00-1.00)	0.96 (0.32-2.95)	**1.94 (0.65-5.75)**	1.15 (0.66-2.00)	1.73 (0.36-8.29)
		Cladosporium	1.00 (1.00-1.00)	0.19 (0.06-0.62)	0.43 (0.20-0.90)	0.83 (0.60-1.14)	0.77 (0.31-1.94)
	Pollen (grain/m^3)	Trees	**0.98 (0.97-0.98)**	0.40 (0.15-1.08)	0.36(0.14-0.96)	0.81 (0.54-1.23)	0.73 (0.22-2.40)
		Weed	**1.07 (1.03-1.00)**	8.45 (1.78-40.1)	1.53 (0.58-4.03)	1.17 (0.71-1.94)	1.29 (0.33-5.08)
		Grass	1.02 (0.98-1.07)	0.22 (0.03-1.80)	0.97 (0.18-5.26)	0.88 (0.35-2.24)	0.29 (0.01-4.33)
Chemical exposures		Annual $PM_{2.5}$ ($\mu g/m_3$): Median IQR	1.26 (1.00-1.59)	1.18 (0.83-1.70)	1.11 (0.69-1.78)	1.00 (0.76-1.33)	0.66 (0.23-1.87)
		Annual NO_2 ($\mu g/m_3$): Median IQR	**2.03 (1.36-3.02)**	2.00 (0.75-5.38)	**2.85 (1.03-7.84)**	1.43 (0.88-2.34)	2.94 (0.73-11.8)

Adjusted for age, sex (ref: female) BMI and atopy; 95% CI: 95% confidence intervals; Bold estimates denote significance at 5% level. OR – odds ratio. 95% CI – 95% confidence interval
Effects estimated per interquartile range increase in annual $PM_{2.5}$ of 4.95 $\mu g/m^3$; and annual NO_2 of 14.22 $\mu g/m^3$

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Table S7: Association between airway obstruction and airway inflammation in relation to biological and chemical pollutants in minimally adjusted multiple logistic regression model

Variables			**FEV_1 < LLN (n=119) OR (95% CI)**	**FVC < LLN (n=75) OR (95% CI)**	**FEV_1/FVC < 0.8 (n=124) OR (95% CI)**	**FEF_{25-75} < LLN (n=128) OR (95% CI)**	**FeNO > 35 ppb (n=67) OR (95% CI)**
Biological exposures	**Fungi (spores/m^3)**	Fungal spores	0.88 (0.59-1.32)	1.18 (0.73-1.91)	0.84 (0.55-1.27)	0.81 (0.55-1.21)	**0.36 (0.18-0.72)**
		Ascospores	0.88 (0.60-1.28)	1.11 (0.71-1.76)	0.83 (0.56-1.22)	0.82 (0.56-1.19)	**0.40 (0.22-0.74)**
		Alternaria	1.23 (0.78-2.03)	1.02 (0.58-1.80)	1.36 (0.85-2.19)	1.43 (0.89-2.27)	**2.86 (1.42-5.78)**
		Cladosporium	0.96(0.74-1.25)	1.20 (0.89-1.62)	0.97 (0.74-1.27)	0.93 (0.72-1.19)	**0.63 (0.41-0.98)**
	Pollen (grain/m^3)	Trees	0.83 (0.59-1.16)	1.11 (0.74-1.64)	0.80 (0.56-1.14)	0.72 (0.51-1.01)	0.33 (0.17-0.64)
		Weed	0.73 (0.47-1.13)	0.70 (0.41-1.16)	0.72 (0.46-1.10)	0.61 (0.40-0.93)	**0.62 (0.35-1.11)**
		Grass	**2.27 (1.06-4.85)**	1.68 (0.69-4.18)	**2.24 (1.01-4.96)**	**4.08 (1.93-8.63)**	**3.69 (1.25-10.91)**
Chemical exposures		Annual $PM_{2.5}$ (μg/m^3): Median IQR	1.00 (0.99-1.01)	0.99 (0.98-1.01)	1.00 (0.99-1.01)	1.00 (0.99-1.01)	1.01 (0.98-1.02)
		Annual NO_2 (μg/m^3): Medina IQR	1.32 (0.89-1.99)	0.92 (0.57-1.51)	1.51 (0.99-2.30)	**1.71 (1.14-2.58)**	**2.66 (1.39-5.10)**

Adjusted for age, sex (ref: female) and atopy and BMI; OR: odds ratios; 95% CI: 95% confidence intervals; Bold estimates denote significance at 5% level. OR – odds ratio. 95% CI – 95% confidence interval; Scale used for biological pollutants is 1 unit log of the biological pollutants

Effects estimated per interquartile range increase in annual $PM_{2.5}$ of 4.95 μg/m^3; and annual NO_2 of 14.22 μg/m^3.Effect estimates were not adjusted-for age and gender as this has already been accounted-for in the calculation of the lower limits of normal (LLN)

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Table S8: Association between reported asthma-related outcomes with biological and chemical pollutants using minimally adjusted logistic regression model stratified by sex.

Variables	Total (n)	Ocular-nasal symptoms (n = 153) OR (95% CI)		Asthma symptom score>=2 (n =83) OR (95% CI)		Doctor diagnosed asthma (n =10) OR (95% CI)	
		Boys (n=81)	Girls (n=72)	Boys (n=41)	Girls (n=42)	Boys (n=3)	Girls (n=7)
Pollen Grass	522	1.46 (0.52-4.11)	1.50 (0.50-4.49)	1.15 (0.31-4.23)	0.69 (0.18-2.66)	0.0053 (0.00-23.21)	0.89 (0.04-19.07)
Annual NO_2 ($\mu g/m^3$)	483	**2.34 (1.32-4.17)**	**1.83 (1.05-3.20)**	2.08 (0.99-4.39)	1.07 (0.55-2.07)	**1.91 (1.80-2.03)**	4.61 (0.71-29.93)

Effect estimated as odds ratio (OR) for age, BMI, atopy, 95 CI: 95% confidence intervals.

Effects estimated per interquartile range increase in annual $PM_{2.5}$ of 4.95$\mu g/m^3$; and annual NO_2 of 14.22$\mu g/m^3$

Scale used for biological pollutants is 1 unit log of the biological pollutants.

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Table S9: Association between airway obstruction, lung function decline and airway inflammation with biological and chemical pollutants using minimally adjusted logistic regression model stratified by sex

Variables	Total (n)	FEV_1 < LLN (n = 119)* OR (95% CI)		FEV_1/FVC < 0.8 (n = 124) OR (95% CI)		FEF_{25-75} < LLN (n =128)* OR (95% CI)		FeNO > 35 ppb (n=67) OR (95% CI)	
		Boys (n=66)	Girls (n=53)	Boys (n=63)	Girls (n=61)	Boys (n=56)	Girls (n=72)	Boys (n=35)	Girls (n=32)
Pollen Grass	522	2.26 (0.79-6.45)	2.45 (0.80-7.48)	**4.54 (1.49-13.92)**	1.10 (0.34-3.51)	**6.18 (2.05-18.67)**	**3.12 (1.11-8.78)**	3.53 (0.82-15.11)	4.06 (0.79-21.03)
Annual NO_2 ($\mu g/m^3$)	483	1.29 (0.71-2.35)	1.34 (0.75-2.39)	1.37 (0.74 -2.52)	1.72 (0.92-3.20)	1.73 (0.93-3.22)	1,68 (0.98-2.89)	**2.87 (1.14-7.24)**	2.50 (0.96-6.53)

Effect estimated as odds ratio (OR) for age, BMI, atopy,

* Age, and BMI have been accounted for already in FEV_1 < LLN, and FEF_{25-75} < LLN, so they were **NOT** included.

Bold estimates denote significance at 5% level. OR – odds ratio. 95% CI – 95% confidence interval.

Effects estimated per interquartile range increase in annual $PM_{2.5}$ of 4.95$\mu g/m^3$; and annual NO_2 of 14.22$\mu g/m^3$

Scale used for biological pollutant is 1 unit log of the biological pollutants.

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Table S10: Association between reported asthma-related outcomes with biological and chemical pollutants using minimally adjusted logistic regression model stratified by Atopy

Variables	Total (n)	Ocular-nasal symptoms (n = 149) OR (95% CI)		Asthma symptom score>=2 (n =80) OR (95% CI)		Doctor diagnosed asthma (n =9) OR (95% CI)	
		Atopic (n=60)	Non-Atopic (n=89)	Atopic (n=31)	Non-Atopic (n=49)	Atopic (n=5)	Non-Atopic (n=4)
Pollen Grass	522	1.35 (0.43-4.18)	1.55 (0.56-4.31)	0.57 (0.13-2.44)	1.13 (0.33-3.91)	0.34 (0.01-12.67)	0.12 (0.00-10.00)
Annual NO_2 (µg/m^3)	483	**1.95 (1.01-3.78)**	**2.05 (1.24-3.40)**	1.25 (0.54-2.86)	1.58 (0.86-2.92)	0.97 (0.18-5.35)	**21.18 (1.27-352.68)**

Effect estimated as odds ratio (OR) for age, sex, BMI, 95 CI: 95% confidence intervals.

Effects estimated per interquartile range increase in annual $PM_{2.5}$ of 4.95µg/m^3; and annual NO_2 of 14.22µg/m^3

Scale used for biological pollutant is 1 unit log of the biological pollutants.

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Table S11: Association between airway obstruction, lung function decline and airway inflammation with biological and chemical pollutants using minimally adjusted logistic regression model stratified by Atopy

Variables	Total (n)	FEV_1 < LLN (n = 114)* OR (95% CI)		FEV_1/FVC < 0.8 (n = 119) OR (95% CI)		FEF_{25-75} < LLN (n =121)* OR (95% CI)		FeNO > 35 ppb (n=62) OR (95% CI)	
		Atopic (n=50)	Non-Atopic (n=64)	Atopic (n=54)	Non-Atopic (n=65)	Atopic (n=52)	Non-Atopic (n=69)	Atopic (n=56)	Non-Atopic (n=6)
Pollen Grass	522	**3.95 (1.26-12.40)**	1.44 (0.51-4.05)	**3.21 (1.01-10.17)**	1.46 (0.47-4.51)	**4.21 (1.36-13.07)**	**3.99 (1.47-10.80)**	**3.32 (1.04-10.54)**	11.19 (0.39-325.24)
Annual NO_2 ($\mu g/m^3$)	483	1.57 (0.79-3.11)	1.18 (0.70-2.01)	1.93 (0.97-3.84)	1.17 (0.65-2.08)	**2.31 (1.15-4.64)**	1.45 (0.87-2.41)	**3.41 (1.61-7.23)**	0.81 (0.15-4.42)

Effect estimated as odds ratio (OR) for age, BMI, sex.

* Age, BMI, and sex have been accounted for already in FEV_1 < LLN, and FEF_{25-75} < LLN, so they were **NOT** included.

Bold estimates denote significance at 5% level. OR – odds ratio. 95% CI – 95% confidence interval.

Effects estimated per interquartile range increase in annual $PM_{2.5}$ of 4.95$\mu g/m^3$; and annual NO_2 of 14.22$\mu g/m^3$

Scale used for biological pollutant is 1 unit log of the biological pollutants.

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Table S12: Association between reported asthma-related outcomes in relation to biological and chemical pollutants using adjusted multiple logistic regression models stratified by sex.

Variables	Total (n)	Ocular-nasal symptoms (n = 153) OR (95% CI)		Asthma symptom score≥2 (n =83) OR (95% CI)		Doctor diagnosed asthma (n =10) OR (95% CI)	
		Boys (n=81)	Girls (n=72)	Boys (n=41)	Girls (n=42)	Boys (n=3)	Girls (n=7)
Pollen Grass (grain/m^3)	522	0.14 (0.39-3.31)	1.49 (0.49-4.48)	1.07 (0.28-4.07)	0.66 (0.17-2.55)	2.99 (0.00-2.82)	0.78 (0.04-1.56)
Annual NO_2 (µg/m^3)	483	1.79 (0.95-3.34)	1.77 (0.91-3.43)	1.82 (0.82-4.03)	0.77 (0.32-1.82)	1.85 (0.13-2.60)	4.53 (0.62-32.84)

Effect estimated as odds ratio (OR) for age, BMI, atopy, paraffin use, visible mould, smoker in the house; 95 CI: 95% confidence intervals.

Effects estimated per interquartile range increase in annual $PM_{2.5}$ of 4.95µg/m^3; and annual NO_2 of 14.22µg/m^3

Scale used for biological pollutants is 1 unit log of the biological pollutants

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Table S13: Association between reported asthma-related outcomes in relation to biological and chemical pollutants using adjusted multiple logistic regression stratified by atopic status

Variables	Total (n)	Ocular-nasal symptoms (n = 149) OR (95% CI)		Asthma symptom score>=2 (n =80) OR (95% CI)		Doctor diagnosed asthma (n =9) OR (95% CI)	
		Atopic (n=60)	Non-Atopic (n=89)	Atopic (n=31)	Non-Atopic (n=49)	Atopic (n=5)	Non-Atopic (n=4)
Pollen Grass (grain/m^3)	522	0.92 (0.28-3.03)	1.53 (0.55-4.24)	0.65 (0.14-2.96)	1.13 (0.32-3.90)	0.18 (0.00-8.52)	0.13 (0.00-8.09)
Annual NO_2 (µg/m^3)	483	1.75 (0.85-3.58)	1.68 (0.92-3.05)	1.18 (0.48-2.89)	1.19 (0.56-2.50)	0.63 (0.07-5.34)	19.16 (0.95-387.15)

Effect estimated as odds ratio (OR) for age, sex: female, BMI, paraffin use, visible mould, smoker in the house; 95 CI: 95% confidence intervals.

Effects estimated per interquartile range increase in annual $PM_{2.5}$ of 4.95µg/m^3; and annual NO_2 of 14.22µg/m^3

Scale used for biological pollutant is 1 unit log of the biological pollutants.

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Appendix 2: Asthma screening questionnaire

(Sections highlighted in yellow in the questionnaires are applicable to this study)

Area number		1-3
Study number		4-8
Sample		9

TO ANSWER THE QUESTIONS PLEASE CHOOSE THE APPROPRIATE BOX
IF YOU ARE UNSURE OF THE ANSWER PLEASE CHOOSE 'NO'

1. Has your child had wheezing or whistling in his/her chest at any time in the last 12 months?	**NO** ☐	**YES** ☐	10
IF 'NO' GO TO QUESTION 2, IF 'YES':			
1.1. Has your child been at all breathless when the wheezing noise was present?	**NO** ☐	**YES** ☐	11
1.2. Has your child had this wheezing or whistling when he/she did not have a cold?	**NO** ☐	**YES** ☐	12
2. Has your child woken up with a feeling of tightness in his/her chest at any time in the last 12 months?	**NO** ☐	**YES** ☐	13
3. Has your child been woken by an attack of shortness of breath at any time in the last 12 months?	**NO** ☐	**YES** ☐	14
4. Has your child been woken by an attack of coughing at any time in the last 12 months?	**NO** ☐	**YES** ☐	15
5. Has your child had an attack of asthma in the last 12 months?	**NO** ☐	**YES** ☐	16
6. Is your child currently taking any medicine (including inhalers, aerosols or tablets) for asthma?	**NO** ☐	**YES** ☐	17
7. Does your child have any nasal allergies including hay fever?	**NO** ☐	**YES** ☐	18
8. What is your child's date of birth?	**DAY** ☐☐	**MONTH** ☐☐ **YEAR** ☐☐	19-24
9. What is today's date?	**DAY** ☐☐	**MONTH** ☐☐ **YEAR** ☐☐	25-30
10. Is your child a male or female?	**MALE** ☐	**FEMALE** ☐	31

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THANK YOU FOR YOUR HELP 32

If you don't mind being telephoned at home or at work by one of the study team, please write your telephone number below: 33 34

(DAY).....(EVE).....

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Appendix 3: Caregiver questionnaire

G1. Date:____/____/____ G2. Study Identification No. | | | | - | | | | - | | | |
Day Month Year

G3. Study Area: _____

Child Caregiver Baseline Survey

Cover Sheet

G4. Relationship to Child:	
G5. Study number of child participating in the study:	
G6. Child's birth date:	____/____/____ Day Month Year
G7. Child's sex:	☐1 Male ☐2 Female
G8. Child's school:	
G9. Interview time started:	Time: __:__ am/pm
G10. [INTERVIEWER: Enter gender of respondent]	☐1 Male ☐2 Female
G11. Are you the person most responsible for care of **[child]** or most familiar with any health problems (s)he has?	
[If answer is "NO" then assess informally whether the person knows enough to complete the questionnaire.]	
G12. How are you related to **[child]**?	☐1 Mother ☐2 Father ☐3 Grandmother ☐4 Grandfather ☐5 Aunt ☐6 Uncle ☐7 Other (**SPECIFY:** _____)

[INTRODUCTION: INTERVIEWER READS TO RESPONDENT]
The purpose of this questionnaire is to collect information about your child's health status. Your answers will help us figure out how to assist you and your child in protecting your child's health. If there is a question you do not want to answer, please let me know and we can skip it. All of your responses are confidential and will not be shown to anyone outside the study team without your written consent.

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A. BIRTH HISTORY

G13. In what country was **[CHILD]** born?	_____ country name
G14. What is the highest grade or year of regular school has **[CHILD]** completed?	Grade_____
G15. How old was the biological mother of **[CHILD]** when **[CHILD]** was born?	_____ years age
G16. Did **[CHILD]'s** biological mother smoke at anytime while she was pregnant with **[CHILD]?**	☐1 Yes ☐2 No ☐8 Don't know
G17. At any time during the pregnancy did **[CHILD]'s** biological mother quit or refrain from smoking for the rest of the pregnancy?	☐1 Yes ☐2 No ☐8 Don't know
G18. Did **[CHILD]** receive any newborn care in an intensive care unit, premature nursery or any other type of special care facility?	☐1 Yes **[if yes: How many days: _____ days]** ☐2 No
G19. How much did **[CHILD]** weigh at birth?	_____grams number ☐8 Don't know
G20. Did **[CHILD]** weigh more than 2.5 kg or less?	☐1 more than 2.5 kg ☐2 2.5 kg or less ☐8 Don't know
G21. Did **[CHILD]** weigh more than 4.0 kg or less?	☐1 more than 4.0 kg ☐2 4.0 kg or less ☐8 don't know

B. DIET

G22. How often does **[CHILD]** eat breakfast: everyday, on some days, rarely, never, or on weekends only?	☐1 everyday ☐2 some days ☐3 rarely ☐4 never ☐5 weekends only
G23. During the past 12 months, has **[CHILD]** changed eating habits to try to lose weight?	☐1 Yes ☐2 No
G24. During the past 12 months, has **[CHILD]** changed what you eat for any medical	☐1 Yes ☐2 No

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reason or health condition?	
G25. What was the medical reason or health condition that caused **[CHILD]** to change what he/she eats? **[Mark all that apply – USE HAND CARD CCG-1]**	☐1 High Blood Pressure/Hypertension ☐2 High Blood Cholesterol ☐3 Allergy ☐4 Diabetes ☐5 Formula-related reason ☐6 Due to infections ☐7 Gastro-intestinal Problems ☐8 Overweight/Obesity ☐9 Nutrition/Anemia ☐10 Other, specify: _____
G26. Do you consider **[CHILD]** to be overweight, underweight, or about the right weight?	☐1 overweight ☐2 underweight ☐3 about the right weight

C. HEALTH SERVICES AND HEALTH IMPAIRMENT

G27. Would you say that **[CHILD]** s health in general is excellent, very good, good, fair, or poor?	☐1 excellent ☐2 very good ☐3 good ☐4 fair ☐5 poor
G28. Is there a particular clinic, health center, doctor's office, or other place that you usually go to if **[CHILD]** is sick, or needs advice about health or for routine care?	☐1 Yes ☐2 No
G29. Is there one particular doctor or health professional that **[CHILD]** usually sees?	☐1 Yes ☐2 No
G30. About how long has it been since you or anyone last saw or talked to a medical doctor or other health professional about **[CHILD]** ? Include doctors seen while a patient in a hospital.	☐1 less than 1 year ☐2 more than 1 year, but less than 2 years ☐3 more than 2 years, but less than 5 years ☐4 5 years or more ☐5 never ☐8 don't know
G34. Since **[CHILD]** was born, how many different times has **[CHILD]** stayed in the hospital overnight or longer? **Do not include the hospitalization when [child] was born**.	☐0 none _____ times number
G35. Is **[CHILD]** able to take part at all in any of the usual kinds of activities done by most children of his/her age?	☐1 Yes ☐2 No

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G36. Is **[CHILD]** limited in the kind or amount of activities can do because of an impairment or health problem?	☐1 Yes ☐2 No
G37. Does any impairment or health problem now keep **[CHILD]** from attending school?	☐1 Yes ☐2 No
G38. Does **[CHILD]** need to attend a special school or special classes because of any impairment or health problem?	☐1 Yes ☐2 No
G39. How long ago was the impairment or health problem first noticed?	_____ number ☐1 months ☐2 years ☐8 don't know
G40. Did a doctor ever say that **[CHILD]** had rheumatic fever/rheumatic heart disease?	☐1 Yes ☐2 No **[GO TO G44]**
If yes:	
G41. How old was **[CHILD]** when he/she first had this illness?	_____ number ☐1 months ☐2 years
G42. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 Don't know
G43. Has **[CHILD]** ever been treated by a Doctor for this illness?	☐1 Yes ☐2 No ☐8 Don't know
G44. Did a doctor ever say that **[CHILD]** had epilepsy/fit/convulsion?	☐1 Yes ☐2 No **[GO TO G48]**
If yes:	
G45. How old was **[CHILD]** when he/she first had this illness?	_____ number ☐1 months ☐2 years
G46. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 Don't know
G47. Has **[CHILD]** ever been treated by a Doctor for this illness?	☐1 Yes ☐2 No ☐8 Don't know
G48. Did a doctor ever say that **[CHILD]** had	☐1 Yes ☐2 No **[GO TO G52]**

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Cerebral palsy?	
If yes:	
G49. How old was **[CHILD]** when he/she first had this illness?	_____ number □1 months □2 years
G50. Does **[CHILD]** still have this illness?	□1 Yes □2 No □8 Don't know
G51. Has **[CHILD]** ever been treated by a Doctor for this illness?	□1 Yes □2 No □8 Don't know
G52. Did a doctor ever say that **[CHILD]** had mental retardation?	□1 Yes □2 No **[GO TO G56]**
If yes:	
G53. How old was **[CHILD]** when he/she first had this illness?	_____ number □1 months □2 years
G54. Does **[CHILD]** still have this illness?	□1 Yes □2 No □8 don't know
G55. Has **[CHILD]** ever been treated by a Doctor for this illness?	□1 Yes □2 No □8 don't know
G56. Did a doctor ever say that **[CHILD]** had muscle weakness or paralysis of the arms?	□1 Yes □2 No **[GO TO G60]**
If yes:	
G57. How old was **[CHILD]** when he/she first had this illness?	_____ number □1 months □2 years
G58. Does **[CHILD]** still have this illness?	□1 Yes □2 No □8 don't know
G59. Has **[CHILD]** ever been treated by a Doctor for this illness?	□1 Yes □2 No □8 don't know
G60. Did a doctor ever say that **[CHILD]** had muscle weakness or paralysis of the legs?	□1 Yes □2 No **[GO TO G64]**
If yes:	

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G61. How old was **[CHILD]** when he/she first had this illness?	_____ number ☐1 months ☐2 years
G62. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 don't know
G63. Has **[CHILD]** ever been treated by a Doctor for this illness?	☐1 Yes ☐2 No ☐8 don't know
G64. Did a doctor ever say that **[CHILD]** had asthma?	☐1 Yes ☐2 No **[GO TO G68]**
If yes:	
G65. How old was **[CHILD]** when he/she first had this illness?	_____ number ☐1 months ☐2 years
G66. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 don't know
G67. Has **[CHILD]** ever been treated by a Doctor for this illness?	☐1 Yes ☐2 No ☐8 don't know
G68. Did a doctor ever say that **[CHILD]** had chronic bronchitis?	☐1 Yes ☐2 No **[GO TO G72]**
If yes:	
G69. How old was **[CHILD]** when he/she first had this illness?	_____ number ☐1 months ☐2 years
G70. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 don't know
G71. Has **[CHILD]** ever been treated by a doctor for this illness?	☐1 Yes ☐2 No ☐8 don't know
G72. Did a doctor ever say that **[CHILD]** had hay fever?	☐1 Yes ☐2 No **[GO TO G76]**
If yes:	
G73. How old was **[CHILD]** when he/she	

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first had this illness?	_____ number □1 months □2 years
G74. Does **[CHILD]** still have this illness?	□1 Yes □2 No □8 don't know
G75. Has **[CHILD]** ever been treated by a doctor for this illness?	□1 Yes □2 No □8 don't know
G76. Did a doctor ever say that **[CHILD]** had hypertension or high blood pressure?	□1 Yes □2 No **[GO TO G80]**
If yes:	
G77. How old was **[CHILD]** when he/she first had this illness?	_____ number □1 months □2 years
G78. Does **[CHILD]** still have this illness?	□1 Yes □2 No □8 don't know
G79. Has **[CHILD]** ever been treated by a doctor for this illness?	□1 Yes □2 No □8 don't know
G80. Did a doctor ever say that **[CHILD]** had high blood cholesterol?	□1 Yes □2 No **[GO TO G84]**
If yes:	
G81. How old was **[CHILD]** when he/she first had this illness?	_____ number □1 months □2 years
G82. Does **[CHILD]** still have this illness?	□1 Yes □2 No □8 don't know
G83. Has **[CHILD]** ever been treated by a doctor for this illness?	□1 Yes □2 No □8 don't know
G84. Has **[CHILD]** ever seen a psychiatrist,	□1 Yes □2 No

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psychologist, or psychoanalyst about any emotional, mental, or behavioral problems?	
G85. During the past 12 months, has **[CHILD]** taken any prescribed medicines or drugs to help control activity or behavior?	☐1 Yes ☐2 No
G86. During the past 12 months, how often did **[CHILD]** complain of headaches? Would you say never, rarely, sometimes, frequently, or always?	☐1 never ☐2 rarely ☐3 sometimes ☐4 frequently ☐5 always
G87. During the past 12 months, how often did **[CHILD]** complain of stomach aches? Would you say never, rarely, sometimes, frequently, or always? [**Do not include menstrual cramps]**	☐1 never ☐2 rarely ☐3 sometimes ☐4 frequently ☐5 always
G88. Does **[CHILD]** have any speech defect, such as stuttering, stammering, or lisping?	☐1 Yes ☐2 No
G89. Has **[CHILD]** ever had anemia, sometimes called "tired blood" or "low blood?"	☐1 Yes ☐2 No ☐8 don't know
G90. Now I will ask about some immunizations that may have received. It may be easier to recall this information if you have a record of **[CHILD]**'s shots. Do you have a vaccinationrecord for **[CHILD]** that I can see?	☐1 Vaccination record available ☐2 Vaccination record NOT available
G91. Has **[CHILD]** ever received a DPT or tetanus shot? A DPT shot is to prevent diphtheria, tetanus, and pertussis or whooping cough. **[Verify with vaccination record if available]**	☐1 Yes ☐2 No **[GO TO G93]** ☐8 don't know **[GO TO G93]**
G92. How long ago was **[CHILD]** 's last DPT or tetanus shot?	_____ number ☐1 months ☐2 years

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G93. During the past 12 months, how many times did **[CHILD]** have an accident, injury or poisoning, excluding lead poisoning, that required medical attention?	□0 None _____ times number □8 don't know

D. RESPIRATORY CONDITIONS AND ALLERGY

Cough	
G94. Does **[CHILD]** usually cough on most days for 3 consecutive months or more during the year?	□1 Yes □2 No
G95. Does **[CHILD]** usually cough first thing in the morning in the winter?	□1 Yes □2 No
if no to the above, SKIP to PHLEGM:	
G96. Does **[CHILD]** usually cough at all during the rest of the day?	□1 Yes □2 No
Ignore an occasional cough	
G97. For how many years has **[CHILD]** had this cough?	_____ Number years
Phlegm	
Count phlegm on first going outdoors. Exclude phlegm from the nose. Count swallowed phlegm.	
G98. Does **[CHILD]** usually bring up any phlegm/sputum/mucus from your chest first thing in the morning in the winter?	□1 Yes □2 No
G99. Does [CHILD] usually bring up any phlegm/sputum/mucus from his/her chest during the day in the winter?	□1 Yes □2 No
if no to above, SKIP to EPISODES OF COUGH and PHLEGM	
G100. Does **[CHILD]** bring up phlegm like this on most days for as much as three months each year?	□1 Yes □2 No
G101. Does **[CHILD]** usually bring up phlegm at all on getting up or first thing in the morning?	□1 Yes □2 No
G102. For how many years has **[CHILD]** had trouble with phlegm?	_____ years
G103. Has **[CHILD]** ever coughed up blood?	□1 Yes □2 No
G104. Was this in the past year?□□	□1 Yes □2 No

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Episodes Of Cough And Phlegm	
G105. Has **[CHILD]** had periods or episodes of (increased) cough and phelgm lasting for 3 weeks or more each year?	□1 Yes □2 No
Breathlessness	
G106. Is **[CHILD]** troubled by shortness of breath when hurrying on level ground?	□1 Yes □2 No
G107. Does **[CHILD]** get short of breath walking with other children of his/her own age on level ground?	□1 Yes □2 No
G108. Does **[CHILD]** have to stop for breath when walking at his/her own pace on level ground?	□1 Yes □2 No
G109. Is **[CHILD]** too breathless to leave the house or breathless on dressing or undressing?	□1 Yes □2 No
Wheezing	
G110. Does **[CHILD]** chest ever sound wheezy or whistling?	□1 Yes □2 No **[GO TO G125]**
[If no, GO to WEATHER] if yes to above, is it:	
G111. When **[CHILD]** has a cold?	□1 Yes □2 No
G112. Occasionally apart from colds?	□1 Yes □2 No
G113. Most days or nights?	□1 Yes □2 No
G114. For how many years has this been Present?	_____ years
G115. How many episodes of wheezing or Whistling has **[CHILD]** had in the past 12 Months?	_____ number
G116. How many times in the past 12 Months was **[CHILD]** hospitalised overnightfor these episodes of wheezing or whistling?	_____ number
G117. Can you estimate the total cost of all these hospitalizations for the past year? **[HELP RESPONDENT FIGURE OUT BY SUMMING ACROSS COST OF EACH HOSPITALIZATION]**	**R**_____,____ □8 Don't know

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G118. How many times in the past 12 Months has **[CHILD]** gone to a doctor's Surgery or hospital emergency room for one of these episodes of wheezing or whistling?	_____ number
G119. Can you estimate the total cost of all these visits for the past year? **[HELP RESPONDENT FIGURE OUT BY SUMMING ACROSS COST OF EACH HOSPITALIZATION]**	**R_____,____** □8 Don't know
G120. Has **[CHILD]** ever had an ATTACK of wheezing that has made him/her feel short of breath?	□1 Yes □2 No
if yes to above	
G121. How old was **[CHILD]** when he/she had your first such attack?	_____ Age in years
G122. Has **[CHILD]** had 2 or more such Episodes?	□1 Yes □2 No
G123. Has **[CHILD]** ever required medicine or treatment for the(se) attack(s)?	□1 Yes □2 No
G124. Is/Was **[CHILD]'s** breathing Absolutely normal between attacks?	□1 Yes □2 No
Weather	
G125. Does the weather affect **[CHILD]'s** chest?	□1 Yes □2 No
Only record "YES" if adverse weather definitely and regularly causes chest symptoms if yes to above	
G126. Does the weather make **[CHILD]** short of breath?	□1 Yes □2 No
G127. What kind of weather?	_____
Other Symptoms And Allergies	
During the past 12 months, has [CHILD] had any episodes of:	
G128. Stuffy, itchy, running nose?	□1 Yes □2 No
G129. Watery, itchy eyes?	□1 Yes □2 No

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G130. During the past 12 months, how many episodes of stuffy, itchy, running nose or watery, itchy eyes has **[CHILD]** had?	□1 none □2 constantly/continuously _____ episodes
Are ANY of the above symptoms (wheezing, whistling, runny nose, watery eyes etc), brought on by:	
G131. Exercise or cold air?	□1 Yes □2 No
G132. Animals?	□1 Yes □2 No
G133. Housedust?	□1 Yes □2 No
G134. pollen?	□1 Yes □2 No
G135. Wool clothing	□1 Yes □2 No
G136. Cigarette smoke	□1 Yes □2 No
G137. Soaps, sprays or detergents	□1 Yes □2 No
G138. Colds or 'flu	□1 Yes □2 No
G139. Air pollution	□1 Yes □2 No
G140. Strong odours/smells	□1 Yes □2 No
G141. Other things	□1 Yes □2 No □9 please specify _____
G142. During which months of the year does pollen make **[CHILD]'s** symptoms worse? **[circle months that apply]**	□1 ALL months J F M A M J J A S O N D
ALLERGY	
G143. Within an hour after eating something, has **[CHILD]** ever had a severe reaction, such as itching all over, trouble breathing, flushing, or swelling of the hands and feet?	□1 Yes □2 No
G144. Within an hour after receiving allergy shots or allergy tests, has **[CHILD]** ever had a severe reaction, such as itching all over, trouble breathing, flushing, or swelling of the hands and feet?	□1 Yes □2 No □3 Never had allergy shots or tests □8 Don't know
G145. **Has** [CHILD] **ever given up or had to avoid a pet because of allergies?**	□1 Yes □2 No

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E. EAR INFECTION

G146. Did **[CHILD]** ever have an ear infection or an earache?	☐1 Yes ☐2 No ☐8 don't know
G147. How many times has **[CHILD]** had an ear infection or an earache?	☐0 never ☐1 once ☐2 twice ☐3 3 – 5 times ☐4 6 or more times ☐8 don't know
G148. How old was **[CHILD]** when had the first ear infection or earache?	☐1 less than 1 year old ⇨ ___months age ☐2 1 year old or older ⇨ _____ years age
G149. Was **[CHILD]** ever treated by a doctor for ear infection(s) or earache(s)?	☐1 Yes ☐2 No ☐8 don't know
G150. Has **[CHILD]** ever had trouble hearing with one or both ears? Do not include any problems which lasted just a short period of time such as during a cold.	☐1 Yes ☐2 No ☐8 don't know
G151. Does **[CHILD]** still have trouble hearing with one or both ears?	☐1 Yes ☐2 No ☐8 don't know
G152. Does **[CHILD]** use a hearing aid?	☐1 Yes ☐2 No ☐8 don't know
G153. How long ago did **[CHILD]** last have hearing tested?	☐1 never ☐2 6 months or less ☐3 more than 6 months, but less than 12 months ☐4 more than 12 months, less than 2 years ☐5 more than 2 years, less than 5 years ☐6 more than 5 years ☐8 don't know

F. SCHOOL ATTENDANCE

G154. During the past 12 months, about how many whole days was **[CHILD]** absent from school because of illness, playing truant, or for other reasons?	☐0 none _____ days number ☐8 don't know
G155. Has **[CHILD]** ever skipped any grades for any reason?	☐1 Yes ☐2 No
G156. Has **[CHILD]** repeated any grades for any	☐1 Yes

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reason?	□2 No
G157. What grade did **[CHILD]** repeat?	1 2 3 4 5 6 7 8
Why did **[CHILD]** repeat the grade(s)? **[USE HAND CARD CCG-2]**	□1 Academic failure □2 Immature/acted too young □3 Frequently absent □4 Moved into a more difficult school □5 Language problem □6 Learning/behavior problem □7 Hearing/vision problem □8 Health problem □9 Relocation problem □10 Language problem □11 Other, specify: _____
G158. Has **[CHILD]** ever been suspended, excluded or expelled from school?	□1 Yes □2 No
G159. How many times has **[CHILD]** been suspended, excluded or expelled from school?	_____ times number
G160. On the average during the school year, how many hours per week does **[CHILD]** work in a paid or unpaid job?	□1 none □2 5 or fewer hours □3 6-9 hours □4 10-14 hours □5 15-19 hours □6 20-24 hours □7 25 or more hours

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G. ASTHMA SEVERITY

G161. Has a *doctor or nurse* ever told you that **[child]** has asthma?	☐1 Yes **[GO TO G162]** ☐2 No **[READ PASSAGE BELOW]** ☐8 Don't know **[READ PASSAGE BELOW]**
If NO or DON'T KNOW say: From now on, when I say asthma, I will be talking about breathing problems such as episodes of wheezing, coughing, tightness of the chest, heaviness in the chest or shortness of breath that **[child]** may sometimes experiences. I understand that [he or she] may or may not be having any problems like this. Okay? **[GO TO G164]**	
G162. How old was **[child]** when a doctor or nurse told you that he/she had asthma?	____ years old
G163. Does **[CHILD]** still have this illness?	☐1 Yes ☐2 No ☐8 don't know
G164. In the past 12 months, how often has your Child had a cough that won't go away? Would you say… **[USE HAND CARD CCG-3]**	☐1 Every day ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 Never
G165. In the past 12 months, how often has your child had wheezing (a whistling sound from the chest) with a cold? **[USE HAND CARD CCG-3]**	☐1 Every day ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to 12 times in whole year ☐5 1 or 2 times in the whole year ☐6 Never
G166. In the past 12 months, how often has your child had wheezing (a whistling sound from the chest) *without* a cold? **[USE HAND CARD CCG-3]**	☐1 Every day ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 Never
G167. In the past 12 months, how often has your child had an attack of wheezing that made it hard for him or her to breathe or catch his or her breath**?** **[USE HAND CARD CCG-3]**	☐1 Every day ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 Never
G168. In the past 12 months, how often has your child wheezed with exercise or running or playing hard? **[USE HAND CARD CCG-3]**	☐1 Every day ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 Never
G169. In the past 12 months, how often has your child coughed with exercise or running or playing hard? **[USE HAND CARD CCG-3]**	☐1 Every day ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 Never

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G170. In the past 12 months, how often has your child complained that his or her chest felt tight or heavy? **[USE HAND CARD CCG-3]**	☐1 Every day ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 Never
G171. In the past 12 months, how often has your child's sleep been disturbed due to wheezing, coughing, chest tightness or shortness of breath?	☐1 Most nights ☐2 More than 2 times per week ☐3 More than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 or 2 times in the whole year ☐6 Never
G172. Are there any particular seasons or months when **[child's]** symptoms are worse?	☐1 YES **[GO TO G173]** ☐2 NO **[GO TO G174]**
G173. During which season (or months) does **[child]** have the most breathing problems? **[CHECK ALL THAT APPLY]**	☐1 Spring (September, October, November) ☐2 Summer (December, January, February) ☐3 Autumn (March, April, May) ☐4 Winter (June, July, August) ☐5 Never has breathing problems
G174. I am going to read a list of things that might bring on wheezing, tightness in the chest, cough, or shortness of breath in some children. I would like to know whether each of these things brings on these symptoms for **[child]**. **[CHECK ALL THE RESPONSES THAT R. MENTIONS, REMEMBER TO REPEAT QUESTION FROM TIME TO TIME]** **[USE HAND CARD CCG-4]**	☐1 Being active (running, playing, swimming, or exercising) ☐2 Sprays or strong smells (such as colognes, perfumes, or cleaning supplies) ☐3 Colds or flu ☐4 Cold air ☐5 Change in weather ☐6 Laughing or crying hard ☐7 Dust ☐8 Pets ☐9 Truck or car exhaust ☐10 Hot summer days ☐11 Pollen, trees, fresh cut grass ☐12 Mold and mildew ☐13 Smoke ☐14 Cockroaches ☐15 Certain foods ☐16 Nothing causes breathing problems ☐99 Other (**SPECIFY:**)
Has a doctor *ever* told you that **[child]** has.... **[READ ALL CHOICES]**	
G175. Allergies	☐1 Yes ☐2 No
G176. Eczema	☐1 Yes ☐2 No
G177. Reactive airway disease	☐1 Yes ☐2 No

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G178. Asthmatic bronchitis	☐1 Yes ☐2 No
G179. Any other lung/breathing condition	☐1 Yes (**SPECIFY:** _____) ☐2 No

H. HEALTH SERVICES UTILIZATION

G180. Not including the emergency room, does **[child]** have a *regular* family doctor or health care provider that you usually go to for his/her health care?	☐1 Yes What is Doctor or Clinic's name _____ ☐2 No **[GO TO G182]**
G181. When is the last time you visited this doctor or clinic?	_____ month/year

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I. ASTHMA MEDICATION

Can you bring all the medications in the home that **[child]** is has ever taken for asthma, wheezing, tightness in the chest, shortness of breath, or cough. This includes those medications that a doctor or clinic has prescribed and those that a doctor did not prescribe, for example, over-the-counter drugs or home remedies. [Ask respondent to bring you all containers of medication in that house that the child has used. Fill in names from containers] **[IF CHILD HAS NEVER TAKEN ANY MEDICATION FOR ASTHMA, SKIP TO HOUSEHOLD ENVIRONMENTAL CHECKLIST]**

Medication Name	Code **[LEAVE BLANK]**	Is the container present and have you seen it...	Is this a. . .	Was this medicine prescribed by a doctor?	How often did the doctor say to take it or use it?		Can you tell me when [child] last used this medicine?	How much does a one month supply of the medication [if taken as prescribed] cost you?	Does **[child]** use this medication less often than needed or prescribed because of the cost?
G182.	**G183**	**G184.** ☐1 container seen ☐2 container not seen	**G185.** ☐1 pill ☐2 liquid (to swallow) ☐3 inhaler/pump ☐4 added to a breathing machine or nebulizer	**G186.** ☐1 Yes ☐2 No	**G187.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G188.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G189.** **R**_____,_____	**G190.** ☐1 Yes ☐2 No

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G191.	**G192.**	**G193.** ☐1 container seen ☐2 container not seen	**G194.** ☐1 pill ☐2 liquid ☐3 inhaler/puffer ☐4 breathing machine or nebulizer	**G195.** ☐1 Yes ☐2 No	**G196.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G197.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G198.** **R_____,_____**	**G199.** ☐1 Yes ☐2 No
G200.	**G201.**	**G202.** ☐1 container seen ☐2 container not seen	**G203.** ☐1 pill ☐2 liquid ☐3 inhaler/puffer ☐4 breathing machine or nebulizer	**G204.** ☐1 Yes ☐2 No	**G205.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G206.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G207.** **R_____,_____**	**G208.** ☐1 Yes ☐2 No
G209.	**G210.**	**G211.** ☐1 container seen ☐2 container not seen	**G212.** ☐1 pill ☐2 liquid ☐3 inhaler/puf	**G213.** ☐1 Yes ☐2 No	**G214.** PRN (as needed) ☐1	____ times/day or	**G215.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month	**G216.** **R_____,_____**	**G217.** ☐1 Yes ☐2 No

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			fer ☐4 breathing machine or nebulizer			____ puffs/day	☐5 more than 1 month ago		
G218.	**G219.**	**G220.** ☐1 container seen ☐2 container not seen	**G221.** ☐1 pill ☐2 liquid ☐3 inhaler/puffer ☐4 breathing machine or nebulizer	**G222.** ☐1 Yes ☐2 No	**G223.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G224.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G225.** **R_____,_____**	**G226.** ☐1 Yes ☐2 No

G227. Are there any other medications that **[child]** has taken in the last month for asthma that aren't here?	☐1 Yes **(GO TO G228)** ☐2 No
Complete chart below	

Medication Name	Code **[LEAVE BLANK]**	Is the container present and have you seen it...	Is this a. . .	Was this medicine prescribed by a doctor?	How often did the doctor say to take it or use it?	Can you tell me when [child] last used this medicine?	How much does a one month supply of the medication [if taken as prescribed] cost you?	Does **[child]** use this medication less often than needed or prescribed because of the cost?

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G228.	**G229.**	**G230.** ☐1 container seen ☐2 container not seen	**G231.** ☐1 pill ☐2 liquid (to swallow) ☐3 inhaler/pump ☐4 added to a breathing machine or nebulizer	**G232.** ☐1 Yes ☐2 No	**G233.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G234.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G235.** R_____,_____	**G236.** ☐1 Yes ☐2 No
G237.	**G238.**	**G239.** ☐1 container seen ☐2 container not seen	**G240.** ☐1 pill ☐2 liquid ☐3 inhaler/puffer ☐4 breathing machine or nebulizer	**G241.** ☐1 Yes ☐2 No	**G242.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G243.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G244.** R_____,_____	**G245.** ☐1 Yes ☐2 No
G246.	**G247.**	**G248.** ☐1 container seen	**G249.** ☐1 pill	**G250.** ☐1 Yes	**G251.** PRN (as	____ times/da	**G252.** ☐1 today ☐2 yesterday	**G253.**	**G254.** ☐1 Yes ☐2 No

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		□2 container not seen	☐2 liquid ☐3 inhaler/puffer ☐4 breathing machine or nebulizer	☐2 No	needed) ☐1	y or ____ puffs/day	☐3 last week ☐4 last month ☐5 more than 1 month ago	**R_____,_____**	

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G255.	**G256.**	**G257.** ☐1 container seen ☐2 container not seen	**G258.** ☐1 pill ☐2 liquid ☐3 inhaler/puffer ☐4 breathing machine or nebulizer	**G259.** ☐1 Yes ☐2 No	**G260.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G261.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G262.** **R_____,_____**	**G263.** ☐1 Yes ☐2 No
G264.	**G265.**	**G266.** ☐1 container seen ☐2 container not seen	**G267.** ☐1 pill ☐2 liquid ☐3 inhaler/puffer ☐4 breathing machine or nebulizer	**G268.** ☐1 Yes ☐2 No	**G269.** PRN (as needed) ☐1	____ times/day or ____ puffs/day	**G270.** ☐1 today ☐2 yesterday ☐3 last week ☐4 last month ☐5 more than 1 month ago	**G271.** **R_____,_____**	**G272.** ☐1 Yes ☐2 No

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G273. Are there other medications that a doctor or clinic has prescribed for asthma, wheezing, tightness in the chest, shortness of breath, or cough, that you have not bought because of the cost?	□1 Yes □2 No
G274. If yes, do you have the prescription still **[copy name and dose from prescription].**	a. _____ b. _____ c. _____ d. _____ e. _____
G275. If you don't have the prescription, do you remember the name of the medication(s)?	a. _____ b. _____ c. _____ d. _____ e. _____
G276. How much would a one-month supply **[of each medication**] cost?	a. R_____,_____ b. R_____,_____ c. R_____,_____ d. R_____,_____ e. R_____,_____ □8 don't know

J. CAREGIVER'S QUALITY OF LIFE

Now, I am going to ask you some similar questions about how your child's asthma has affected you and also some questions about your health.

G363. In the past 3 months, how often did you wake up or lose sleep because of **[child's]** asthma? would you say you woke up or lost sleep……	☐1 most nights ☐2 more than 2 times per week ☐3 more than 2 times per month ☐4 1 or 2 times per month ☐5 no nights
G364. Is there a particular season or month when you wake up or lose sleep most because of **[child's]** asthma?	☐1 YES ☐2 NO **[SKIP TO G366]**
G365. During what season or month do you wake up or lose sleep most because of **[child's]** asthma? **[CHECK ONE]**	☐1 Spring (September, October, November) ☐2 Summer (December, January, February) ☐3 Autumn (March, April, May) ☐4 Winter (June, July, August)
G366. During the last **[USE ANSWER FROM G365]**, how often did you wake up or lose sleep because of **[child's]**	☐1 Most nights ☐2 More than 2 times per week ☐3 More than 2 times per month

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asthma?	☐4 1 or 2 times per month ☐5 No nights
G367. In the past 3 months, how often did you have to change your daytime or evening plans because of [**child's**] asthma? Would you say it was…..	☐1 Most days/evenings ☐2 More than 2 times per week ☐3 More than 2 times per month ☐4 1 or 2 times per month ☐5 No days/evenings
G368. Is there a particular season or month when you have to change your daytime or evening plans most because of **[child's]** asthma?	☐1 YES ☐2 NO **[SKIP TO G371]**
G369. During what season or month do you have to change your daytime or evening plans most because of **[child's]** asthma? **[CHECK ONE]**	☐1 Spring (September, October, November) ☐2 Summer (December, January, February) ☐3 Autumn (March, April, May) ☐4 Winter (June, July, August)
G370. During the last **[USE ANSWER FROM G369]**, how many days or nights per week do you have to change your daytime or evening plans because of **[child's]** asthma? Would you say it was…	☐1 Most days/evenings ☐2 More than 2 times per week ☐3 More than 2 times per month ☐4 1 or 2 times per month ☐5 No days/evenings

K. HOUSEHOLD ENVIRONMENTAL CHECKLIST

G371. Do you rent or own your home or neither?	☐1 rent ☐2 own **[skip to G373]** ☐3 neither **[skip G373]**
G372. In general, how easy or difficult would you say that it is to get your landlord to make repairs when they are needed? Would you say . . . **[READ CHOICES]**	☐1 Very easy ☐2 Somewhat easy ☐3 Neither easy or difficult ☐4 Somewhat difficult ☐5 Very difficult
At any time during the year is there standing water or puddles located in. . . **[READ EACH CHOICE]**	
G373. [Child's] sleeping room?	☐1 Yes ☐2 No
G374. The sitting room?	☐1 Yes☐2 No ☐3 No sitting room
G375. The kitchen?	☐1 Yes☐2 No
G376. Another place I did not specify?	☐1 Yes (**specify** _____) ☐2 No
In the past year have you had any other problem with water damage or leaking water in your home, such as from a leaking roof or leaky plumbing	
G377. [Child's] sleeping room?	☐1 Yes ☐2 No
G378. The sitting room?	☐1 Yes☐2 No ☐3 No sitting room
G379. The kitchen?	☐1 Yes☐2 No

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G380. Another place I did not specify?	☐1 Yes (**specify**_____) ☐2 No
G381. How many pets of each type come inside the home?	Dog? _____ Cat? _____ Other pets (**SPECIFY:** _____) ☐1 No pets in the house **[GO TO G384]**
[IF ANY CATS OR DOGS OR OTHER PETS WITH FUR ARE PRESENT, ASK G382. AND C383.]	
G382. Do any of these pets spend any time in child's bedroom?	☐1 Yes ☐2 No ☐8 Don't Know
G383. Are the pets put out of the house at night?	☐1 Yes ☐2 No ☐3 Sometimes ☐8 Don't Know
G384. Are there cockroaches in your Home?	☐1 Yes ☐2 No ☐8 Don't know
G385. Have you had any problems with cockroaches in your home during the past year?	☐1 Yes ☐2 No ☐8 Don't know
G386. Have you or someone else (your landlord, another family member, a professional) treated your home for cockroaches in the past year?	☐1 Yes ☐2 No **[GO TO G389]** ☐8 Don't know **[GO TO G389]**
G387. When was the last time it was treated ?	☐1 Within last month ☐2 1 to 3 months ago ☐3 3 to 6 months ago ☐4 6 to 12 months ago ☐5 More than 12 months ago ☐8 Don't know
G388. What was used to treat your home for roaches? **[READ EACH CHOICE AND CHECK ALL THAT APPLY]**	☐1 Dry powder ☐2 Spraying ☐3 Gel ☐4 Roach bait trap (**SPECIFY:** _____) ☐5 Boric acid ☐6 Other (**SPECIFY:** _____) ☐8 Don't know
G389. Have you had any problems with mice or rats in your home during the past year?	☐1 Yes ☐2 No ☐8 Don't know
G390. Have you or someone else (your landlord, another family member, a professional) treated your home for rats or mice in the past year?	☐1 Yes ☐2 No **[GO TO G393]** ☐8 Don't know **[GO TO G393]**
G391. When was the last time?	☐1 Within last month ☐2 Between 2 and 6 months ago

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	☐3 Between 6 and 12 months ago ☐4 More than 12 months ago ☐8 Don't know
G392. How is your home treated for rats or mice? [**READ EACH CHOICE AND CHECK ALL THAT APPLY**]	☐1 Spring traps ☐2 Glue traps ☐3 Poison ☐4 Other (**SPECIFY:** _____) ☐8 Don't know
G393. During the past 12 months was a room heater used to heat one or more rooms in this house?	☐1 Yes ☐2 No ☐8 Don't know
G394. Was this heater fueled by	☐1 Paraffin ☐2 Gas ☐3 Electricity ☐4 Wood ☐5 Coal ☐6 Charcoal ☐7 Cattle manure/animal dung ☐8 Solar (sun) heat ☐9 Other, Please Specify: _____ ☐88 Don't Know
G395. During the past 12 months was one or more wood stoves used in this house?	☐1 Yes ☐2 No ☐8 Don't know
G396. During the past 12 months was a fireplace used to heat the rooms in this house?	☐1 Yes ☐2 No ☐8 Don't know
G397. During the past 12 months was the stove or oven ever used to heat this house?	☐1 Yes ☐2 No ☐8 Don't know
G398. Is a stove or oven used for cooking in this house?	☐1 Yes ☐2 No ☐8 Don't know
G399. What is the primary source of heat for this stove or oven?	☐1 Paraffin ☐2 Gas ☐3 Electricity ☐4 Wood ☐5 Coal ☐6 Charcoal ☐7 Cattle manure/animal dung ☐8 Solar (sun) heat ☐9 Other, Please Specify: _____ ☐88 Don't Know

L. BEHAVIOR CHANGE TO REDUCE ENVIRONMENTAL HAZARDS

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The purpose of these questions is to look at the environment in your home and how it relates to your child's asthma as well as the health of other household members.

G400. Is there anyone whose paying job is working around chemicals (such as pesticides, paints) or dust living in the home?	☐1 Yes ☐2 No ☐8 Don't know
G401. If yes, do they usually wear their work clothes home?	☐1 Yes ☐2 No ☐8 Don't know
G402. Is there anyone whose informal job (includes working with chemicals) is in or near the home?	☐1 Yes ☐2 No ☐8 Don't know
G403. During the last 2 weeks, how many times was the room in which **[child]** sleeps dusted?	☐1 None ☐2 1 ☐3 2 ☐4 3 ☐5 4 or more ☐8 Don't know
G404. What do you use when you dust?	☐1 Dry cloth ☐2 Damp cloth ☐9 Other (**SPECIFY:** _____)
G405. During the last 2 weeks, how many times were other rooms in the house dusted?	☐1 None ☐2 1 ☐3 2 ☐4 3 ☐5 4 or more ☐8 Don't know
G406. How often do you change the **[child's]** Bedding? **[DO NOT READ RESPONSE CATEGORIES TO RESPONDENT; CHOOSE CATEGORY WHICH FITS RESPONSE]**	☐1 once a week or more ☐2 more than every two weeks ☐3 more that once a month ☐4 once a month or less ☐9 Other (**SPECIFY:** _____) ☐8 Don't know
G407. When the child's bedding are machine washed, what temperature is usually used for the wash cycle? **[WE ARE INTERESTED IN THE WASH CYCLE ONLY. THIS IS THE FIRST CYCLE OF THE WASHING MACHINE. EXAMPLE: SOMEONE USES THE WARM-COLD SETTING, YOU WOULD RECORD WARM.]**	☐1 Hot ☐2 Warm ☐3 Cold ☐4 Not applicable [**DO NOT READ**] ☐8 Don't know

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G408. When the child's bedding is hand washed, what temperature is usually used for the wash?	☐1 Hot ☐2 Warm ☐3 Cold ☐4 Not applicable [**DO NOT READ**] ☐8 Don't know
G409. Do you or any member of your family add anything to the wash to help get rid of dust mites? **[PROMPT: "Such as eucalyptus oil."]**	☐1 Yes (**SPECIFY:** _____) ☐2 No ☐8 Don't know
G410. How often does the cover on your child's bed get washed (i.e. bedspreads/comforters)? **[RECORD THE CATEGORY CLOSEST TO THE RESPONSE. IF RESPONDENT UNSURE, READ RESPONSES]**	☐1 Once a week or more often ☐2 More than once a month ☐3 More often that every 3 months (4 times a year) ☐4 More often that every six months (2 times a year) ☐5 Less often than every six months ☐8 Don't know
G411. Does **[child]** have stuffed animals in his or her bedroom?	☐1 Yes ☐2 No **[GO TO G407]** ☐8 Don't know
G412. Do **[child's]** stuffed animals get washed?	☐1 Yes ☐2 No **[GO TO G407]** ☐8 Don't know or not applicable **[GO TO G407]**
G413. How many times per year do **[child's]** stuffed animals get washed? **[DO NOT READ RESPONSE CATEGORIES TO RESPONDENT; CHOOSE CATEGORY WHICH FITS RESPONSE]**	☐1 Once a week ☐2 Once a month ☐2 Every three months (4 times a year) ☐3 Every six months (2 times a year) ☐4 Less than once a year ☐5 Once a year ☐9 Other (**SPECIFY:** _____) ☐8 Don't know
Have you done any of the following things around the house because of **[child's]** asthma?	
G414. Removed visible mold growth?	☐1 Yes ☐2 No
G415. Removed pets from the home?	☐1 Yes ☐2 No ☐3 Never had pets
G416. Changed cigarette-smoking rules in the home?	☐1 Yes, no one allowed to smoke ☐2 Yes, reduced amount of smoking ☐3 Yes, limited smoking to one room. ☐4 No ☐5 Never any smokers
G417. Attempted to control or eliminate cockroaches?	☐1 Yes ☐2 No ☐3 Never a problem

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G418. Attempted to control or eliminate mice or rats?	☐1 Yes ☐2 No ☐3 Never a problem
G419. Did you do any other things around the house because of [**child's**] asthma?	(**SPECIFY:** _____)
Now I'm going to ask you a few questions about smoking. These questions concern smoking of cigarettes.	
G420. How many people who live in [child's] home smoke? **[INCLUDE RESPONDENT IF SMOKER.]**	_____ people ☐1 None **[GO TO G418]**
G421. Do you smoke cigarettes, even occasionally?	☐1 Yes ☐2 No **[GO TO G418]**
G422. About how many cigarettes a day do you now smoke?	_____ cigarettes
G423. How often do you go outside the home to smoke?	☐1 Always ☐2 Sometimes ☐3 Rarely ☐4 Never ☐8 Don't know
G424. Does **[child]** smoke cigarettes?	☐1 Yes ☐2 No ☐8 Don't know
G425. Do any frequent visitors smoke?	☐1 Yes ☐2 No ☐8 Don't know
G426. Many people have difficulties keeping their children away from cigarette smoke. Do you have problems keeping **[child]** away from people who are smoking?	☐1 Yes ☐2 No
G427. How frequently is your child around people who are smoking? Would you say. . . . **[READ CHOICES]**	☐1 Daily ☐2 Several times a week ☐3 Several times a month ☐4 Never ☐8 Don't know

M. HOUSEHOLD PESTICIDE USAGE

G428. Do you or any one in your house use pesticides in the garden or in your home?____ (Yes/ No)

G429. If yes, what do you use? _____

G430. Do pesticide contaminated clothes get washed at home? ____ (Yes/ No)

G431. If yes, does it get washed with the rest of the washing? _____ (Yes/ No)

G433. Do you eat fruit or vegetables from your garden ? _____ (Yes / No)

G434. Do you use empty pesticide containers at home for domestic purposes? ____(Yes/ No)

G435. If yes, what do you use them for? _____

N. ISAAC STANDARD QUESTIONS

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G436. Has **[child]** ever had an itchy rash which was coming and going for at least six months?	☐1 Yes ☐2 No **[GO TO G426]**
G437. Has **[child]** had this rash at any time in the past 12 months?	☐1 Yes ☐2 No **[GO TO G426]**
G438. Has this itchy rash at any time affected any one of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?	☐1 Yes ☐2 No **[GO TO G426]**
G439. Has this rash cleared up completely at any time during the past 12 months?	☐1 Yes ☐2 No **[GO TO G426]**
G440. In the past 12 months, how often, on average, has **[child]** been kept awake at night by this itchy rash?	☐1 Never in the past 12 months ☐2 Less than one night per week ☐3 One or more nights per week
G441. Has **[child]** ever had a problem with sneezing, or a runny or blocked nose when he/she DID NOT have a cold or flu?	☐1 Yes ☐2 No **[GO TO G428]**
G442. In the past 12 months, how much did this nose problem interfere with **[child's]** daily activities?	☐1 not at all ☐2 a little ☐3 a moderate amount ☐4 a lot
G443. Has **[child]** ever had hayfever?	☐1 Yes ☐2 No

N. SCREENING AND SEVERITY QUESTIONS

G444. In the past 12 months has your child taken any medications, nebulisers, or inhalers (pumps) prescribed by a doctor for any of CHEST conditions?	☐1 Yes ☐2 No
G445. Does your child take **any of these doctor-prescribed medications every day**, even when he/she is **not** having trouble breathing?	☐1 Yes ☐2 No ☐8 Does not apply
G446. In the past 12 months how many times has your child **had to make an unplanned visit to a doctor's office for breathing problems?**	☐1 0 times ☐2 1 time ☐3 2 times ☐4 3 or 4 times ☐5 5 or 6 times ☐6 7 times or more
G447. In the past 12 months how many times has your child **been to the emergency room** (but not stayed overnight in the hospital) **for breathing problems?**	☐1 0 times ☐2 1 time ☐3 2 times ☐4 3 or 4 times

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	□5 5 or 6 times □6 7 times or more
G448. In the past 12 months how many times has your child **had to stay in the hospital for one night or more because of breathing problems?**	□1 0 times □2 1 time □3 2 times □4 3 or 4 times □5 5 or 6 times □6 7 times or more

G449. What is the highest grade or year of school you completed? **[READ CHOICES]**	☐1 Never attended school or only pre-school ☐2 Class 1 – Std 6 (Grades 1 through 8) ☐3 Std 7 – Std 9 (Grades 9 through 11- Some high school) ☐4 Std 10 / Matric (Grade 12 - High school graduate) ☐5 College / technikon / university (1 year to 3 years - Some college or technical school) ☐6 College 4 years or more (College graduate) ☐9 Refused to answer

O. RESIDENTIAL HISTORY

G450. Since when does your child live in his/her current home?	(State month and year)
G451. List all the places where your child has lived in his/her lifetime and the dates that that he/she stayed at each place (start with the place before the current home and work backwards until the place of birth).	

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE!

END: Thank you for helping us!
Interview completed at: Time: _ _:_ _ am / pm

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Appendix 4: Ethical approval for the main study

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 404 7682 • **Facsimile** [021] 406 6411
Email: nosi.tsama@uct.ac.za
Website: www.health.uct.ac.za/research/humanethics/forms

18 November 2014

HREC REF: 697/2014

A/Prof A Dalvie
CIDER
Public Health & Family Medicine

Dear A/Prof Dalvie

PROJECT TITLE: AN EPIDEMIOLOGICAL COHORT STUDY OF SCHOOL CHILDREN INVESTIGATING ASTHMA AND OF ADULTS INVESTIGATING CARDIOPULMONARY OUTCOMES

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee dated for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30TH November 2015.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/research/humanethics/forms)

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC reference no in all your correspondence.

Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

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Appendix 5: Ethical approval for the sub-study

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.10.2024
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee	Signed by candidate	Date Signed	17/10/2023

Note: Please email this form and supporting documents (if applicable) in a combined pdf file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

HUMAN RESEARCH ETHICS COMMITTEE
17 OCT 2023
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

Comments to PI from the HREC

Thank you for your Study Deviation

Signed by candidate

HREC Chair Signature

Date: 17/10/2023

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	16 October 2023		
HREC REF Number	322/2022	Current Ethics Approval was granted until	30 June 2023
Protocol title	MODIFICATION BY SEX AND ATOPY OF THE EFFECT MODIFICATION OF CHEMICAL AIR POLLUTIONS AND BIOLOGICAL AIR POLLUTANTS INCLUDING POLLEN AND FUNGAL SPORES ON CHILDREN RESPIRATORY HEALTH IN THE WESTERN CAPE, CAPE TOWN, SOUTH AFRICA.		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes	✓☐ No	
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Prof MA Dalvie		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

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Department / Office Internal Mail Address	School of Public Health and Family Medicine, room 4.31, 4th floor, Falmouth Building, Health Sciences Faculty

1.1 Does this protocol receive US Federal funding?	☐ Yes	✓☐ No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-submission@uct.ac.za)	☐ Yes	☐ No

If yes in 1.2 please complete section 1.3 below for invoicing purposes

1.3 Ethics Renewal Fee

Please **(tick ✓)**appropriate box for billing purposes:

Submission Type	***Description***	***New fee (Vat Incl.)***	***tick ✓***
Research funded solely from UCT departmental/ divisional/group budget	Annual evaluation of research progress report for re-certification	**R0,00**	☐
Non-sponsored student research for degree purposes at UCT/Other Universities & Colleges	Annual evaluation of research progress report for re-certification	**R0,00**	✓☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R7000,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Expedited review	**R3 710.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National grant funded research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R6000.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National Grant funded research for Annual evaluation of research progress report for re-certification for Expedited review	**R1 500,00**	☐

NB: Protocols funded by UCT (e.g. departmental funding / student research) and by certain grant funding organizations (e.g. MRC, NRF, CANSA,) are exempt from these charges.

Please provide details for Invoicing, either complete section 1 or 2 :

1. Invoice billing – Directly to Sponsor

Sponsor's name	
Billing Address of Sponsor:	
Vat Number:	

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

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Contact person	
Telephone number	
Email Address	
2. **Internal Journal Billing:**	
Fund Number:	
Cost Centre Number:	
Account Holder Name:	
Division of Account Holder:	

2. List of documentation for approval

3. Protocol status (tick ✓)

☐	Open Enrolment
✓ ☐	Closed to enrolment (tick ✓)
☐	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
☐	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	600
Number of participants enrolled, since last HREC Progress report (continuing review)	0
Additional number of participants still required	0

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	0

6. Cumulative summary of participants

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

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Total number of participants who provided consent	600
Number of participants determined to be ineligible (i.e. after screening)	0
Number of participants currently active on the study	0
Number of participants completed study (without events leading to withdrawal)	600
Number of participants withdrawn at participants' request (i.e. changed their mind)	0
Number of participants withdrawn by PI due to toxicity or adverse events	0
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	0
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	0
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	0

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:
There have been no progress since the last approval but data analysis will commence soon.

8. Protocol violations and exceptions (tick ✓ all that apply)

✓☐	No prior violations or exceptions have occurred since the original approval
☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

✓☐	No Prior amendments have been made since the original approval
☐	Prior amendments have been reported since the last review and have already been approved

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

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☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.
N/A

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
☐ Yes	☐ No	✓☐ Not applicable
If yes, please describe:		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	☐ No	✓☐ Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	☐ No	✓☐ Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	✓☐ Not applicable
		DSMB report attached	☐ Yes	☐ No	✓☐ Not applicable

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	✓☐ No
If yes, please explain:	

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

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12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased
✓☐	Shown no change
If there has been a change, please explain:	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.

13. Insurance

Please confirm that valid no fault insurance is still in place? **(tick ✓)**			
☐ Yes	☐ No		☐✓ Not Applicable – N/A
If yes, please complete the following:			
Insurer's name:			
Policy no.		*Coverage Period:	
For UCT sponsored studies please liaise the Insurance office via fhs.sponsorship@uct.ac.za regarding the required documentation and information required obtain a renewed UCT No-fault Insurance Certificate.			

14. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	✓☐ No
If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

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15. Signature

My signature certifies that the above is complete and correct.			
Signature of PI	Signed by candidate	Date	16/10/2023

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Appendix 6: Permission letter to schools

UNIVERSITY OF CAPE TOWN

CENTRE FOR OCCUPATIONAL AND ENVIRONMENTAL HEALTH RESEARCH

Department of

Public Health

Faculty of Health Sciences

Anzio Road

Observatory 7925

SOUTH AFRICA

Telephone: (27 21) 4066719

Date

Dear Principal

Re: An epidemiological cohort study of school children investigating asthma outcomes

We would like to ask for your permission to include Grade 4 pupils at your school in the important study above conducted by the University of Cape Town at the request of the Department of Environmental Health and Development Planning in the Western Cape. The study will investigate the effect of air pollution and other environmental pollutants on asthma in school children. The study will be conducted during this year (2015), with a follow up into 2016. The study will benefit residents in the Western Cape exposed to air pollution and other environmental pollutants. Participation by your school involves identification of all Grade 4 classes at the school, making available a copy of the class lists with date of birth if possible, distributing letters to all Grade 4 parents (copy enclosed) asking them for permission to include their child in the study and arranging an appropriate venue on the school on the days of testing during 2015-2016. Participating children will be required to undergo an interview and produce urine and a blood sample every year during 2015-2016. A respiratory test will be included during 2015 and 2016. Additionally, in 2016 the child will be required to undergo continuous respiratory testing every day for a two weeks period once during winter and once during summer. Further details on the risks of testing is detailed in the attached consent form that parents must sign.

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The results of the study would help in further planning in reducing environmental exposures in residential areas in the Western Cape.

Yours sincerely

Associate Professor MA Dalvie (Principle Investigator)
Cellphone number: 0827863781

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Appendix 7: Permission letter to parent

UNIVERSITY OF CAPE TOWN

CENTRE FOR OCCUPATIONAL AND ENVIRONMENTAL HEALTH RESEARCH

Department of Public Health

Faculty of Health Sciences

Anzio Road

Observatory 7925

Date

Dear Parent/Guardian

Re: An epidemiological cohort study of school children investigating asthma outcomes

We would like to ask for your permission to include your child or ward in the important study above conducted by the University of Cape Town at the request of the Department of Environmental Health and Development Planning in the Western Cape. The study will investigate the effect of air pollution and other environmental pollutants on asthma in school children. The study will be conducted during this year (2015), with a follow up into 2016. The study will benefit residents in the Western Cape exposed to air pollution and other environmental pollutants.

Participating children will be required to undergo an interview in the form of a questionnaire and produce urine and a blood sample every year during 2015-2016. A respiratory test will be included during 2015 and 2016. Additionally, later in 2016 the child will be required to undergo continuous respiratory testing every day for a two weeks period once during winter and once during summer. We would like to kindly request that your child or ward does not take any anti-asthmatic inhalers (12 hours before) or oral asthma medications (48 hours before) prior to the test. Further details on the risks of testing is detailed in the attached consent form that you must sign if you agree the participation of your child or ward in the study.

The results of the study would help in further planning in reducing environmental exposures in residential areas in the Western Cape.

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Yours sincerely

Associate Professor MA Dalvie (Principle Investigator)
Cellphone number: 0827863781

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Appendix 8: Caregiver informed consent

Consent to participate in a survey investigating asthma in school children due to exposures to ambient air pollution and other environmental pollutants in the Western Cape

1. **Title of research project**

An Epidemiological Cohort of school children investigating Asthma Outcomes Following Exposure to Ambient Air Pollution and Other Environmental Pollutions

2. **Name of researchers**

Mohamed Aqiel Dalvie (BSc, Honours, MSc, PhD)
Mohamed Jeebhay (MbChB, MMED, PhD)
Rajen Naidoo (MbChB, MMED, PhD)
Toyib Adedamola Olaniyan (BSc Hons., MSc)

Purpose of the research project

The Department of Environmental Health and Development Planning, is conducting this survey investigating the effect of air pollution and other environmental pollutants on asthma in school children. This study is necessary and important because air pollution in the Western Cape is significant and could result in health effects especially in children. In order to effectively study the health effects from air pollution, it is important to follow participants for a long time. This study is therefore very important as no previous long term study following children for three years has been done before in the Western Cape. We would like to interview you about your child's health and conduct testing on your child during this year (2015), with a follow-up into 2016. The study will benefit residents in the Western Cape exposed to air pollution and other environmental pollutants.

3. **Description of the research project**

If you agree for you and your child to participate, we will interview you once during 2015 and 2016 while your child will be undergoing two sets of tests at school during these years. The testing in the study includes:

Your interview: A member of our study team will interview you at your home for about 15 minutes once during 2015 and 2016. You will be asked questions about your child's breathing problems, your child's medication and use of health services, your current and previous employment history; smoking habit (if any); home and environment.

Set 1 testing procedure of your child at school during one day each in 2015 and 2016:

a) **Complete questionnaires:** A member of our study team will interview your child in privacy at the school for about 5 minutes. Your child will be asked about any breathing or chest problems.
b) **Breathing tests**

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- Your child will be asked to blow several times into a machine which measures how well your child's lungs are working. This test will last for about 30 minutes.

- Your child will also be asked to blow two times into a NIOX MINO machine, which measures nitric oxide produced by the airways. This machine is used to detect if a person has allergic airway inflammation which is present in asthma or rhinitis. This test will last for about 30 minutes.

c) **Urine test:** We will collect a urine sample (in privacy) in a plastic container from your child. The sample will be analyzed for pesticides.

d) **Blood test:** A nurse will draw 9 ml blood from a vein on your child's arm. The blood will be analyzed to test your child's allergy status.

Set 2 testing procedure of your child at school during summer 2016 and winter 2016 for two weeks

a) **Breathing test:** Your child will be asked to blow 3 times a day into a handheld device every day for 2 weeks each in summer and winter to measures how well your child's lungs are working. The disruption of school activities will be minimal as measurements will be taken in the morning before school starts, during the first break and then once during classes. Your child will be trained to use the device and a researcher will be present at the school to assist. This test will last for about 10 minutes each.

b) **Symptoms log:** On each day before the 2nd and 3rd breathing test, your child will be asked to complete a brief questionnaire on asthma symptoms and your child's activities during the previous 2 hours. This questionnaire will last for about 5 minutes each.

4. Risks and discomforts of the research

a) From the blood tests. A single needle stick will be felt when the blood is taken. Sometimes a small bruise may occur from the needle stick, but this is minor and will heal quickly. The total amount of blood taken is quite small and the body will quickly replace it. Blood samples will be used only to measure allergy and will be destroyed at the end of the study.

b) From the questionnaires and urine tests.

There are minimal risks associated with completing the questionnaire and from the urine test. The only risk is a loss of confidentiality about personal information but the data will be seen only by study personnel. All reports will present aggregate data in which individuals will not be identifiable.

c) From breathing tests: There is a small chance that the initial breathing tests could cause your child to become light-headed or faint. Having your child complete the test in a seated position under the observation of trained personnel greatly reduces the chance of your child having such a problem. Your child will be given medicine (salbutamol) to breathe in that works to open your child's lungs. Although very rare, this medication can briefly cause a fast heartbeat, tremor, nervousness or chest pain. A nurse knowledgeable in the treatment of such problems will be immediately available.

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6. **Expected benefits to you and to others**

You will be provided with a detailed clinical report, results and interpretation of your child's lung function test, and referral to your family practitioner or local clinic for further management if any problems are found through testing.

The results of the study would help you and others know the risks associated with various occupational and environmental pollutions for adults and children. This would further allow you to manage and/or reduce your risk. A copy of the final report of the study will be made available at the school that your child attends. Additionally, an information sheet on the risks of air pollution and how to manage these risks will be distributed to school staff and caregivers of students. A seminar on the results of the study and the managing of the risks of air pollution will be held at the school after the completion of the study.

The results obtained from this questionnaire at large would help the government of the Western Cape know the degree to which health is affected by environmental pollution. This would further help in further planning in reducing environmental exposures in residential areas.

7. **Costs to you resulting from participation in the study**

The study is offered at no cost to you. In the event a problem is discovered and you wish to be seen by a doctor for it, we can recommend to you who to see. However, the study cannot pay for these additional medical visits or treatments.

The University of Cape Town (UCT) has insurance cover for the event that research-related injury or harm results from your child's participation in the study. The insurer will pay all reasonable medical expenses in accordance with the South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI) in the event of an injury or side effect resulting directly from your participation in the study. You will not be required to prove fault on the part of the University.

The University **will not be liable** for any loss, injuries and/or harm that your child may sustain where the loss is caused by:

- The use of unauthorized medicine or substances during the study.
- Any injury that results from your child not following the protocol requirements or the instructions that the study nurse may give.
- Any injury that arises from inadequate action or lack of action to deal adequately with a side effect or reaction to the study medication.
- An injury that results from negligence on your child's part

By agreeing to participate in this study, you do not give up your right to claim compensation for injury where you can prove negligence, in separate litigation. In particular, your right to pursue such a claim in a South African court in terms of South African law must be ensured. Note, however, that you will usually be requested to accept that payment made by the

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University under the SA GCP guideline 4.11 is in full settlement of the claim relating to the medical expenses.

An injury is considered study-related if, and to the extent that, it is caused by study activities. You must notify the study nurse immediately of any side effects and/or injuries during the study, whether they are research-related or other related complications.

UCT reserves the right not to provide compensation if, and to the extent that, your child's injury came about because your child chose not to follow the instructions that your child were given while taking part in the study. Your right in law to claim compensation for injury where you prove negligence is not affected.

8. **Confidentiality of information collected**

Your and your child's name will not appear in any reports on this study. The records from the questionnaires will be kept completely confidential and will be seen only by members of the study team.

9. **Documentation of the consent**

One copy of this signed document will be kept together with our research records for this study. A copy of the information sheet about the study will be given to you to keep.

10. **Contact person**.

You may contact one of the following persons for answers to further questions about the research, your rights, or any injury you may feel is related to the study.

Name of person: MA Dalvie (The principal investigator) telephone 021 4066610
Name of person: Lamees Emjedi (Ethics administrator) telephone 021 4066492

11. **Voluntary nature of participation**

You and your child's participation in this project is voluntary. Subsequent to your consent, you and/or your child may refuse to participate in or withdraw from the study at any time without penalty or loss of benefits to which you may otherwise be entitled.

9. **Consent of the participant**

I have read the information given above, or it has been read to me. I understand the meaning of this information. By signing this form, I hereby consent for me and my child to participate in the study. I also understand that I am free to withdraw myself and my child from the study at any time without penalty.

_____ _____

Printed name of caregiver Signature, Mark, or Thumb Print

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Interviewer's name (Print)	Signature
Witness (Print) (If caregiver is illiterate)	Signature

DATE: _____

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Appendix 9: Child assessment form

1. Title of research

An Epidemiological Cohort of Adult investigating Cardiopulmonary Outcomes Following Exposure to Ambient Air Pollution and Other Environmental Pollutions

2. **Name of researchers**

Mohamed Aqiel Dalvie (BSc, Honours, MSc, PhD)
Mohamed Jeebhay (MbChB, MMED, PhD)
Rajen Naidoo (MbChB, MMED, PhD)
Toyib Adedamola Olaniyan (BSc Hons., MSc)

Purpose of the research project

The Department of Environmental Health and Development Planning, is conducting this survey investigating the effect of air pollution and other environmental pollutants on asthma in school children and cardiopulmonary health in adults. We would like to interview you about your own respiratory health and conduct testing on you during this year (2015), with a follow-up into 2016. The study will benefit residents in the Western Cape exposed to air pollution and other environmental pollutants.

3. **Description of the research project**

You will be required to do a respiratory test, produce urine and a blood sample and complete a questionnaire on one day each in 2015 and 2016. Also in 2016 you will be required to do a continuous respiratory testing every day for a two weeks period once during winter and once during summer. The testing will include:

a) **Questionnaires:** A member of our study team will interview you in privacy to complete a questionnaire. You will be asked about any breathing or chest problems.
b) **Respiratory test:** A nurse will perform a respiratory test on you.
c) **Urine sample:** You will produce a urine sample (in privacy) in a plastic container and give it to the nurse. The sample will be analyzed for pesticides.
d) **Blood sample:** A nurse will draw 9 ml blood from a vein on your arm. The blood will be analyzed to test your allergy status.

5. Risks and discomforts of the research

d) From the blood tests. A single needle stick will be felt when the blood is taken. Sometimes a small bruise may occur from the needle stick, but this is minor and will heal quickly. The total amount of blood taken is quite small and the body will quickly replace it. Blood samples will be used only to measure allergy and will be destroyed at the end of the study.

e) From the questionnaire.

There are minimal risks associated with completing the questionnaire. The only risk is a loss of confidentiality about personal information but the data will be seen only by study personnel. All reports will present aggregate data in which individuals will not be identifiable.

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6. **Expected benefits to you and to others**

The results of the study would help you and others know the risks associated with various occupational and environmental pollutions for adults and children. This would further allow you to manage and/or reduce your risk. The results obtained from this questionnaire at large would help the government of the Western Cape know the degree to which health is affected by environmental pollution. This would further help in further planning in reducing environmental exposures in residential areas.

7. **Costs to you resulting from participation in the study**

The study is offered at no cost to you. In the event a problem is discovered and you wish to be seen by a doctor for it, we can recommend to you who to see. However, the study cannot pay for these additional medical visits or treatments.

9. **Confidentiality of information collected**

Your name will not appear in any reports on this study. The records from the questionnaires will be kept completely confidential and will be seen only by members of the study team.

9. **Documentation of the consent**

One copy of this signed document will be kept together with our research records for this study. A copy of the information sheet about the study will be given to you to take home.

10. **Contact person**.

You may contact one of the following persons for answers to further questions about the research, your rights, or any injury you may feel is related to the study.

Name of person: MA Dalvie (The principal investigator) telephone 021 4066610
Name of person: Lamees Emjedi (Ethics administrator) telephone 021 4066492

11. **Voluntary nature of participation**

Your participation in this project is voluntary. Subsequent to your consent, you may refuse to participate in or withdraw from the study at any time without penalty or loss of benefits to which you may otherwise be entitled.

12. **Consent of the participant**

I have read the information given above, or it has been read to me. I understand the meaning of this information. By signing this form, I hereby consent for me to participate in the study. I also understand that I am free to withdraw myself from the study at any time without penalty.

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_____	_____
Printed name of participant	Signature, Mark, or Thumb Print
_____	_____
Interviewer's name (Print)	Signature
_____	_____
Witness (Print) (If care giver is illiterate)	Signature

DATE: _____

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Appendix 10: Instructions for authors

Environmental Pollution - Guide for authors

Introduction

Before you begin

Preparation

After acceptance

Author inquiries

Your Paper Your Way

We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article.
To find out more, please visit the Preparation section below.

Introduction

Environmental Pollution is an international peer-reviewed journal that publishes high quality research papers and review articles about all aspects of environmental pollution and its effects on ecosystems and human health. The journal welcomes high-quality **process-oriented and hypothesis-based** submissions that report results from original and novel research and contribute new knowledge to help address problems related to environmental pollution at a regional or global scale.

Subject areas include, but are not limited to:
• Sources and occurrences of pollutants that are clearly defined and measured in **environmental compartments, food and food-related items, and human bodies**;
• Interlinks between **contaminant exposure** and biological, ecological, and human health effects, including those of climate change;
• Contaminants of **emerging concerns** (including but not limited to antibiotic resistant microorganisms or genes, microplastics/nanoplastics, electronic wastes, light, and noise) and/or

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their biological, ecological, or human health effects;
• Laboratory and field studies on the remediation/mitigation of environmental pollution **via new techniques and with clear links to biological, ecological, or human health effects**;
• **Modeling of pollution processes, patterns, or trends** that is of clear environmental and/or human health interest;
• **New techniques** that measure and examine environmental occurrences, transport, behavior, and effects of pollutants within the environment or the laboratory, provided that they can be clearly used to address problems within **regional or global environmental compartments**.

Papers focusing on the following areas are likely to be returned to the authors without review:
• Routine surveys or monitoring programs primarily of local or regional interest;
• Descriptions of well-known contaminants, such as legacy pollutants, in yet another location;
• Studies relating to waste treatment that do not have specific relevance to pollution within the environment;
• Synthesis/fabrication of new materials solely for remediation and/or mitigation of pollution without any direct environmental relevance;
• Nitrogen or phosphorus deposition or biogeochemical processes with little or no relation to environmental consequences and/or climate change;
• Studies on eutrophication and secondary pollution by eutrophication without illuminating their governing mechanisms and factors;
• Studies within which the concentrations of toxicants used are higher than those that are typically found in an environmental pollution context. Authors of toxicology studies must justify the concentrations that they are using by reference to environmentally relevant concentrations that have been reported in the literature.

Please DO NOT ask the Editors-in-Chief for permission before submitting a manuscript. Kindly check the guidelines to determine whether your manuscript is within the scope of the journal; if yes, please go ahead and submit it.

The resubmission of manuscripts previously rejected by the journal is by invitation only.

Types of article

A covering letter is required for all article types. This must explicitly express how the submission fits the Aims and Scope of *Environmental Pollution*, and should establish the ramifications of the research findings with regards to environmental quality, ecological health, and/or human health.

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Failure to include such justifications in the cover letter may result in returning the paper to the author.

Research Papers: Full Research Papers should not exceed 8000 words (including abstract, figures, and tables but excluding references). Please note that small tables and figures each count as 300 words, and large tables or figures with multiple panels may count for 600 or more words. There should be no more than nine figures and tables (e.g., 5 figures and 4 tables maximum) in the main text. Any additional figures and tables should be placed in Supplementary Material.

The abstract (up to 300 words), highlights and conclusions of papers in this journal must contain clear and concise statements. A graphical abstract is mandatory.

Review Articles: Authors may submit manuscripts that provide in-depth critical review of a special subject. These reviews must provide a Synthesis and Critical Evaluation of the state of the knowledge of the subject and indicate research directions. The Editors also periodically invite review articles. Manuscripts should not exceed 10,000 words, as defined above.

We also encourage Systematic Reviews that provide a comprehensive summary and critical appraisal of existing evidence. They relate to answering a research question, and are conducted using methods which seek to minimize risk of bias in results and conclusions. A systematic review should include a meta-analysis, which uses statistical techniques to pool the results of multiple individual studies into a combined summary result.

Short Communication: Short Communications may be submitted that express opinions and concerns, suggest research priorities and question conventional methodologies and conclusions. Manuscripts should include an Abstract, Introduction, Presentation of the Concerns or Analysis and Conclusions. References, Tables and Illustrations should be used sparingly. The manuscript should not exceed 12 double-spaced pages. The Editors will evaluate all manuscripts for suitability of publication, including peer review.

Correspondence: Readers are encouraged to write to any of the Editors (Letter to Editor) and raise issues and concerns about papers published in the journal. Editors or authors will be invited to submit a **Reply** to respond to points raised. The Editor will decide on the publication of Correspondence and Replies based on scientific merit, importance to the raised issues, and interest to the general audience. Correspondence and Replies of an unprofessional or unscientific nature, or containing personal invective, will not be considered.

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Corrigendum: Authors may submit Corrigenda to alert the readership to errors discovered in their articles post-publication.

Special Issues: Proposals for Special Issues of Full Research Papers that focus on a specific topic or theme will also be considered. Special Issues will be published on emerging thematic issues and innovative conferences. An Editor or Associate Editor should be contacted early in the conference planning process to get approval and for guidelines on special issues of the journal. Furthermore, the Editors or Associate Editors will invite leading experts as Guest Editors for Special Issues. **Editorials** for Special issues are submitted by invitation only through the Editorial Manager portal.

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
• E-mail address
• Full postal address

All necessary files have been uploaded:
Manuscript:
• Include keywords
• All figures (include relevant captions)
• All tables (including titles, description, footnotes)
• Ensure all figure and table citations in the text match the files provided
• Indicate clearly if color should be used for any figures in print
Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations
• Manuscript has been 'spell checked' and 'grammar checked'
• All references mentioned in the Reference List are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Internet)

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• A competing interests statement is provided, even if the authors have no competing interests to declare
• Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

Finally...

As mentioned above, a covering letter must be sent with the manuscript and must include:
•The novelty, scientific significance and importance of the manuscript.
•The name and e-mail address [institutional email addresses ONLY] of the corresponding author.
•The resubmission of manuscripts previously rejected by the journal is by invitation only.

Before you begin

Ethics in publishing

Please see our information on Ethics in publishing.

Studies in humans and animals

If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The manuscript should be in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. The terms sex and gender should be used correctly.

The author should ensure that the manuscript contains a statement that all procedures were performed in compliance with relevant laws and institutional guidelines and have been approved by the appropriate institutional committee(s). This statement should contain the date and reference number of the ethical approval(s) obtained. Authors should also include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

The journal will not accept manuscripts that contain data derived from unethically sourced organs or tissue, including from executed prisoners or prisoners of conscience, consistent with recommendations by Global Rights Compliance on Mitigating Human Rights Risks in

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Transplantation Medicine. For all studies that use human organs or tissues authors must provide sufficient evidence that they were procured in line with WHO Guiding Principles on Human Cell, Tissue and Organ Transplantation. The source of the organs or tissues used in clinical research must be transparent and traceable. Authors of manuscripts describing organ transplantation must additionally declare within the manuscript:

Declaration of competing interest

Corresponding authors, on behalf of all the authors of a submission, must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. All authors, including those *without* competing interests to declare, should provide the relevant information to the corresponding author (which, where relevant, may specify they have nothing to declare). Corresponding authors should then use this tool to create a shared statement and upload to the submission system at the Attach Files step. **Please do not convert the .docx template to another file type. Author signatures are not required.**

Declaration of generative AI in scientific writing

The below guidance only refers to the writing process, and not to the use of AI tools to analyse and draw insights from data as part of the research process.

Where authors use generative artificial intelligence (AI) and AI-assisted technologies in the writing process, authors should only use these technologies to improve readability and language. Applying the technology should be done with human oversight and control, and authors should carefully review and edit the result, as AI can generate authoritative-sounding output that can be incorrect, incomplete or biased. AI and AI-assisted technologies should not be listed as an author or co-author, or be cited as an author. Authorship implies responsibilities and tasks that can only be attributed to and performed by humans, as outlined in Elsevier's AI policy for authors.

Authors should disclose in their manuscript the use of AI and AI-assisted technologies in the writing process by following the instructions below. A statement will appear in the published work. Please note that authors are ultimately responsible and accountable for the contents of the work.

Disclosure instructions

Authors must disclose the use of generative AI and AI-assisted technologies in the writing process

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by adding a statement at the end of their manuscript in the core manuscript file, before the References list. The statement should be placed in a new section entitled ‘Declaration of Generative AI and AI-assisted technologies in the writing process’.

Statement: During the preparation of this work the author(s) used [NAME TOOL / SERVICE] in order to [REASON]. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

This declaration does not apply to the use of basic tools for checking grammar, spelling, references etc. If there is nothing to disclose, there is no need to add a statement.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify compliance, your article may be checked by Crossref Similarity Check and other originality or duplicate checking software.

The editors take ethics seriously and check the CrossCheck reports for ALL incoming submissions. Any evidence of excessive overlap with previously published papers could lead to instant rejection for offending authors and the possibility of additional, more severe sanctions in the worst cases.

Preprints

Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Preprint posting on SSRN

In support of Open Science, this journal offers its authors a free preprint posting service. Preprints provide early registration and dissemination of your research, which facilitates early citations and collaboration.

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During submission to Editorial Manager, you can choose to release your manuscript publicly as a preprint on the preprint server SSRN once it enters peer-review with the journal. Your choice will have no effect on the editorial process or outcome with the journal. Please note that the corresponding author is expected to seek approval from all co-authors before agreeing to release the manuscript publicly on SSRN.

You will be notified via email when your preprint is posted online and a Digital Object Identifier (DOI) is assigned. Your preprint will remain globally available free to read whether the journal accepts or rejects your manuscript.

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