

**Effects of Air Pollution on the Respiratory
Health and the Respiratory Immune System.
Studies in Ecuadorian Children**

Bertha Estrella Cahueñas



The studies described in the thesis were performed at the Department of Immunology, Central University of Ecuador, Quito, Ecuador in collaboration with the institutes indicated in the various chapters.

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**Effects of Air Pollution on the Respiratory
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Studies in Ecuadorian Children**

**Effecten van luchtvervuiling op de gezondheid van de
luchtwegen en het immuunsysteem van de luchtwegen.
Studies onder Ecuadoraanse kinderen**

Thesis

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To my Mother, my Sisters, and my Brothers here on the earth, and to my Father and Pepi in the infinity.

Para mi Madre, mis Hermanas y Hermanos aquí en la tierra y para mi Padre y Pepi en el infinito.

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Chapter 1

Introduction

INTRODUCTION

This thesis addresses the effect of certain environmental pollutants on respiratory health of Ecuadorian children, and studies the influence of public policy aimed at controlling the vehicular emissions in Quito on respiratory health. The investigations are based on studies carried out at both the community level and in a hospital setting in Quito, Ecuador.

This introduction provides a general view of air pollution and respiratory illnesses in Quito; evidence on the effects of several pollutants on respiratory health with an emphasis on the innate immune response; and finally an overview of potential solutions and measures to improve air quality.

AIR POLLUTION CONDITIONS AND RESPIRATORY INFECTIONS IN QUITO, ECUADOR

Air pollution conditions

Quito, the capital of Ecuador is a developing city with ~ 2.700.000 inhabitants of relative homogeneous ethnicity (1). The city is situated on the equatorial line on average 2828 meters above sea level in the Andean Region. While the general climate of Ecuador is hot and subtropical, the climate of the city of Quito is defined by its mountainous location. The monthly temperatures are stable throughout the year averaging 15°C (59°F) (2) and the seasons are defined by precipitation. The dry (summer) season generally extends from June to September, with the rest of the months reserved for rainy (winter) season (2). The city sits on a long, narrow valley and is surrounded by high mountains that impede wind flow that could disperse pollutants, causing them to remain in the environment for a long time. The altitude of the city results in greater solar radiation that photochemically transforms the pollutants into oxidants (3). Its topography also favors thermal inversions where a hot air roof traps and concentrates pollutants within the city. Furthermore, some of the surrounding mountains are active volcanoes (Guagua Pichincha and Reventador) that from time to time intensify their activity with the emission of large amounts of volcanic ash. For example, In October of 1999 and April of 2000 the stratovolcano Guagua Pichincha located 13 km west of Quito became active after 340 years of dormancy. Concluding, due to its geographic location, topography, and climatology, Quito is very vulnerable to stagnant air and high air pollution.

In addition, the rapid urban development of the city in the last two decades has deteriorated the quality of the air due to an increase of pollutants from industry, deforestation, and vehicle exhaust. It is important to mention that vehicle exhaust is the most significant source of pollutants in Ecuador. In fact, the number of circulating vehicles in Quito has increased by 7% per year or ~ 30,000 vehicles per year between 1998 and 2014 (4), and that residential

population has grown in the peripheral areas. This growing number of commuters entails a steady increase in heavy vehicular circulation, especially public transportation buses that run on diesel (5) are making Quito the most polluted city in Ecuador.

No routine regular monitoring of air pollution was conducted in Quito before 2004. Based on ad hoc official report, ambient carbon monoxide (CO) concentration levels in five consecutive months (August to December) of the years 2001 and 2002 were higher than those in 2003. In 2002, such concentrations exceeded the national standard (Figure 1) (6).

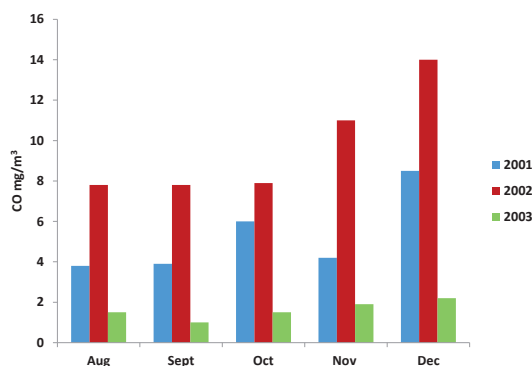


Figure 1. Annual comparison of average concentrations of 8 h carbon monoxide (CO), Quito

The monitoring system started in 2004 through the Metropolitan Atmospheric Monitoring Network of Quito (REMMAQ). It consists of eight online and one additional backup station. These stations are equipped with automatic analyzers for particulate matter (PM)_{2.5}, carbon monoxide (CO), Ozone (O₃), nitric oxide (NO_x), and sulphur dioxide (SO₂) with the capacity to operate permanently and continuously 24 hours a day, 365 days a year (7). Between 2004 and 2017, the annual averages of PM_{2.5}, CO, O₃, and NO₂, show a downward trend with measures below the national standard for all except PM_{2.5} (Table 1) (8). The high levels of PM_{2.5} could be related to an increase in the number of cars in Quito during that period since vehicular exhaust is the principal source of this pollutant.

Within this situation, the residents of Quito, especially children, may be prone to multiple health problems including respiratory illness.

Respiratory infections in Ecuadorian children related to air pollution

One of the main public health problems globally is environmental air pollution, which has been gradually deteriorating air quality in most cities of the world. Air quality deterioration is due to the rapid urbanization and industrialization of cities, forest fires (9), volcanic eruptions (10), and particularly the increase of circulating vehicles (11, 12), which produce irritating substances and toxic gases.

Table 1. Annual average concentration of pollutants in Quito 2004 and 2017

Ambient Pollutant	2004	2017	National standard
CO (mg/m ³)*	5.5	2.4	10
PM _{2.5} (ug/m ³)**	22.5	17	15
O ₃ (ug/m ³)*	29.5	27	40
NO ₂ (ug/m ³)**	26	23	40

*annual octo-hour average

**annual average

According to the World Health Organization (WHO), globally 570,000 children under the age of five die every year as a result of respiratory infections caused by indoor and outdoor air pollution and exposure to environmental tobacco smoke (13). Although in Ecuador respiratory infections, mainly pneumonia, remain the leading cause of morbidity and death in young children (14, 15), accurate data on the effects of air pollution on respiratory infections in children are scarce.

In this thesis we present evidence on the relationship between exposure to different air pollutants and the presence of respiratory infection in children of Quito. In chapter 2 we compare the incidence of acute respiratory infections (ARIs) in school-age children living in three communities that differ in traffic intensity in urban and suburban areas of Quito, and examine the relationship between ARI occurrence and individual carboxyhemoglobin (COHb) concentrations, as a measure of CO exposure. In chapter 3 we document the rates of emergency room visits for acute upper and lower respiratory infections and asthma-related conditions in children living in Quito, Ecuador associated with the eruption of the Guagua Pichincha volcano in April of 2000. In chapter 4, we assess the simultaneous effects of traffic air pollution and anemia as risk factors for pneumonia in Ecuadorian children.

AIR POLLUTION AND RESPIRATORY SYSTEM: IMMUNOLOGICAL ASPECTS

There is accumulating evidence to suggest that people chronically exposed to gaseous pollutants (CO, SO₂, O₃, and NO_x) and PM have a higher risk of developing respiratory infections (16-20), and asthma (21-24). These respiratory conditions may be mediated through effects of air pollutants on different immune lung cells, among them a novel group of cells named the innate lymphoid cells (ILCs).

Although ILCs play an important role in pathogen containment and airway hyperresponsiveness, the effects of air pollutants on them are poorly understood. In chapter 5 of this thesis we conduct a systematic review to evaluate available evidence on the effect

of the major air pollutants on the lung ILC subsets, specifically type 1 (ILC1) and type 2 ILCs (ILC2).

In order to a better understanding of how pollutants affect other lung immune cells in human, a brief review is presented below:

Particulate matter

Particulate matter is a complex and heterogeneous mixture of solid particles and liquid matters suspended in the atmosphere. Its composition varies according to region, season, and time (25, 26). The most deleterious are the smallest inhalable particles capable of depositing throughout the airways up to the alveoli. Inhaled particles with diameters between 2.5 and 10 micrometers (μm) (PM_{10}) are deposited mainly in the nose and large conducting airways; fine particles (0.1-2.5 μm) with a diameter of 2.5 μm , but larger than 0.1 ($\text{PM}_{2.5}$) can reach the small airways and alveoli; and ultrafine PM (UFPM) fraction $\text{PM}_{0.1}$ (particles < 0.1 μm) can deposit in the pulmonary tissue and even translocate from the alveoli to the pulmonary circulation (27). All of these particles have been associated with impairment of the innate immune respiratory system in humans, with effects varying across size, mass, composition of the particles. Exposure to PM alters the function of several lung innate immune cells, as outlined in more detail below and in figure 2.

- **Effects on lung epithelial cells (EC):** PM exposure causes oxidative stress and activation of multiple cell death pathways (28), deregulates the ability to express the antimicrobial peptides human β -defensin 2 (HBD-2) and HBD-3 (29), increases interleukin (IL)-6 and IL-8 production (30, 31) as well as matrix metalloproteinase (MMP)-9 and cyclooxygenase (COX)-2 (31). More recently, it has been shown that $\text{PM}_{2.5}$ exposure alters the mitochondrial structure, including mitochondrial dynamic, DNA biogenesis and morphological alteration of bronchial epithelial cells via reactive oxygen species (ROS) formation (32).

- **Effects on Alveolar macrophages (AMs):** AMs are activated by PM to produce increased proinflammatory cytokines (IL-6 and $\text{TNF-}\alpha$), and to generate a pro-oxidant state (33-35). Paradoxically, these particles have also been reported to lower the capacity of pulmonary macrophages to secrete IL-6 and interferon beta ($\text{IFN-}\beta$) (36) as well as IL-8, $\text{TNF-}\alpha$ and PGE_2 (37). In addition, a decrease in cell viability (33, 38), suppression of phagocytic activity (39) and reduction of antibacterial function (40) have been reported.

- **Effects on Dendritic cells (DC):** Human DCs exposed to PM improve their maturation and activation (41); increase cell-surface expression of co-stimulatory molecules (CD40), major histocompatibility complex (MHC) class II, and vascular endothelial growth factor (VEGF) (42); and enhance the production of $\text{TNF-}\alpha$, IL-6, $\text{IFN-}\gamma$, IL-12 (41, 42), IL-13, Granzyme A and Granzyme B (41).

- **Effects on adaptive lymphocytes (AL):** PM from diesel exhaust can act directly on T Helper (Th) 1 reducing cytokine production, and inducing autophagic lysosomal blockage and

mitochondrial membrane perturbations (43). PM also affects AL indirectly through activation of DC and macrophages. For example, PM-activated DCs produce great quantity of IL-6 which enhance CD4⁺ T cell proliferation with low IFN- γ secretion (44). In addition, DCs enhance priming of naive CD8⁺ T lymphocytes, and induce resting memory CD4 T cells to secrete IFN- γ and IL-13, and to expand into Th1, Th2, and Th17 inflammatory effector cells (45). These Th17 cells co-express IL-17A with IFN- γ , GM-CSF, and granzyme B (46). PM-exposed macrophages induce human CD4⁺ and CD8⁺ T cells to upregulate protein levels of interferon IFN- γ , interleukin IL-10, IL-17, and IL-21 production as well as granzyme A and granzyme B expression (47). In the reviewed literature, no studies on the effect of pollutants on B lymphocytes were found.

Carbon monoxide

Carbon monoxide (CO) is a gas that results from incomplete combustion whenever carbon-containing material is burning (48). In general, high CO concentrations result from natural events such as volcanic eruptions or wild forest fires (49). In urban settings, high CO concentrations primarily occur due to incomplete fuel combustion and in areas with heavy traffic congestion. The main indoor sources of environmental CO are smoking and domestic fuel combustion with inadequate stoves and furnace ventilation (48). A direct effect of high CO exposure on innate immune cells has not been reported, but it is known that CO binds tightly to hemoglobin to form Carboxyhemoglobin (COHb), a compound that prevents hemoglobin from delivering oxygen throughout the body (50), which causes several effects on subsets of innate immune cells (Figure 2).

- **Effects on macrophages and lung epithelial cells:** A hypoxic environment has been associated with an inhibitory effect on the recruitment, differentiation, and phagocytic activity of AMs (51, 52) as well as with the upregulation of monocyte/macrophage pro-inflammatory responses (53, 54). In addition, hypoxia induces the alveolar epithelium to increase surfactant production, causes disruption of cytoskeleton integrity, and triggers apoptosis in these cells (55).

- **Effects on Dendritic cells:** It has been reported that hypoxic immature DCs exhibit low bacterial phagocytosis and increased migratory capacity towards secondary lymphoid organs (56), and that hypoxic mature DC showed raised gene expression of pro-inflammatory cytokines and chemokines (57).

Ozone

Ozone (O₃) is a pale blue reactive gas comprised of three oxygen atoms. It is found naturally in the earth's stratosphere where it creates a protective layer that shields the planet from UV rays. However, Ozone can also occur at ground level. This tropospheric ozone is not emitted naturally into the air but rather created by chemical reactions between oxides

of nitrogen (NO_x), volatile organic compounds (VOCs) and CO in the presence of sunlight (58, 59). This newly-formed tropospheric ozone can reach the lining of the respiratory tract and react with several molecules causing inflammation (Figure 2).

- **Effects on epithelial cells:** The principal harmful effects of O₃ is the disruption of the airway epithelial integrity (60) which facilitates access of different external antigens, and the impairment of microbial phagocytosis (61). On human bronchial epithelial cells O₃ induces an increased expression of inflammatory marker genes (IL-8 and COX-2) (62), and production of IL-8, IL-6, IL-1 α , IL-1 β (63-67), TNF- α , E-selectin and PGE2 (68). O₃ exposure also induces oxidative stress (64), DNA damage, apoptosis and cytotoxicity of epithelial cells (69). Furthermore, O₃ exposed bronchial epithelial cells play an important role in driving signals in macrophages to increase markers of alternative activation, enhance cytotoxicity, and reduce phagocytosis (70).

- **Effects on phagocytic cells:** Human alveolar macrophages exposed to O₃ exhibit impaired phagocytosis, superoxide production, and increased levels of pro-inflammatory cytokines (61, 70, 71). Ozone also induces neutrophilic inflammation of the lower airways but with defective neutrophil phagocytosis, intracellular killing and production of superoxide radicals (61). High levels of polymorphonuclear cells (PMN), elevated expression of IL-1 β and IL-8, increased innate immune function and minimal activation of the immune cell trafficking pathways are seen in responders to O₃, while opposite changes are seen in non-responders (72).

- **Effects on Lymphocytes:** Little has been published on the effect of O₃ exposure on human lymphocytes. One study showed that lymphocyte numbers of human bronchoalveolar (BAL) fluid decreases by 3.1-fold after ozone exposure compared to air exposure (73) while other found suppression of IgG production in O₃-exposed mitogen-stimulated lymphocytes (74).

Nitric oxide

Nitrogen is released during fuel combustion and it combines with oxygen to create nitric oxide (NO), which in a new reaction with oxygen forms nitrogen dioxide (NO₂) (75). In areas of high motor vehicle traffic, such as in large cities, the amount of nitrogen oxides emitted into the atmosphere as air pollution can be significant. Similarly to CO, NO_x (particularly NO), reduces oxygen saturation through the formation of methemoglobin (76, 77). While high concentration of NO₂ is considered to cause inflammation of the airways (78, 79), few reports demonstrate its effects on lung innate immune cells. For example, it has been shown that NO₂ interacts with the lung epithelial fluid and epithelial cell membranes to produce reactive oxygen and nitrogen species (80). The epithelial cells exposed to NO₂ undergo apoptotic cell death at an early stage and exhibit cell membrane damage at a later time as well as changes in expression levels of adhesion molecules with the consequent increase of cell interaction with PMN (81). It has been described that the percentage of macrophages in

sputum increases depending on the exposure level, but no changes in cytokine production have been seen (82).

Volcanic emissions

The composition of volcanic emissions varies depending on the geochemistry of the individual volcano. Nevertheless, in general, they contain a mixture of fine particle matter of pulverized rock (mainly silica, aluminum, and iron) and superheated gases (mainly water vapor, CO₂, and SO₂) (83).

- **Effects on epithelial cells:** Volcanic gases, especially SO₂, induce the respiratory epithelium to produce mucus and secrete the pro-inflammatory cytokine IL-13 (84). Volcanic ash induces human alveolar type-1 like epithelial cells (TT1) to release MCP-1, IL-6, and IL-8, produces acute cytotoxicity of those cells (85), but does not induce alveolar epithelial cell death even at high concentrations (86).

- **Effects on macrophages:** Human AMs exposed to volcanic ashes increase the release of lactate dehydrogenase, β -glucuronidase, and β -N-acetylglucosaminidase enzymes (87); decrease production of TNF- α mRNA with a subsequent decrease of killing ability (86). Additionally, volcanic ashes activate the NLRP3 Inflammasome (88), induce cytotoxicity of AMs (87), and interfere with the autophagy process (86). Furthermore, volcanic ash has been proved to be highly reactive in the lung since the fracture of crystalline silica produces surface radicals and also can generate reactive oxygen species (ROS) in aqueous suspensions such as the superoxide radical (O₂⁻) and hydroxyl radical (HO⁻), but the main cause of reactivity is the removable divalent iron (Fe²⁺), which is present in abundance on the surfaces of the particles (89).

POTENTIAL SOLUTION TO IMPROVE AIR QUALITY

According to WHO, ambient air pollution affects developed and developing countries alike, but low- and middle-income countries experience the highest burden of disease (90). Mortality and morbidity related to environmental exposures could be diminished if countries reduce the principal sources of ambient pollution through the establishment of regulatory policies and strong investments in energy efficacy projects or sustainable practices; surely, it is most likely that developed countries could meet this goal sooner than developing ones. In developed countries, limited trials are demonstrating a benefit of air pollution reduction policies, and other actions implemented to improve air quality on the respiratory health of children. Overall, declining trends in several air pollutants were associated with large reductions in respiratory infections and asthma cases (91-93), as well as with an improvement

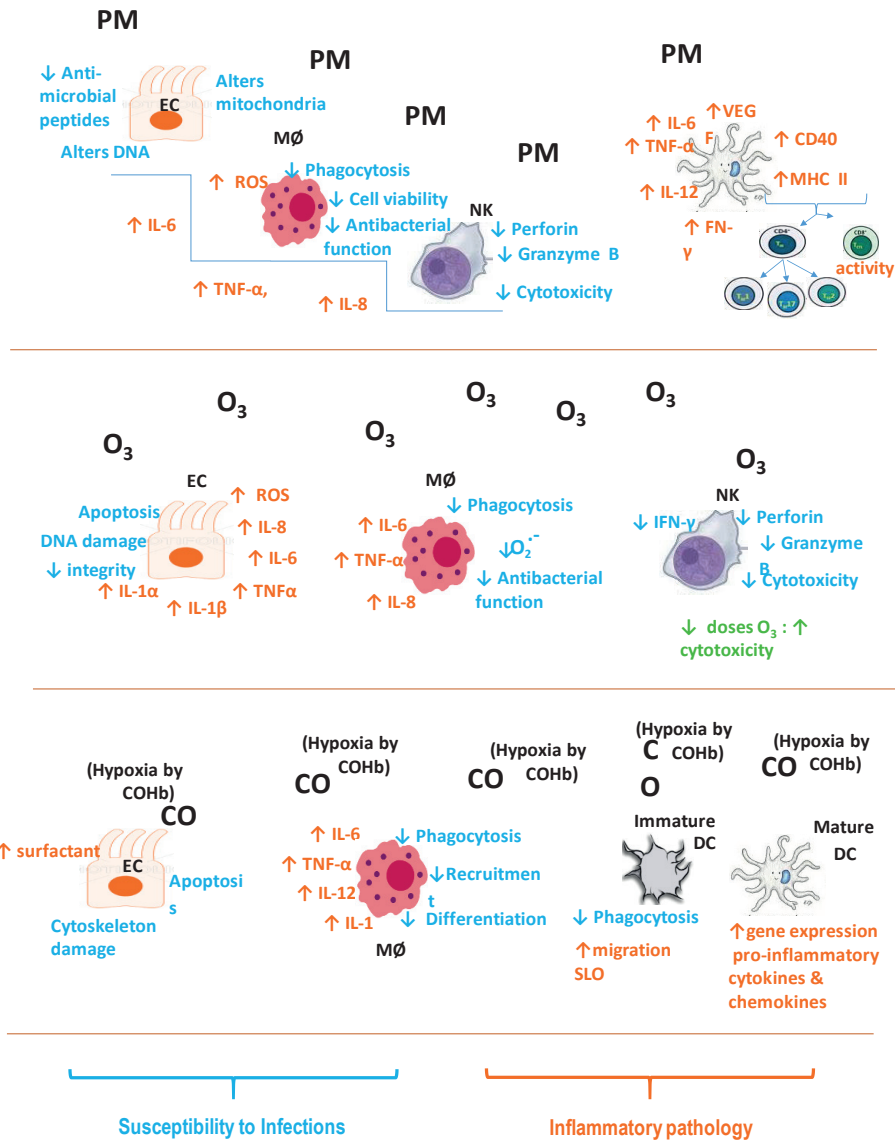


Figure 2. Effects of main contaminants on immune cells.

Particulate Matter (PM), Ozone (O₃), and carbon monoxide (CO) through carboxyhemoglobin (COHb) formation decrease some functions (in blue) and increase others (in orange) of epithelial cells (EC), macrophages (Mφ), natural killer cells (NK) and dendritic cells (DC). Decreased phagocytosis, and antibacterial function of Mφ, DC, and EC, and cytotoxic function of NK result in susceptibility to infections. Inflammatory pathology is due to increased cytokines (IL-1, IL-6, IL-8, IL-12, TNF-α), chemokines (IL-8), and reactive oxygen species (ROS) production. Increased activation of DC results in CD4⁺ T cell activation and differentiation.

of lung function (94-96) among children. However, other studies did not show definitive effects of air pollution regulation on respiratory illnesses in children (97, 98).

Although some Latin American countries have established air pollution abatement policies, there are no studies evaluating the impact on respiratory health in children. In Quito, the first policy for air pollution abatement started in 2002 and consisted in controlling the emission of exhaust gas from gasoline engine vehicles, and the gradual removal of outdated carburetor-containing vehicles from the circulation; however, its effectiveness has not been assessed.

We aimed to evaluate the effect of air pollution control on the occurrence of acute respiratory illnesses in school children five years later by comparing two studies conducted at the same location in Quito. Findings from this study are presented in chapter 6 of this thesis.

THE AIMS OF THIS THESIS

In summary the aims of this thesis are:

1. To study the relation between carbon monoxide exposure and respiratory diseases in children.
2. To document the elevated rates of emergency room visits for acute upper and lower respiratory infections and asthma-related conditions in children living in Quito, Ecuador associated with the eruption of the Guagua Pichincha volcano in April of 2000.
3. To assess the simultaneous effects of traffic air pollution and anemia as risk factors for pneumonia in Ecuadorian children.
4. To conduct a systematic review to evaluate available evidence on the effect of the major air pollutants on the lung ILC subsets, specifically type 1 (ILC1) and type 2 ILCs (ILC2).
5. To evaluate the effect of a citywide 5-year air pollution control program on the occurrence of acute respiratory illness in children.

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Chapter 2

Acute respiratory diseases and carboxyhemoglobin status in school children of Quito, Ecuador

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ABSTRACT

Outdoor carbon monoxide comes mainly from vehicular emissions, and high concentrations occur in areas with heavy traffic congestion. CO binds to hemoglobin, forming carboxyhemoglobin (COHb), and reduces oxygen delivery. We investigated the link between the adverse effects of CO on the respiratory system using COHb as a marker for chronic CO exposure. We examined the relationship between acute respiratory infections (ARIs) and COHb concentrations in school-age children living in urban and suburban areas of Quito, Ecuador. We selected three schools located in areas with different traffic intensities and enrolled 960 children. To adjust for potential confounders we conducted a detailed survey. In a random subsample of 295 children, we determined that average COHb concentrations were significantly higher in children attending schools in areas with high and moderate traffic, compared with the low-traffic area. The percentage of children with COHb concentrations above the safe level of 2.5% were 1, 43, and 92% in low-, moderate-, and high-traffic areas, respectively. Children with COHb above the safe level are 3.25 (95% confidence interval (CI), 1.65–6.38) times more likely to have ARI than children with COHb < 2.5%. Furthermore, with each percent increase in COHb above the safety level, children are 1.15 (95% CI, 1.03–1.28) times more likely to have an additional case of ARI. Our findings provide strong evidence of the relation between CO exposure and susceptibility to respiratory infections.

Keywords: acute respiratory infections, carbon monoxide exposure, carboxyhemoglobin, children, Ecuador, traffic-related pollution.

INTRODUCTION

Numerous studies have found a strong association between respiratory illness and exposure to traffic-related air pollution (Choudhury et al. 1997; Hajat et al. 2002; Polosa et al. 2002; Romieu et al. 2002; Shamsiiarov et al. 2002; Spinaci et al. 1985). Traffic-related nitrogen monoxide, nitrogen dioxide, black fumes, and ammonia particulate have been linked to an increase in respiratory symptoms and a decrease in pulmonary function in school-age children (Boussin et al. 1990; Keiding et al. 1995; Lercher et al. 1995; Lwebuga-Mukasa et al. 2003; Quian et al. 2000; Steerenberg et al. 2001; van Vliet et al. 1997; Wjst et al. 1993; Yang et al. 1998, 2002). Carbon monoxide, a toxic product of incomplete combustion, can also impair respiratory function. High CO concentrations may occur in areas with heavy traffic congestion, especially in urban settings with insufficient emission regulation. The main indoor sources of environmental CO are smoking and domestic fuel combustion with inadequate stoves and furnace ventilation (Collings et al. 1990; Kleinman 2000; Puente-Maestu et al. 1998). Although the physiology and adverse effects of acute CO poisoning on the respiratory system are well documented, very few studies have been conducted to understand the effects of chronic low-dose CO exposures on susceptibility to respiratory infections.

Carboxyhemoglobin (COHb), a marker for CO exposure, reflects the binding of CO to the hem portion of hemoglobin capturing oxygen. A concentration of COHb < 2.5% is currently considered safe (Kleinman 2000). The lowest level of COHb, at which adverse effects are observed, ranges from 2.9 to 3% (U.S. Environmental Protection Agency (EPA) 2000). COHb concentrations of 5–10% serve as an indicator of acute CO poisoning and are associated with impaired visual function, task performance, and maintaining alertness (Raub and Benignus 2002). Even a relatively low CO exposure may increase COHb levels in human peripheral blood (Raub and Benignus 2002). Higher levels of COHb have been observed in smokers compared with nonsmokers (Behera et al. 1991). In addition, children living in households with smokers or wood/coal/gas heating systems exhibit slight increases in COHb levels (Vazquez et al. 1997).

The effects of CO exposure at high altitudes may be more detrimental than exposure at sea level. In the presence of high CO concentrations, a compensatory mechanism of adaptation to low oxygen saturation in high altitude that leads to increased production of red blood cells may be insufficient. High-altitude residents have a greater initial body burden of COHb and may attain the COHb level associated with the U.S. National Ambient Air Quality Standard for CO more quickly than sea-level residents (McGrath 1989). The respiratory effects of chronic exposure to CO in high-altitude populations have not been explored.

The objectives of this pilot study were a) to compare the incidence of acute respiratory infections (ARIs) in school-age children living in three communities that differ in traffic intensity in urban and suburban areas of Quito, b) to examine the relationship between ARI

occurrence and individual COHb concentrations, and c) to examine the joint effect of COHb levels (a measure of CO exposure) and hematocrit levels (a measure of a compensatory oxygen-delivery function) on the incidence of ARI. To achieve these goals, we conducted a 12-week prospective study of 960 children attending elementary schools in the early spring of 2000 in Quito, the capital of Ecuador. Quito is a rapidly developing city with > 1 million residents of relatively homogeneous ethnicity. It is located 2,825 m above sea level and enjoys a mild climate year round, but is challenged by heavy air pollution, 82% of which is due to vehicle exhaust. Substantial human morbidity and mortality in Quito are likely linked to environmental factors.

MATERIALS AND METHODS

Study design

From January through April of 2000, we conducted a prospective study in young children attending Quito's public elementary schools. First, three schools were selected that were comparable with respect to the type of school building (with concrete walls and roofs and cemented playgrounds) and the number of children per class (~ 45 children, $p = 0.56$) but differed by traffic intensity in surrounding areas. One school was located north of Quito, in the suburban area of Nayon and represents a low-traffic area (LT-school). The second school was located in a moderate-traffic area of Quito (MT-school). The third school was located in a heavy-traffic area in down-town Quito (HT-school). Next, an initial screening was performed in each school to identify a pool of children eligible for the study. During the screening period, detailed information about the study was delivered to the teachers and to the parents of each child. Children with chronic respiratory illnesses and major congenital and/or chest deformities interfering with the respiratory tract were excluded from enrollment. In each school, 320 children, 6–11 years of age (age was confirmed by birth certificate), who had formal written consent freely signed by their parents, were randomly selected and enrolled in the study. Finally, from the total 960 children enrolled 295 were randomly selected to obtain blood measurements.

Primary outcome, ARI

During the 12-week study period, each child was visited in the school twice weekly by a pediatrician who examined the child's respiratory signs and symptoms to determine the presence of upper and lower ARIs. For each child, the number of episodes of upper and lower ARIs observed over the study period was determined, considering a 2-week period to be free of infections. We adapted ARI case definitions proposed by Sempértegui et al. (1999). Upper ARI was defined as the presence of two or more of the following signs/

symptoms: cough, nasal secretion, fever $> 37.5^{\circ}\text{C}$ (auxiliary temperature), inflammation of pharynx, and anterior cervical lymphadenitis. Presence of otitis (local pain, aural pus, and eardrum congestion) was also considered as upper ARI. Lower ARI was defined as tachypnea (respiratory rate > 20) and/or lower respiratory tract secretions (alveolar or bronchoalveolar) assessed by thoracic auscultation, with one or more of the following: fever, cough, and chest retractions.

Anthropometric measurements

On the first day of the study, weight and height for all enrolled children was measured by standard procedures using the instruments calibrated by the Ecuadorian Institute of Normalization (Quito, Ecuador). Weight was measured with a DETECTO balance (DETECTO, Webb City, Missouri, USA) and recorded to the nearest 0.1 kg. Height was obtained with a calibrated scale using a fiberglass tape measure and recorded in centimeters. Weight-for-age Z-score (WAZ), height-for-age Z-score (HAZ), and body mass index (BMI) values were calculated.

Survey

Baseline measurements for confounders, including household heating and cooking conditions (the use of kerosene or wood), the presence of smokers, and household crowdedness (number of persons/number of rooms), were collected via household surveys. On the first week of the study, a survey was sent to the parents of each child. After 2 weeks, 715 surveys (77%) were returned.

Blood measurements

COHb and hematocrit levels were measured on the first day of the study. Venous blood was drawn with plastic syringes and placed into EDTA-treated tubes. Blood was immediately transported for analysis. COHb was measured by spectrometry and expressed as a percentage of plasma hemoglobin. Hematocrit was obtained by centrifuging whole blood in microtubes and expressed as a percentage.

Statistical analysis

Data entry and management were performed using Epi-Info 6.04c software (CDC, Atlanta, Georgia, USA). SPSS 11.5 (Lead Technologies Inc. SPSS Inc., Chicago, Illinois, USA) and S-plus 6.0 (Insightful Inc., Seattle, Washington, USA) were used for statistical analysis.

For each child, we estimated the number of ARI episodes observed during the study period and the number of weeks a child had attended the school. The primary health outcome—the annual ARI rate—was expressed as the number of ARI episodes per year per 1,000 children. Descriptive statistics for the primary health outcome, the blood measurements, all baseline

measurements, and variables collected via surveys were calculated. Because the blood measurements were not available for all children, we compared descriptive statistics in both subsets, with and without the blood measurements, using t-test or test of proportions as appropriate.

To examine the effect of traffic-related pollution on COHb and ARI, we estimated the average COHb concentration and the average rate of ARI for each school and assessed the differences using analysis of variance, hypothesizing that children attending the school located at in the high-traffic area would have the highest level of COHb and the highest incidence of ARI compared with children attending LT- or MT-schools.

To test the hypothesis that children with COHb concentrations above the safe level of 2.5% are more susceptible to ARI, we created two binary variables: one to reflect the occurrence of ARI (0, no ARI; 1, at least one case of ARI), and the second variable to reflect the level of COHb (0, COHb \leq 2.5%; 1, COHb $>$ 2.5%). A logistic regression model including a set of confounders for adjustment (age, sex, HAZ, WAZ, type of domestic fuel (kerosene or wood), smoking, crowdedness, and hematocrit level) was then applied. Because the household information was not available for all the children in the study, we repeated this model excluding variables on household confounders. The results of modeling were expressed as risk ratios with their confidence intervals (CIs).

To assess the association between the recurrence of ARI episodes and high COHb concentrations, we employed a log-linear Poisson regression model. In this model we predicted the observed number of ARI in a given child by an individual COHb measurement that exceeds the safety level. The model included the same set of confounders as the logistic model. Results were expressed as an adjusted relative risk with its CIs.

To examine interactions between COHb concentration, hematocrit level, and the incidence of ARI, we applied a generalized additive model (GAM) with nonparametric spline smoothing (Hastie and Tibshirani 1990). In this nonlinear model, we regressed the number of cases of ARI against individual levels of COHb and hematocrit. The result of the model was displayed using a three-dimensional surface with x- axes reflecting COHb concentration, y-axes reflecting hematocrit level, and axes reflecting the predicted numbers of ARI episodes.

RESULTS

Of 960 enrolled children, 910 (95%) completed the study (294 in the LT-school, 303 in the MT-school, and 313 in the HT-school). Fifty children were lost in the follow-up because of local migration. A total of 10,729 child-weeks of observation were accumulated in the study (3,382 child-weeks in the LT-school, 3,560 child-weeks in the MT-school, and 3,777

child-weeks in the HT-school). Of 910 children, 715 (78%) completed the household survey. The blood tests were available for a subset of 295 children.

Over the 12-week study period, 848 cases of ARI were detected. Twenty-four percent of the children suffered recurrent ARIs. The overall incidence rate of ARIs was 78.6 cases per 1,000 child-weeks of observation or 4.05 cases per 1,000 children annually.

We estimated the descriptive statistics for ARI rates, baseline characteristics, survey responses, and COHb and hematocrit measurements for the entire study population and for the two subsets, with and without the blood measurements (Table 1). The ARI incidence and all other measurements, except the percentage of stunted children, did not differ between the two subsets.

Next, we estimated and compared the descriptive statistics for ARI incidence and other measurements by school (Table 2). The schools were comparable in nutritional status as measured by the proportion of underweight children. However, children attending the

Table 1. The incidence of ARIs and exposure measurements for the entire study population as well for the COHb substudy contrasted with the remaining study participants

	Total (n=910)	COHb Substudy (n=295)	Remaining (n=615)
Measure			
Children with ARI (%)	49.56	50.50	50.40
No. of ARI episodes	848	285	563
Annual Rate of ARI	4.05	4.19	3.98
Baseline characteristics (n)	910	295	615
Age (years (Mean \pm SD))	8.5 \pm 1.2	8.4 \pm 1.2	8.6 \pm 1.2
Females (%)	43.3	40.68	44.6
Weight (kg (Mean \pm SD))	26.3 \pm 5.8	25.9 \pm 1.0	26.4 \pm 5.7
Underweight children, WAZ < -2SD (%)	3.4	4.2	3.1
Height (cm (Mean \pm SD))	125.2 \pm 9.2	124.6 \pm 9.4	125.6 \pm 9.1
Stunted, HAZ < -2SD (%)	16.1	20.7*	13.8
BMI (Mean \pm SD)	16.6 \pm 2.3	16.6 \pm 2.2	16.6 \pm 2.3
Survey response (n)	715	233	482
Completed Survey (%)	78.6	78.4	79
Crowdedness (Mean \pm SD)	1.29 \pm 1.1	1.28 \pm 0.85	1.29 \pm 0.83
Households with kerosene use (%)	2.6	1.8	3.1
Households with firewood use (%)	5.3	5.4	5.2
Smokers(%)	25.5	23.5	26.5
Children with history of asthma (%)	2.5	2.6	2.5
Blood tests (n)		295	
COHb (%) (Mean \pm SD))		2.81 \pm 2.19	
COHb > 2.5%(%)		46.4	
Hematocrit (%) (Mean \pm SD))		43.26 \pm 2.6	

The blood tests were performed only for 295 children (our substudy). To avoid redundancy we provided the values only for the substudy. The total will have identical values.

*Significant difference at $p = 0.02$ between groups with and without COHb measurements

Table 2. Incidence of ARIs and exposure measurements for children attending LT-, MT-, and HT-schools.

	Low-Traffic (n=294)	Moderate- Traffic (n=303)	High-Traffic (n=313)	Significance
Measure				
Children with ARI (%)	48.6	29.7	69.6	*, **, ***
No. of ARI episodes	238	114	496	*, **, ***
Annual Rate of ARI	3.49	1.63	6.89	*, **, ***
Baseline characteristics (n)				
Age (years (Mean \pm SD))	8.3 \pm 1.6	8.9 \pm 0.8	8.3 \pm 1	*, **, ***
Females (%)	51.7	49.5	29.4	
Weight (kg (Mean \pm SD))	23.9 \pm 5.5	27.7 \pm 5.2	27.0 \pm 5.9	*, **, ***
Underweight children, WAZ < -2SD (%)	4.4	2.3	3.2	
Height (cm (Mean \pm SD))	120.4 \pm 9.5	128.5 \pm 7.6	126.4 \pm 8.5	*, **, ***
Stunted, HAZ < -2SD (%)	28.2	9.6	8.6	*, **, ***
BMI (Mean \pm SD)	16.4 \pm 1.8	16.7 \pm 2.4	16.8 \pm 2.3	*, **, ***
Survey response (n)				
Completed Survey (%)	176	301	258	
Crowdedness (Mean \pm SD)	60	99	76	*, **, ***
Households with kerosene use (%)	1.9 \pm 1.1	1.2 \pm 0.6	0.8 \pm 0.4	*, **, ***
Households with firewood use (%)	4.1	1.7	2.9	
Households with firewood use (%)	18.1	1.3	0.5	*, **, ***
Smokers(%)	25.5	30.5	17.6	***
Children with history of asthma (%)	1.1	3.6	2.1	
Blood tests (n)				
COHb (%) (Mean \pm SD))	99	90	106	
COHb > 2.5%(%)	0.70 \pm 1.17	2.52 \pm 1.12	5.09 \pm 1.7	*, **, ***
Hematocrit (%) (Mean \pm SD))	1	43	92	*, **, ***
	41.6 \pm 2.0	44.4 \pm 2.4	43.8 \pm 2.5	*, **, ***

aSignificance at $p < 0.05$: *LT-school versus MT-school; **LT-school versus HT-school; ***MT-school versus HT-school

LT-school were significantly more stunted than were children in the other areas. Crowdedness and the use of firewood fuel were significantly higher, and the average hematocrit level was significantly lower, in the suburban than in the urban area. The HT-school had fewer girls than did the other schools. The presence of smokers in the households was significantly higher in the MT-school, and children in this school were slightly older than the rest of the children.

The incidence of ARI was also significantly different among three schools ($p < 0.01$). The highest incidence rate of ARI of 6.89 cases per 1,000 child-years was observed in children attending the HT-school. Children attending the MT-school had the lowest incidence rate of ARI of 1.63 cases per 1,000 child-years. Children attending the LT-school had an incidence rate of ARI of 3.49 cases per 1,000 child-years.

The average COHb concentrations were also significantly different among three schools ($p < 0.001$). Children attending the HT-school had the highest COHb level ($5.1 \pm 1.7\%$), and 92% of those children had COHb levels above 2.5%. Children from the MT-school had significantly lower levels of COHb ($2.5 \pm 1.1\%$), although 43% of children had high COHb. The lowest average concentration of COHb ($0.7 \pm 1.2\%$) was observed in children attending the LT-school, where only one child had a COHb concentration exceeding the safety level. The significant differences in COHb concentrations among the schools indicate a strong gradient of CO exposure in the studied areas.

Next, we examined the effects of household smoking and cooking fuel use on the average COHb concentration. In each school, we compared average COHb levels in children living in households with and without smokers (Table 3), as well as in households with kerosene and firewood cooking fuel. Neither smoking nor cooking fuel significantly altered the area-related COHb concentration pattern.

The results of the logistic regression models suggest that children with COHb > 2.5% are 3.25 (95% CI, 1.65–6.38; adjusted) or 2.06 (95% CI, 1.3–3.2; crude) times more likely to have ARI than children with COHb < 2.5%. Except for COHb level, the included variables (age, sex, weight, height, BMI, hematocrit levels, and child's previous history of asthma) as well as the presence of smokers, kerosene and/or firewood use for cooking, and the level of crowdedness at households did not exhibit significant associations with ARI occurrence. Furthermore, the results of the log-linear model indicate that with each percent increase in COHb above the safety level of 2.5%, children are 1.15 (95% CI, 1.03–1.28) times more likely to have an additional case of ARI.

The interactive effect of COHb and hematocrit level on occurrence of ARI examined by the GAM model is shown in Figure 1. Low COHb concentrations (< 2.5%) were associated with a low rate of ARI (0.6 episodes per child per 12 weeks). As COHb level increases, there is a steep increase in the likelihood of occurrence of ARI. COHb concentrations that exceeded 5% were associated with at least 1.5 ARI episodes per child per 12 weeks of observation. Hematocrit level did not affect the observed relationship between individual COHb concentration and ARI occurrence.

Table 3. Average COHb concentrations in children attending LT-, MT-, and HT-schools and living in households with or without smokers, and with or without firewood/kerosene use.

	n	COHb (% (mean ± SD))	% COHb > 2.5%
LT-school			
Households with smokers	15	0.76±0.59	0.00
Households without smokers	44	0.60±0.29	2.27
MT-school			
Households with smokers	26	2.52±1.1	42.3
Households without smokers	62	2.55±1.25	41.93
HT-school			
Households with smokers	11	4.55±1.75	90.9
Households without smokers	63	5.27±1.62	93.6
LT-school			
Households with firewood/kerosene use	13	0.59±0.26	0.00
Households without firewood/kerosene use	47	0.75±0.58	2.21
MT-school			
Households with firewood/kerosene use	1	2.15	0.00
Households without firewood/kerosene use	86	2.49±1.15	41.9
HT-school			
Households with firewood/kerosene use	1	3.24	100
Households without firewood/kerosene use	73	5.19±1.64	93.2

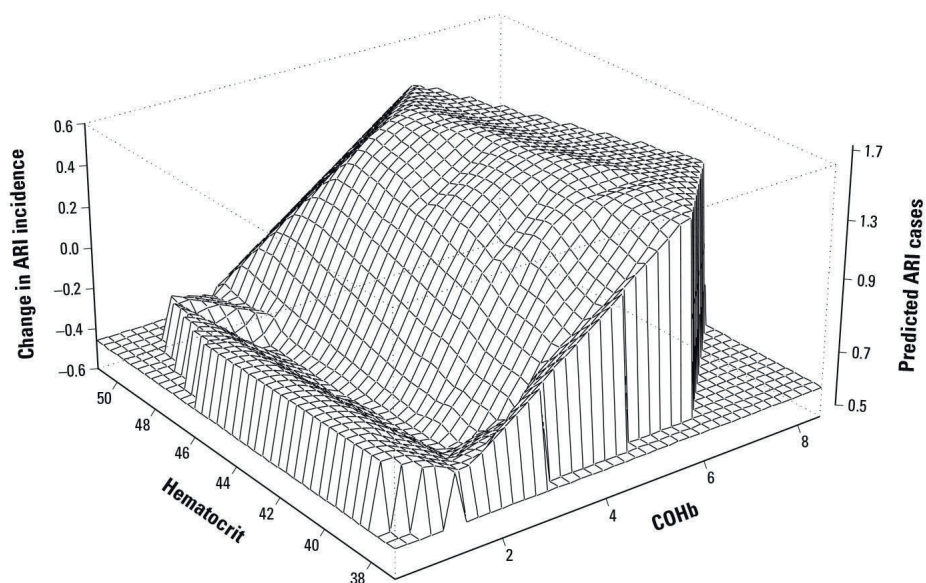


Figure 1. The GAM-predicted joint effect of COHb concentration (x-axes) and hematocrit level (y-axes) on the occurrence of ARIs during the 12-week study period in young school-age children in Quito, Ecuador

DISCUSSION

The main finding of the study was that COHb concentrations elevated because of traffic pollution correlate with the occurrence of ARIs in young children. A high COHb level was associated with at least one additional case of ARI in a 12-week period or a 3-fold increase in the annual rate of ARI incidence. These associations remain after adjusting for age, sex, weight, height, BMI, hematocrit level, previous history of asthma, presence of smokers, kerosene and/or wood use for cooking, and level of crowdedness. Our findings imply that exposure to a high level of CO, the primary reason for increased COHb concentration, may lead to increased susceptibility to ARIs. Information on chronic CO exposure and the incidence of respiratory disease in a sensitive subpopulation such as children residing in areas with high micronutrient and oxygen deficiency is novel.

The observed COHb level in the studied population was very high. Even the average concentrations exceeded a safe level of 2.5%, mostly due to elevated COHb in children from the area with high traffic volume. Almost half of those children had a COHb level consistent with acute CO poisoning. Unfortunately, routine monitoring for CO in Quito was not conducted at the time of the study. The absence of ambient CO measurements did not allow direct assessment of the relations between COHb concentrations and exposure to CO in the studied

population. The sparse CO and particle matter monitoring, performed by the Departamento de Control de la Calidad del Aire (Department of Air Quality Control, Quito) in the central part of Quito in 1995–1999, has demonstrated that the median monthly concentrations of both pollutants consistently exceeded U.S. and European standards (Southgate et al. 1995). Furthermore, in December and January, CO concentrations exceeded the standards three times. It is plausible that the study inception in early January 2000 coincided with the seasonal peak in CO exposure, which contributed to elevated COHb levels.

Indoor CO comes predominantly from smoking and heating/cooking fuel combustion. A gradient of COHb levels in children with different type of heating systems has been observed, $0.88 \pm 1.34\%$ for wood and coal heating, $0.58 \pm 0.97\%$ for gas heating, and $0.28 \pm 0.4\%$ for electric system, although it was not significant (Vazquez et al. 1997). It has been demonstrated that CO inhaled during cooking in high-altitude conditions results in a 1% increase in COHb concentration but does not reach clinically unsafe levels (Keyes et al. 2001). Because of the warm climate of Quito, with air temperature ranging from 10°C (50°F) at night to 25°C (77°F) at noon averaging at 15°C (64°F) year around, the heating systems are not necessary. In our study, we did not observe significant effects of smoking or kerosene and/or wood use for cooking on individual COHb concentrations. Therefore, we concluded that the dominant factor for the significant COHb gradient observed in the study was traffic-related pollution, which was different in the three school locations.

It is important to note that children attending the school located in the central historical district of Quito with the heaviest traffic had the highest levels of COHb and the highest incidence rate of ARIs. Surprisingly, children from the area with low traffic had the lowest COHb levels but did not experience the lowest incidence of ARI. This finding could be explained by the potential influence of other factors that we were not able to consider, such as anemia, immune status, or the presence of local outbreaks of infection. Although the regression analysis demonstrated that the confounders considered were not associated with ARI, it is possible that other factors might influence health outcomes for these suburban children. It is also possible that the slight “ridge” of ARI incidence in Figure 1 at very low COHb levels occurred because the children who had low COHb concentrations (most of them were attending the LT-school) were also more chronically malnourished (stunted) than the children from the two other schools (28 vs. 9 and 8%, respectively) (Table 2). The observed differences in stunting might be indicative of frequent diarrheal diseases and/or malnutrition that occurred in early childhood (Freire et al. 1988). Despite our effort to select schools with similar socioeconomic status, we suspect that the students in the suburban area came from families with lower literacy levels (considering the lowest percentage of completed surveys) and poorer household’s conditions (considering the high rate of crowdedness and more frequent use of firewood, the cheapest cooking fuel) than the rest of the students. Nevertheless, the subanalysis of potential interactions between stunting and COHb levels

in children residing in the low-traffic area revealed that the risk of ARI in children who were stunted and had high COHb level was 3.37 (95% CI, 1.001–11.26), but the sample size was too small to draw a strong conclusion. Therefore, more detailed studies are needed to disentangle the effect of CO exposure from the effect of malnutrition and socioeconomic conditions on ARI.

Although we did not find reports on susceptibility to respiratory infections and elevated COHb, similar associations between COHb level and inflammatory pulmonary diseases has been described (Yasuda et al. 2002). A biologic plausibility for the effect of high CO exposure on bronchial-alveolar system impairing local immune reactivity has been proposed (McGrath 2000); however, direct investigations of CO effects on innate and adaptive immunity are scarce. The following mechanism might explain a local defect of immunoreactivity leading to a high susceptibility to respiratory infection. Given that the mobility of immune cells and the motility of a ciliar epithelium that lines bronchial-alveolar system are highly ATP- dependent processes, the high CO content might directly compete with free radicals for the binding site on cytochrome C during oxidative phosphorylation. This might deprive dendritic cells, B-cells, and T-cells of ATP- dependent cytokine production, immuno- globulin synthesis, and the killing of virus- infected cells (Bona et al. 1999; Caldwell et al. 2001; Loeffler et al. 1990). Considering hemoglobin dependency, the oxygen binding might affect the proliferation of dendritic cells and T- and B-cell reactivity in the lymphoid organs (Shimizu et al. 1996; Taneja et al. 2000). The division of immune cells, primed in bronchial lymph nodes, might be affected because of their high sensitivity to hypoxia even when the inhibitory effect CO on the neural system is not yet detected.

Our pilot study contributes to the body of literature that demonstrates the harmful effect of traffic-related pollution in urban settings. In the last decade, the air quality has been rapidly decreasing in Quito because of urbanization and increasing exhaust from public transportation. According to the Dirección Nacional de Tránsito, Quito, the number of cars registered in Quito rose from 174,875 in 1995 to 209,757 in 1998. Diesel is used in the vast majority of trucks and buses and in 6% of the cars (Jurado 1991). The city lies in a narrow valley with a north–south orientation of encircling mountains and environmental conditions challenged by high volcanic activity and El Niño effects. The polluted air in the city is often stagnant, and its clearance depends mainly on prevailing wind and precipitation. Strict emission regulations, as much as economic development, are crucial to the nation of Ecuador. Unfortunately, economic capacities to address the issues of health and the environment at the municipal and national levels, are severely constrained by harsh economic situations and shifting political factors. Nonetheless, receptivity to environmental concerns is evolving rapidly.

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Chapter 3

Emergency room visits for respiratory conditions in children increased after Guagua Pichincha volcanic eruptions in april 2000 in Quito, Ecuador observational study: time series analysis

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ABSTRACT

Background: This study documented elevated rates of emergency room (ER) visits for acute upper and lower respiratory infections and asthma-related conditions in the children of Quito, Ecuador associated with the eruption of Guagua Pichincha in April of 2000.

Methods: We abstracted 5169 (43% females) ER records with primary respiratory conditions treated from January 1 – December 27, 2000 and examined the change in pediatric ER visits for respiratory conditions before, during, and after exposure events of April, 2000. We applied a Poisson regression model adapted to time series of cases for three non-overlapping disease categories: acute upper respiratory infection (AURI), acute lower respiratory infection (ALRI), and asthma-related conditions in boys and girls for three age groups: 0–4, 5–9, and 10–15 years.

Results: At the main pediatric medical facility, the Baca Ortiz Pediatric Hospital, the rate of emergency room (ER) visits due to respiratory conditions substantially increased in the three weeks after eruption ($RR = 2.22$, $95\%CI = (1.95, 2.52)$ and $RR = 1.72$ $95\%CI = (1.49, 1.97)$ for lower and upper respiratory tract infections respectively. The largest impact of eruptions on respiratory distress was observed in children younger than 5 years ($RR = 2.21$, $95\%CI = (1.79, 2.73)$ and $RR = 2.16$ $95\%CI = (1.67, 2.76)$ in boys and girls respectively). The rate of asthma and asthma-related diagnoses doubled during the period of volcano fumarolic activity ($RR = 1.97$, $95\%CI = (1.19, 3.24)$). Overall, 28 days of volcanic activity and ash releases resulted in 345 ($95\%CI = (241, 460)$) additional ER visits due to respiratory conditions.

Conclusion: The study has demonstrated strong relationship between ash exposure and respiratory effects in children.

Keywords: emergency room, respiratory conditions, Guagua Pichincha, eruption

INTRODUCTION

The danger of living near active volcanoes has been well documented since ancient times, from the ruins of Pompeii to the recent satellite images of volcano eruptions. Volcanic activity is typically associated with toxic emissions; pyroclastic flows (the mixture of rock fragments and superheated gases, which can achieve speeds over 100 km/hr and temperatures over 300°C); the release of ash; volcanic gases containing carbon dioxide, water vapor, sulfur dioxide, hydrogen chloride, hydrogen sulphide, hydrogen fluoride, etc.; and polycyclic aromatic hydrocarbons produced whenever any complex organic material is burned by hot pyroclastic flows (1,2). Although approximately 455 million people worldwide live within potential exposure range of an active volcano, information on the acute and chronic respiratory health effects of volcanic emissions is sparse (3,4).

The 1980 eruption of Mt. St. Helens (5) led to numerous studies of health effects of volcanic emissions. The acute effects of volcanic ash fall and gases on respiratory conditions vary from undetected to well-defined (2,3). Transient acute irritant effects of volcanic ash and gases on the mucous membranes of upper respiratory tract and exacerbation of chronic lung diseases during and shortly after eruptions with heavy ash fall have been documented (6,7). Such relationships have been found following the more recent eruption in Cerro Negro, Nicaragua in 1992 (8), Mt. Sakurajima in Japan (9), and Mt. Tungurahua in Ecuador (10).

Quito, the capital of Ecuador, located 2800 m above sea level, is surrounded by four active volcanoes: Guagua Pichincha, Cotopaxi, Antisana, and Tungurahua (11). The stratovolcano Guagua Pichincha is located 13 km (7 mi) west of Quito and became active in 1998 after 340 years of dormancy with emissions of vapor, ashes, and fumes. In the spring of 2000, volcanic ashes containing silica, sulfurs, and particulate matter were again reported and yellow alerts were issued for the city of Quito. Nestled in a long, narrow valley between the base of the mountain range to the west and the precipitous canyon of the Machángara River to the east, Quito possesses an unmatched setting: a dangerous chamber created by the nature and enhanced by humans (Figure 1). Over the last two decades, the rapid urbanization and sprawl has resulted in hazardously high levels of air pollutants (12,13). Quito's unique geographical location, with its topology and the pattern of prevailing wind, traps stagnant contaminated air. At that altitude, where the demand for oxygen is high, even a small increase in air pollution may trigger a substantial increase in ER visits for respiratory conditions. In fact, our own studies indicate strong effects of air pollution on respiratory health in Quito's children (14).

Recent work has emphasized a complex spectrum of health problems associated with direct and indirect effects of displacement, disruptions in infrastructure (15,16), soil, air, and water contamination (17,18). Studies stress the need for implementing efficient surveillance systems to monitor the health effects associated with various environmental



Figure 1. Aerial photograph of Quito, the capital of Ecuador.

Foot- notes: The Pichincha volcano located west of Quito is in the central background of the picture. Along its base is the narrow strip with an air pollution haze stretching from left to right (south to north) across the city

and socio-economic factors, especially in the most vulnerable populations (8). In the absence of such surveillance systems, we have made an attempt to characterize the respiratory health outcomes that often reflect the most severe conditions requiring urgent ER visits and hospitalization. In the presented study we describe the change in pediatric ER visits for respiratory conditions before, during, and after ash falls in Quito due to Pichincha volcano eruptions which occurred in April, 2000. We believe that the results of this study provide important information for environmental public health policy and better understanding of the short term effects of environmental exposure to volcanic ashes on human health.

DATA AND METHODS

Study population and outcomes

This study was conducted at the Baca Ortiz Children's Hospital, Quito's largest government-subsidized facility, which provides care to approximately 307,000 children under 15 years of age residing in the Quito Metropolitan area and surrounding communities (19). During 2000, 72,039 children were treated at the hospital's outpatient departments, 22,353 children were assisted at the hospital's emergency room, and about six per cent of children visited ER were treated for respiratory illnesses, mainly pneumonia (Department of Statistics, Baca Ortiz Hospital, 2000, unpublished data). The Ethical Committee of Ecuador and the Tufts IRB granted permission for abstraction of de-identified records.

We abstracted 5169 (43% females) ER records with primary respiratory conditions treated from January 1 – December 27, 2000. For each case the following information was recorded: age, sex, residential location (barrios), admission date, temperature, weight, respiratory rate,

and primary diagnosis. For 4416 (85%) children nutritional status was determined using EPI/Info software (20) and classified as underweight if the weight-for-age z-score was more than two standard deviations below the age adjusted mean and normal otherwise. Based on the primary diagnosis given in the ER and similar to previous study (14) we classified records into three non-overlapping categories: acute upper respiratory infection (AURI), which includes conditions coded as pharyngitis, tonsillitis, sinusitis, rhinitis, laryngitis, and their combinations; acute lower respiratory infection (ALRI), which includes conditions coded as pneumonia, bronchopneumonia, tracheobronchitis, bronchitis; and asthma-related conditions (2392, 2319, and 431 cases in each group respectively). We estimated the annual incidence rate for ER visits (per 1000 children) in boys and girls for three age groups: 0–4, 5–9, and 10–15 years using the estimates of population served calculated from census data (18).

The timing of eruption

We abstracted information on Guagua Pichincha activity from various sources, including Instituto Geofísico de la Escuela Politécnica Nacional, Volcano Ash Advisory Archive, maintained by the NOAA Satellite and Information Service (21), and the main local news media, and created a timeline of exposure-related volcanic activity. Figure 2 illustrates the compiled timeline superimposed on the time series of daily counts of respiratory infections in the studied population. In the spring of 2000 Pichincha was active; in February, plumes rose to 1–2 km often carrying noticeable ash, and a few ash falls were seen near the vent. During January to April, fumarolic activity was moderate but variable, often most noticeable a week before eruptions (shown as a pink bar in Figure 2). Between April 9 and 12 (days 99–102 in the Figure 2), the volcano erupted approximately 18 times. Ash clouds rose 0.8 km into the air. More than 100 seismic tremors were recorded during the eruptions. A yellow alert was issued for the city on April 12. On April 16, the volcano sent clouds of vapor 0.5 km into the sky over Quito. We defined four periods for analysis: fumarolic activity period – Period 1, one week post eruption – Period 2, two weeks post eruption – Period 3, and three weeks post eruption – Period 4. We also abstracted daily measures for temperature and precipitation, obtained from the Instituto Nacional de Meteorología e Hidrología of Ecuador (22).

Models and analysis

Six time series of daily counts of ER visits were compiled based on the date of admission: for males and females in each of the three age categories. In 52 (1%) records, a missing day was replaced with a mid-month value. Pair-wise Spearman correlations between time series for boys and girls ranged from 0.4 to 0.7 (all significant) indicating similarity of temporal patterns. Three time series of daily counts of ER visits for each diagnostic group for all children were created.

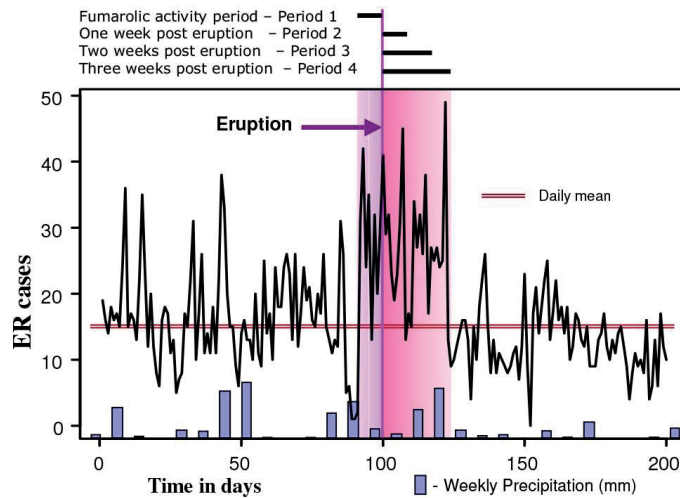


Figure 2. The time line of the Guagua Pichincha volcano activity, daily ER visits, and weekly precipitation.

Footnotes: Time series of daily cases of ER visits for first 200 days are shown. The most noticeable fumarolic activity was observed a week prior eruptions (shown as a pink bar). On April 10th and 11th (days 100 and 101), the volcano erupted approximately 18 times. More than 100 seismic tremors were recorded during the eruptions. Ash clouds rose 0.8 km into the air on April, 10th and then another cloud of vapor was seen on April 16th. A yellow alert was issued for the city Quito. The study periods (2, 3, and 4) are indicated as a red bar.

We also compiled a set of binary time series to indicate the timing of volcano eruptions including one, two, and three-week post-eruption periods (shown as red bar in Figure 2) and one week prior eruption (shown as pink bar in Figure 2). To examine the relationship between the timing of the volcanic eruption and ER visits we applied a Poisson regression model adapted to time series data adjusted for effects of the day of the week, official holidays and potential effect of replaced missing dates, and meteorological factors: $\log(Y(t)) = \beta_0 + \beta_1E + \beta_2D + \beta_3H + \beta_4P + \epsilon(t)$, where $Y(t)$ is a time series of cases, and β s are regression parameter for the variables reflecting presence of exposure (E), weekly cycle (D), holidays and mid-month indicator (H), and weekly precipitation level (P). The predicted daily rate of ER visits per 1000 children; the relative risks (RR), and their 95% confidence intervals (95%CI) were estimated for boys and girls in three age groups for all ER visits and for three diagnostic groups in all children. To examine if the higher rate of ER visits in malnourished children was associated with exposure we performed the χ^2 -test.

To characterize the spatial distribution of ER cases we geocoded 4786 (93.4% of all records) and categorized patients' residential locations into three regions: the City of Quito, the city suburbs, and the area outside the city metropolitan boundaries. Locations of Quito residents were further subdivided into sixteen parishes and five large suburban areas. The spatial distribution of cases and its change during the eruption period were examined.

RESULTS

The descriptive characteristics of population served by the Baca Ortiz Hospital and annual incidence rates of ER visits due to respiratory infections are shown in Table 1. The mean age for girls and boys was 3.23 ± 3.10 years and 3.18 ± 3.08 years, respectively. The annual rates of infections were consistently higher in males in every age category.

Based on the results of the regression model we estimated the daily means of ER visits for a period that include time before and three weeks after the eruption (344 days). We also estimated the relative risks of the increase in the daily mean ER visits during the four defined periods of volcanic activity (28 days). These estimates are shown in Table 2. Overall, children under 4 years of age were treated at the ER three times more often than older children. At base-line, the daily mean ER visits was significantly higher in the youngest children compared to children older than 5 y.o. (0.092 ; $95\%CI = (0.088, 0.098)$ vs. 0.024 ; $95\%CI = (0.021, 0.027)$, $p < 0.0001$). The daily means of ER visits in the youngest children were two times higher during all four defined periods of volcanic activity than pre and post eruption after adjusting for the baseline differences. The relative risks for ER visitations were similar in boys and girls (e.g. during one-week post eruption: $RR = 2.21$, $95\%CI = (1.79; 2.73)$ and $RR = 2.16$, $95\%CI = (1.69; 2.76)$, respectively). These findings indicate that increases in daily cases relative to the annual average (e.g. Figure 2– red horizontal line) were significant for all ER visits and each disease group.

Table 1. Annual incidence rates of ER visits for three specific diagnostic groups

		Age groups			
		0 – 4	5 – 9	10 – 15	all ages
Population served ^a	females	49860	50964	61267	162091
	males	51332	52160	61709	165201
Annual rate ^b	females	32.83 ^c	8.37	2.57	13.70
	males	42.43	11.38	2.88	17.85
Annual rate of AURI ^c	females	16.02	3.21	0.92	6.28
	males	21.39	4.37	0.78	8.32
Annual rate of ALRI	females	15.53	3.36	1.00	6.21
	males	19.47	4.72	1.19	7.98
Annual rate of Asthma	females	1.11	1.78	0.64	1.14
	males	1.43	2.24	0.90	1.49

^a Values are estimated from the Census 2000, Ecuador.

^b Annual rates are estimated per 1000 children served by the Baca Ortiz Hospital.

^c Estimated as: (number of cases/population served) \times 1000

Table 2. Predicted daily rates of ER visits and the relative risks associated with the volcanic activity

Age Groups	Males			Females		
	estimate	LCI	UCI	estimate	LCI	UCI
Daily rates ^a						
0–4	0.104	0.099	0.110	0.080	0.076	0.086
5–9	0.027	0.024	0.030	0.020	0.018	0.023
10–15	0.007	0.006	0.009	0.007	0.005	0.008
All	0.043	0.041	0.045	0.040	0.039	0.042
Relative Risks ^b						
Fumarolic activity period – Period 1						
0–4	2.008	1.610	2.504	1.953	1.509	2.527
5–9	1.698	1.075	2.681	1.358	0.746	2.472
10–15	1.180	0.438	3.180	1.681	0.690	4.098
All	1.927	1.587	2.339	1.799	1.458	2.220
One week post eruption – Period 2						
0–4	2.212	1.791	2.732	2.156	1.685	2.758
5–9	1.331	0.797	2.222	1.106	0.571	2.140
10–15	2.102	0.987	4.476	0.996	0.318	3.121
All	1.965	1.621	2.381	1.799	1.458	2.220
Two weeks post eruption – Period 3						
0–4	1.995	1.699	2.344	2.280	1.913	2.718
5–9	1.434	1.004	2.048	1.108	0.691	1.777
10–15	1.820	1.013	3.270	0.488	0.156	1.530
All	1.795	1.552	2.076	1.894	1.632	2.199
Three weeks post eruption – Period 4						
0–4	2.056	1.799	2.350	2.096	1.798	2.442
5–9	1.579	1.188	2.100	1.067	0.717	1.586
10–15	1.625	0.972	2.716	0.879	0.431	1.790
All	1.891	1.678	2.130	1.807	1.591	2.052

^a Estimated baseline together with 95% confidence interval (LCI, UCI) for cases per day per 1000 served population before and after volcanic eruption.

^b Relative risks together with 95% confidence interval (LCI, UCI) for cases per day per 1000 served population for effects of the day of the week, official holidays and weekly precipitation.

On average, the predicted rates of ER visits for acute upper and lower respiratory tract infections were significantly higher (from 1.65 to 2.32 times) during all four predetermined periods compared to the rates in pre and post eruption periods (Table 3). The rate of asthma and asthma-related diagnosis was two times higher during the period of fumarolic activity $RR = 1.97$, $95\%CI = (1.19, 3.24)$, but not during other time periods. Eight percent of children admitted to ER were underweight. No difference in the rates of ER visits between underweight and normal children associated with exposure was observed (χ^2 -test; $p = 0.24$).

An observed 2.25-fold increase in daily ER visits was present in all parts of the city (Table 4 and Figure 3). The suburb residents accounting for 20% of ER visits experienced a 3-fold increase (3.05) in daily counts of ER visits during the volcano eruption period. Overall, during 28 days of volcanic activity and ash releases on average 138 girls $95\%CI = (104, 207)$, and 206 boys $95\%CI = (137, 252)$, were treated in ER in addition to typically observed level of 6.17 and 8.18 cases per day in girls and boys respectively.

Table 3. The relative risks associated with the volcanic activity for three diagnostic groups.

	ALRI (n = 2392)			AURI (n = 2319)			ASTHMA (n = 431)		
	RR ^a	LCI	UCI	RR	LCI	UCI	RR	LCI	UCI
Period 1 ^b	2.056	1.665	2.538	1.654	1.314	2.082	1.966	1.193	3.239
Period 2 ^c	2.318	1.899	2.830	1.677	1.334	2.107	1.586	0.913	2.754
Period 3 ^d	2.125	1.827	2.473	1.808	1.541	2.122	1.089	0.679	1.746
Period 4 ^e	2.221	1.959	2.518	1.716	1.497	1.968	0.963	0.638	1.453

^a The relative risks and their 95% confidence intervals (LCI, UCI for all ages combined) of an increase in the daily mean ER visits during the defined periods for three diagnostic groups.

^b Fumarolic activity period – Period 1

^c One week post eruption – Period 2

^d Two weeks post eruption – Period 3

^e Three weeks post eruption – Period 4

Table 4. Geographical distribution of residential locations of patients visited emergency room visits at two periods

Region	Parishes	Pre and post eruption (334 days)		During eruption (28 days)		Ratio	Percent age
		Total cases	Cases per day	Total cases	Cases per day		
City of Quito (n = 405,206) ^a							
Turubamba (South)		443	1.33	85	3.04	2.29	11
	Guamani	64	0.19	8	0.29	1.49	2
	Chillogallo	219	0.66	43	1.54	2.34	5
	Las Cuadras	120	0.36	26	0.93	2.58	3
	El Beaterio	40	0.12	8	0.29	2.39	1
Urinsaya (South Central)		902	2.70	173	6.18	2.29	22
	Villaflora	333	1.00	69	2.46	2.47	8
	La Magdalena	140	0.42	25	0.89	2.13	3
	Chimbacalle	141	0.42	24	0.86	2.03	3
	Eloy Alfaro	288	0.86	55	1.96	2.28	7
Yavirac (North Central)		1095	3.28	234	8.36	2.55	28
	San Roque	321	0.96	64	2.29	2.38	8
	Santa Prisca	180	0.54	36	1.29	2.39	5
	EL Batan	66	0.20	14	0.50	2.53	2
	San Blas	528	1.58	120	4.29	2.71	14
Anansaya (North)		936	2.80	162	5.79	2.06	23
	La Concepcion	168	0.50	24	0.86	1.70	4
	Cotocollao	201	0.60	43	1.54	2.55	5
	Carcelen	333	1.00	53	1.89	1.90	8
	El Inca	234	0.70	42	1.50	2.14	6
Quito suburbs (n = 136,466) ^a		422	1.26	108	3.86	3.05	11
	Valle Tumabco (East)	79	0.24	24	0.86	3.62	2
	San Antonio (North)	48	0.14	11	0.39	2.73	1
	Calderon (North East)	159	0.48	41	1.46	3.08	4
	Valle de los Chillos (South East)	136	0.41	32	1.14	2.81	4
	Amaguana (South)	10	0.03	2	0.07	2.39	0.3
Outside of Quito Metropolitan Area		169	0.51	45	1.61	3.18	4
Total geocoded cases		3977	11.91	809	28.89	2.43	100

^a The numbers in parenthesis represent the population of children under 15 years of age.



Figure 3. A schematic map of the city of Quito.

Footnotes: Names of parishes from where geocoded residential locations of patients that visited the emergency room of the Baca Ortiz Hospital in 2000 correspond to Table 4.

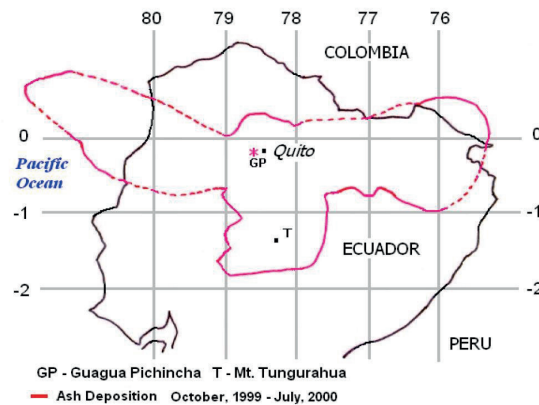


Figure 4. A composite map of ash deposition from Pichincha eruptions (October 1999 – July 2000) demonstrating the extensive area affected by ashfalls.

Footnotes: The map has been modified from the images of the Volcano Ash Advisory Archive (21), courtesy of Dr. Yepes, the Geophysical Institute of Ecuador.

DISCUSSION

This study documented elevated rates of ER visits for acute upper and lower respiratory infections and asthma-related conditions in the pediatric population of Quito associated with the eruption of Guagua Pichincha in April of 2000. Although Pichincha activity during

the year 2000 was generally more moderate than in 1999, the rate of pediatric ER visits due to respiratory conditions following volcanic eruptions in April of 2000 doubled. The rate of asthma and asthma-related diagnosis was two times higher during the period of fumarolic activity of Pichincha. The largest increase in ER visitations was observed in the youngest children.

We demonstrated the potential of ER record utilization for monitoring the health effects associated with volcanic activity. Our study illustrates a set of analytical tools useful as the basis for pre-disaster planning and preparedness in areas where explosive and effusive volcanic eruptions are relatively common in proximity to vulnerable populations. Observed associations imply a potential scenario for compositions of immediate and delayed health effects following single or multiple exposure releases (23). The onset of respiratory symptoms can manifest as three distinct waves. The first wave is composed of symptoms associated with the transient acute irritant effects of volcanic ash and gases on the mucous membranes of the eyes and upper respiratory tract, evidenced by the increase in asthma-related diagnosis which doubled during the period of fumarolic activity. The second and the third waves reflect conditions apparent with a specific delay and include acute upper and lower respiratory infections.

Volcanic ash is capable of inducing acute respiratory problems in susceptible people especially children and those with history of respiratory illness such as asthma or bronchitis (6,3,24,25). The precise mechanism of those respiratory problems has not been well defined; however, its complexity has been outlined (3,26). The basic etiology of lung inflammation begins with deposition of the particles into the lung. The location of the particle's deposition can be influenced by breathing pattern, the branched morphology of the airways, particle size and shape (1). If a particle lodges in the main airways, it is cleared by the mucociliary escalator (27). If it penetrates into the non-ciliated alveolar level, it may be cleared by the phagocytic alveolar macrophages. However, in the latter scenario, the particles may impair macrophage-mediated removal and these particles are not cleared. Some particles may directly penetrate the alveolar epithelium and reach the lung's deep lymph node drainage. Macrophages have also been shown to become inundated with inert particles, which subsequently halt the clearance of all particles at the alveolar level (28). Macrophages release toxins which are utilized to destroy particles; however the released IL-1, IL-6, tumor necrosis factor (TNF), fibroblast growth factor, and the affluence of polymorphonuclear neutrophils damage the host's lung tissue (1). The short-term effect of the above processes is lung inflammation, which manifests itself as difficulty in breathing and increased susceptibility to respiratory infections. Long-term prognoses include possibly fibrosis and carcinogenesis (29).

The irritant effect of volcanic ash on human airways depends on the physical and chemical properties of the ash including particulate size (6,30), the concentration of respirable ash particles, mineralogical composition, and duration of exposure (3). It also depends on main

features of the respiratory tract such as ventilation rate, the nasal filtration efficiency, and the mucociliary clearance rate (30). Several *in vitro* and *in vivo* experiments on different lung cells and animals suggest that inhaled volcanic ash is less toxic to the lung than other compounds like fine and coarse ambient particulate matter, quartz, or free crystal line silica. Some observations support the notion that volcanic ash is not a potent stimulus to lung inflammation since it does not stimulate the release of IL-8, a chemotactic factor for neutrophils, nor does it depress γ -interferon and TNF- α secretion from either human alveolar macrophages or normal human bronchial epithelial cells (31,32). However, it has been shown that physical immunologic barriers such as ciliary beating frequency and mucous lining can be altered after a short exposure to volcanic ash (33). It has been reported that humoral immunologic parameters can also be affected by volcanic ash; for instance, workers exposed to volcanic ash had significantly lower C3 and C4 levels as well as marked decrease in serum IgG levels when compared to unexposed controls (34). Immunological assays have shown that IgA, IgG, and albumin, airways proteins, play a protective role in mediating the effect of inhaled dust in human lungs (30). Therefore, we hypothesize that young and malnourished children with pre-existing low levels of immunoglobulins, could be at higher risk for respiratory problems than healthy children. We did not find a difference in respiratory distress between underweight and normal children, potentially due to low sensitivity of weight-for-age measures in older children, but the age difference was well pronounced.

Moreover, some rodent models suggest that exposure to different particulate air pollutants including volcanic ash, stimulates immunocompetent cells, e.g. monocytes, more strongly than alveolar macrophages, to produce oxidative response (35). Exposure to volcanic ash was also found to be related to an impairment of stimulated superoxide production while resting superoxide anion production is normal suggesting that volcanic ash might pose a risk for infection by compromising phagocyte antibacterial functions (36). Such mechanisms may explain why individuals with underlying lung impairment including chronic inflammation are more susceptible to harmful effect of air pollution than healthy individuals. At Quito's altitude, where the demand for oxygen is high, even a small increase in CO, SO₂, CO₂, and similar compounds that might affect hematocrit level (and are already known to be associated with asthma exacerbation) may trigger a substantial increase in ER visits for asthma exacerbation especially during volcano's fumarolic activity.

Very little is known on the effects of "fresh" fractured silica particles on the developing lung tissue in young children. One may speculate that rough edges of silica particles due to micro-abrasion may damage the epithelial lining and promote pathogen colonization. Together with the high susceptibility for viral infections, which often followed by bacterial pneumonia, this may explain an increase in acute lower respiratory infections of bacterial origin in young children.

We believe that the observed effect most likely reflects the increase in severity of acute respiratory infections and exacerbation of preexisting asthma-related conditions. In the absence of surveillance systems that can continuously and efficiently monitor the health effects associated with various environmental and socio-economic factors, we were using health outcomes that often reflect the most severe conditions, which require urgent ER visits and hospitalizations. We also anticipate that young children with pneumonia and asthma-related conditions are more likely to be hospitalized than older children or children with acute upper respiratory infection. In our recently completed four-year longitudinal study of Quito's children aged from 6 to 36 months, we recorded 4,450 cases of AURI and 518 physician-diagnosed cases of pneumonia, out of which six (1.2%) cases required hospitalization (Sempertegui et al, in preparation). Furthermore, of all the AURI episodes, 102 (2.3%) progressed into ALRI. Therefore, we anticipate that for one episode of hospitalization due to pneumonia there are approximately 86 cases of mild pneumonia. This observation helped us to assert the severity of respiratory conditions. In fact the observed effect was very similar with reported increase in AURI (2.6 times), ALRI (2.5 times) and asthma (2.1 times) eruptions of Tungurahua in 1999 (37).

The observed increase in respiratory infections in April of 2000 was most likely triggered by aerosols and ash falls produced by Pichincha eruptions. However, we recognize that the main limitation of our analysis is the absence of direct measures of exposure. It is also plausible that ash particles deposited during 1999 ash falls can be re-suspended in the air without elevated volcanic activity. Unfortunately, in Quito, systematic monitoring of air quality for critical air pollution was initiated after 2000, so we can only rely on indirect proxies such as satellite imagery records for ash emissions and daily records on seismic activity of Pichincha to establish the timing of high exposure (21). We assessed the relations between ER visits and timing of high exposure in a manner of ecological analysis, limitations of which pose caution on our conclusions. First of all, health effects of volcanic aerosols and ambient traffic-related pollution are difficult to disentangle. Secondly, volcanic activity is a continuous process, in which the delayed health effects of one event may overlap with others. Thirdly, a seasonal elevation in incidence of respiratory infections and pneumonia could coincide with the eruption and therefore bias the results. Finally, temporal variations in ER visits could be prompted by a number of social factors (e.g. weekends, holidays, strikes) as well as by changes in perception associated with volcano alerts. Although potential confounding by air pollution, continuous volcanic activity, seasonal infection rates and reporting bias could have affected our study, we believe that their role is not substantial.

Volcanic ashes worsened the already poor air quality in Quito (14). Typically, the chemical composition of ashes could be distinguished from by-products of man-made air pollutants in urban settings. However, one of the most predominant and routinely measured components, particular matter of 10 micron in diameter and larger (PM₁₀), are in both volcanic ashes

and traffic-related products of incomplete combustion. In fact, a few samples of PM₁₀ concentrations collected in urban Quito in the late-fall of 1999 demonstrated an extremely high level of PM₁₀ in both the northern and southern parts of the city. For instance, during the 1999 Pichincha eruption PM₁₀ increased progressively from 58 µg/m³ in September 30, to 407 µg /m³ in October 6–7, to 1487 µg /m³ in November 26, 1999, exceeding the allowed level by 8 to 28 times (25). Unfortunately, specific information of ash composition, transport, and deposition is limited, especially for eruptions prior to 2000 (39). It has been reported that the ash contained substantial respirable material and an elevated cristobalite content (26,40). An elementary description of Guagua Pichincha ash given by Baxter (26) for three consecutive eruptions in November 1999, July 2000, and February 2002, is based on percentage of PM₁₀ in ash that ranged from 7.9 to 13.6, not too dissimilar to Mount St Helens 1980 ash composition (5). The data from two other Ecuadorian eruptions of Tungurahua and El Reventador volcanoes in 2001 suggested that in Quito area the ash layer was mainly composed of medium to fine ash particles with about 20% free crystals (41). This study also indicates that the ash re-suspended during the windy afternoons caused ocular irritation, respiratory troubles, and other health problems.

Quito was affected by several episodes of ash emission in October – December of 1999, April 2000, and July 2000 (see Figure 4). It is possible that our estimates of the ER rates before and after the eruptions in April could have been elevated by these prior eruptions, and then we probably underestimated the effect. It is also plausible, that we were able to detect the effect of volcanic activity due to little or no rain, which would have cleansed the air during the days of eruption (see Figure 1). Again, additional studies are needed to better quantify the effect of ash fallouts and related pollutants.

The seasonal variations in respiratory infections in Ecuador, and specifically in Quito, are poorly documented. In our own research, we noticed that the incidence of acute upper respiratory infections has no pronounced seasonal variations. The incidence of pneumonia however had a defined seasonal peak in December, January, and February with the lowest level in May, June, and July (Naumova, unpublished data). We believe that an increase of ER visits observed in late April and early May is unlikely to be obscured by typical seasonal variations in the incidence of respiratory infections.

This study was conducted in a government-subsidized hospital, easily accessible by public transportation, providing free care exclusively for children. Although, the quality of medical care utilization may depend on socio- economic status, the main routines of ER visitations are fairly standard in city hospitals. The change in attitudes and perceptions of potential risks associated with volcano eruption in patients, their parents, and physicians could potentially affect the pattern of ER visitations (38). However, little is known about behavioral aspects of medical service utilization in Quito. Important studies performed by the University of South Florida suggest that attitudes of high resilience, e.g. the ability to withstand stresses are

quite dominant in small Ecuadorian towns affected by volcanic activity of Tungurahua and Chimborazo (10). Furthermore, before the April eruptions, a series of yellow alerts (which assumed to be prepared to close schools and businesses and to evacuate when necessary, to know the plan of alternative and evacuation routes, locations of shelters and hospitals) (11) could have warned both physicians and families disrupted by the disaster, and therefore over-utilization is somewhat implausible. Strikes of medical personnel or closing of hospitals (events that could artificially lower the rate of ER visits) can occur in Quito, however the days with extremely low counts are not systematic and no records of strikes in Baca Ortiz or any city hospitals before or after the April eruption of 2000 are known. We believe that the observed increase in ER visits at the Baca Ortiz Hospital after the volcano eruption reflect a plausible health effect on children from families with low and moderate income living in all communities of Metropolitan Quito.

CONCLUSION

We documented significantly increased levels of respiratory disease in Quito children after a volcanic eruption using hospital records. Sound environmental decisions require estimates of the burden of disease or other externalities suffered by the population. In Ecuador, continuous monitoring of air quality is in its infancy, population based surveillance data relevant to environmentally linked diseases are scanty, and often not usable except for descriptive purposes. The quality of passively reported information obtained by the authorities is variable. Quito hospitals do not collect data from emergency room visits or hospitalizations in a manner suitable for detailed analysis electronically and records abstraction was performed manually. Nevertheless, the fraction of missing data in the compiled database is ~5%). It is feasible to prospectively collect highly specific information in ER of Baca Ortiz Hospital in electronic format and at low cost. The next natural steps of this study are to abstract historical ER records that will cover a longer time span including dramatic volcanic eruptions of 1999, the period of volcano dormancy and initiate prospective data collection with the emphasis on linking health and environmental information. We believe that at this stage remote sensing data can provide highly valuable information regarding the characterization of ash deposition (42). However, remotely sensed data on ash transport and deposition might have some limitations. For example, current techniques are not uniformly effective in classifying volcanic ash and may falsely interpret meteorological clouds as volcanic ash clouds and conversely (43). Future interdisciplinary studies are required to convert remote sensing data and other data related to volcanic activity into information relevant to public health needs. The efforts to prospectively acquire human health and disease information from sentinel populations, or from representative populations, will build

disease surveillance capacity which will greatly improve our knowledge and decision making at many levels.

ABBREVIATIONS

particular matter (PM), emergency room (ER), acute upper respiratory infection (AURI), acute lower respiratory infection (ALRI), relative risks (RR), confidence intervals (CI), lower boundary of confidence interval (LCI), upper boundary of confidence interval (UCI), tumor necrosis factor (TNF).

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Chapter 4

Air pollution and anemia as risk factors for pneumonia in Ecuadorian children: a retrospective cohort analysis

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ABSTRACT

Background: Ambient air pollution and malnutrition, particularly anemia, are risk factors for pneumonia, a leading cause of death in children under five. We simultaneously assessed these risk factors in Quito, Ecuador.

Methods: In 2005, we studied two socioeconomically similar neighborhoods in Quito: Lucha de los Pobres (LP) and Jaime Roldos (JR). LP had relatively high levels of air pollution (annual median PM_{2.5} = 20.4 µg/m³; NO₂ = 29.5 µg/m³) compared to JR (annual median PM_{2.5} = 15.3 µg/m³; NO₂ = 16.6 µg/m³). We enrolled 408 children from LP (more polluted) and 413 children from JR (less polluted). All subjects were aged 18-42 months. We obtained medical histories of prior physician visits and hospitalizations during the previous year, anthropometric nutrition data, hemoglobin levels, and hemoglobin oxygen saturation via oximetry.

Results: In anemic children, higher pollution exposure was significantly associated with pneumonia hospitalization (OR = 6.82, 95%CI = 1.45-32.00; P = 0.015). In non-anemic children, no difference in hospitalizations by pollution exposure status was detected (OR = 1.04, NS). Children exposed to higher levels of air pollution had more pneumonia hospitalizations (OR = 3.68, 1.09-12.44; P = 0.036), total respiratory illness (OR = 2.93, 95% CI 1.92-4.47; P < 0.001), stunting (OR = 1.88, 1.36-2.60; P < 0.001) and anemia (OR = 1.45, 1.09-1.93; P = 0.013) compared to children exposed to lower levels of air pollution. Also, children exposed to higher levels of air pollution had significantly lower oxygen saturation (92.2% ± 2.6% vs. 95.8% ± 2.2%; P < 0.0001), consistent with air pollution related dyshemoglobinemia.

Conclusions: Ambient air pollution is associated with rates of hospitalization for pneumonia and with physician's consultations for acute respiratory infections. Anemia may interact with air pollution to increase pneumonia hospitalizations. If confirmed in larger studies, improving nutrition-related anemia, as well as decreasing the levels of air pollution in Quito, may reduce pneumonia incidence.

Keywords: anemia, pneumonia, air pollution, children

INTRODUCTION

Pneumonia is the leading cause of childhood death (1, 2). The pathogenesis of pneumonia includes environmental and host factors, such as air pollution and malnutrition (2). Air pollutants are respiratory irritants and increase susceptibility to acute respiratory infections (ARI) (3, 4). Air pollution increases morbidity and mortality largely from respiratory diseases (5), and reducing ambient air pollution increases lifespan (6). Particulate air pollution (PM), especially particles $< 10 \mu\text{m}$ (PM_{10}) and $< 2.5 \mu\text{m}$ ($\text{PM}_{2.5}$) in aerodynamic diameter, and nitrogen oxides (NOx) are specifically linked to childhood ARI (7-9). Outdoor (ambient) air pollution, and indoor pollution from biofuel use, have been identified as major pneumonia risk factors (2, 3).

Globally, many malnourished children are exposed to high levels of air pollution. Also, because malnourished children are known to be at increased risk for pneumonia (1, 2,10,11), a synergistic relationship between ambient air pollution and malnutrition has been postulated (2). Previous studies of pneumonia have examined either air pollution or malnutrition (e.g. (12-15)) but not both risk factors simultaneously. We explored the relationship between air pollution and malnutrition in Quito, Ecuador where both risk factors are prevalent. Our primary objective was to determine if interaction between malnutrition and air pollution was evident. To our knowledge this is the first study of possible interaction effects between ambient air pollution and malnutrition as risk factors for pneumonia in children.

METHODS

Ethics Approval

This study was approved by the Tufts University Institutional Review Board and the Ethical Committee of the Corporación Ecuatoriana de Biotecnología (CEB).

Study Site

Quito is a high-altitude (2830 m) city with significant ambient air pollution largely due to vehicular traffic and combustion (16). The city is flanked by volcanic mountains that act to trap air pollutants by reducing atmospheric mixing (17). Quito's urban slums have high rates of pneumonia and malnutrition (15,18,19). We identified two neighborhoods, Jaime Roldos (JR) and Lucha de los Pobres (LP), of closely comparable socioeconomic status (SES) (Figure 1, Table 1), but which differ in levels of ambient air pollution. The Instituto Nacional de Estadística y Censos (INEC) provided SES and census data for 2001 and the Corporación Para el Mejoramiento del Aire de Quito (CORPAIRE) provided hourly air pollution measurements for 2005 for two monitoring stations located adjacent to the studied neighborhoods.

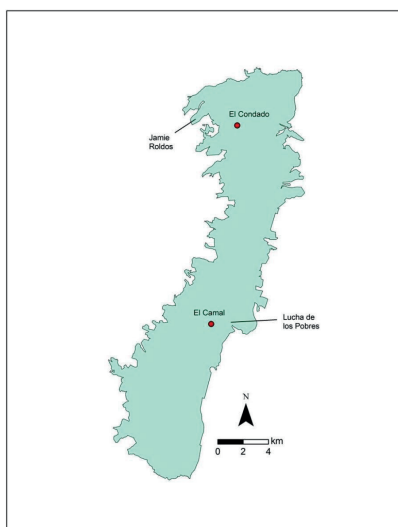


Figure 1. Map of Quito showing the two neighborhoods (Jaime Roldos and Lucha de los Pobres) where the study was conducted.

El Condado and El Camal are sites adjacent to the two study areas where hourly air monitoring data were collected by CORPAIRE during 2005.

Table 1. Socioeconomic indicators in LP (higher air pollution) and JR (lower air pollution).

Indicator	LP	JR	OR (95%CI)	P-value
Population	11751	9009		
Area (hectares)	95	74		
Male	5888	4451	1.03 (0.97-1.09)	0.32
Female	5863	4558	0.97 (0.92-1.03)	0.32
Illiterate (%)	5.3	5.2	1.02 (0.90-1.16)	0.73
Education ^a				
None (%)	37.7	36.6	0.97 (0.83-1.13)	0.72
Primary (%)	37.7	36.6	1.05 (0.99-1.11)	0.10
Secondary (%)	37.7	37.9	1.05 (0.99-1.11)	0.10
Superior and above (%)	4.3	4.0	1.08 (0.94-1.24)	0.28
Household electricity (%) ^b	98.0	98.0	1.00 (0.82-1.22)	0.99
Absence of poverty (%)	4.7	5.2	0.90 (0.79-1.02)	0.14
Working age population (%)	72.6	72.5	1.01 (0.94-1.07)	0.88
Employment by economic sector (persons) ^c			$\chi^2 = 3.23$ (2df)	0.20
Primary	125	87		
Secondary	1437	1226		
Tertiary	3296	2633		

Data provided by INEC. OR, X², and P-values indicate differences between higher and lower air pollution neighborhoods.

^a Educational achievement as reported by INEC; unknown or unreported status for 16.9% of residents of LP and 18.0% of JR.

^b Based on 3231 households in LP and 2379 households in JR.

^c Primary sector = agriculture, secondary sector = industry, tertiary sector = services.

81 of 8885 individuals were not assigned by INEC to employment sectors.

Study Design

Between February and May 2006 we enrolled 408 and 413 children aged 18-42 months in LP and JR, respectively, after using a neighborhood census to identify eligible children. For each child we obtained a medical history using a standardized, pre-tested questionnaire for parents asking if the child was hospitalized or received medical attention for a respiratory illness in the prior year, including diagnoses and treatments. Anthropometric measurements included height, weight, and mid-upper arm circumference (MUAC). Length (age < 24 months) or height (age ≥24 months) was measured (nearest millimeter) using a nondistensible plastic tape glued to a rigid board. Weight was measured (closest 0.1 kilogram) using Detecto® Health-o-meters balances and scales (Webb City, IA, USA). MUAC was measured using a nondistensible plastic tape (closest millimeter). Height and weight were converted into weight-for-age (WAZ) and height-for-age (HAZ) Z-scores using Centers for Disease Control (CDC) reference values in the EpiInfo software package (20). A sterile lancet was used to draw fingerstick blood into a microcuvette analyzer (Hemocue AB, Angelholm, Sweden). Pulse-oximetry blood hemoglobin oxygen saturation (SpO₂) was assessed via an index finger sensor (opposite hand from fingerstick) after sensor readings stabilized. Anemic children were offered iron supplements. Altitude was measured at each house using a GPS receiver (Garmin, Olathe, KS, USA).

Analyses

Statistical analysis was performed using EpiInfo (CDC, EpiInfo for Windows, version 3.4.1) and SPSS for Windows (SPSS Incorporated, SPSS Release 14.0). Demo-graphic and illness data were compared using Pearson χ^2 analysis and Fisher's exact test. Logistic regression models were used for the multivariate analyses of both outcomes: hospitalization due to pneumonia and outpatient consultations due to acute respiratory illness (ARI). Associations were assessed by calculation of the odds ratio (OR) with 95% confidence intervals (CI). Following international conventions, children with height for age (HAZ) z-scores ≤ 2 standard deviations below the mean age and gender adjusted means were termed stunted. Anemia was defined as an adjusted blood hemoglobin < 11.0 g/dL after subtracting 1.9 g/dL to adjust for altitude (21). In order to quantitatively check our assumption of significant air pollution differences by neighborhood, median air pollution levels were tested using the Mann-Whitney test.

RESULTS

There were no significant differences in population density, educational achievement, illiteracy, overall poverty, and other SES indicators between Lucha de los Pobres and Jamie Roldos (Table 1). There was a total of 7808 hourly NO₂ measurements from LP and 8065 from

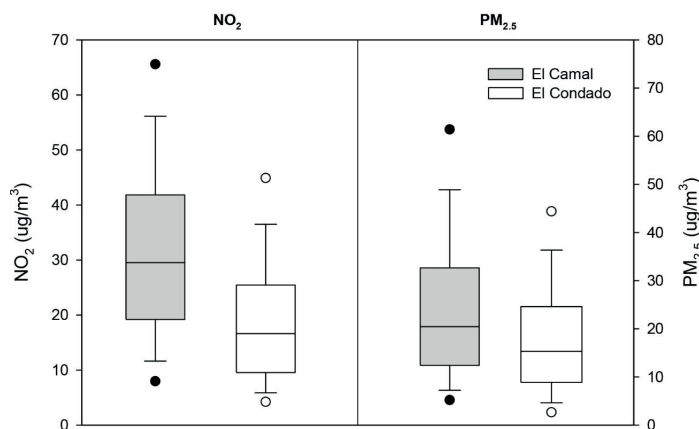


Figure 2. Median PM_{2.5} in µg/m³ and median NO₂ in µg/m³ during 2005 at the air pollution monitoring sites adjacent to the study neighborhoods.

Median PM_{2.5} and NO₂ levels were each significantly lower ($P < 0.001$) at El Condado than at El Camal. El Condado is adjacent to JR, the lower air pollution site, and El Camal is adjacent to LP, the higher air pollution site.

JR, while for PM_{2.5} there were 8449 measurements from LP and 6157 from JR. The annual median NO₂ level for LP was 29.5 µg/m³ and for JR it was 16.6 µg/m³. The annual median concentration of PM_{2.5} for LP was 20.4 µg/m³ and for JR it was 15.3 µg/m³. Box plots of the data are shown in Figure 2. The nonparametric Mann-Whitney test for equal median levels demonstrated highly significant ($P < 0.001$) differences for both pollutants. We considered Lucha de los Pobres to be a community with relatively higher air pollution levels compared to Jamie Roldos, a community with relatively lower air pollution levels.

Complete demographic and anthropometric data were available for 798 (97%) children (Table 2). Neither enrollee age nor gender proportions differed by neighborhood. Children in LP were significantly more mal-nourished (lower HAZ, WAZ, and hemoglobin) than children in JR (Table 2). Likewise, stunting and anemia were significantly more prevalent in LP children than in JR children (Table 2), with odds ratios (ORs) of 1.88 and 1.45, respectively.

High exposure children had significantly lower SpO₂ than low exposure children ($92.2\% \pm 2.6\%$ vs. $95.8\% \pm 2.2\%$; $P < 0.001$; Figure 3). The high exposure households were at a greater mean altitude than the low exposure households (2979 ± 79 versus 2852 ± 47 m, $P < 0.001$), corresponding to 527.2 and 535.7 Torr of atmospheric pressure, respectively. However, this minor pressure difference is not enough to account for the SpO₂ difference (22).

Hospitalizations due to pneumonia, physician consultations for ARI, and air pollution exposure

All 22 hospitalizations were for pneumonia. Of the hospitalized children, 7 resided in the lower air pollution exposure neighborhood while 15 resided in the higher air pollution

Table 2. Demographic, nutritional, and environmental information for children exposed to higher (LP), and lower (JR), air pollution.

	LP N = 401 mean (sd)	JR N = 397 mean (sd)	P
Demographic characteristics			
Age (months)	31.25 (7.51)	31.25 (7.51)	0.94
Males (number, %)	209 (50.6)	224 (54.5)	0.26
Nutritional characteristics			
HAZ (height-for-age)	-1.46 (1.00)	-1.28 (0.87)	0.006
Presence of stunting ^a	125/397	78/401	< 0.001
WAZ (weight-for-age)	-1.31 (1.09)	-1.12 (0.97)	0.010
MUAC (cm)	15.44 (1.09)	15.43 (1.07)	0.900
Adjusted hemoglobin (g/dL) ^b	10.45 (1.39)	10.74 (1.14)	0.002
Presence of anemia ^c	253/401	214/397	0.013
Environmental measures			
SpO ₂ (%)	92.2 (2.60) ^d	95.8 (2.20)	< 0.001
Mean household altitude (m)	2979 (72) ^e	2852 (47)	< 0.001

P-values represent the significance level of t-test comparisons unless otherwise noted.

^a Stunting was defined as a HAZ z-score ≤ -2 (i.e., 2 standard deviations below the mean age- and sex-adjusted height).

^b Hemoglobin was adjusted for altitude by subtracting 1.9 g/dL from the measured value (21).

^c Anemia was defined as an adjusted blood hemoglobin < 11.0 g/dL.

^d n = 400 for the high exposure children (LP), and 397 for lower exposure children (JR).

^e n = 398 for the high-exposure children (LP), 397 for lower exposure children (JR)

exposure neighborhood. Parents were able to recall a specific diagnosis for 97% of physician consultations for all ailments in 2005 and this percentage did not vary between neighborhoods. The most common outpatient diagnoses were asthma, bronchitis, cough, influenza, pharyngitis, pneumonia, sinusitis, and tonsillitis. A total of 172 of 397 children (43%) with lower air pollution exposure required one or more consultations versus 224 of 401 children (56%) with higher air pollution exposure.

Children with higher air pollution exposure were significantly more likely to have had a pneumonia hospitalization (Table 3) or ARI requiring physician consultation (Table 4) than children exposed to lower air pollution exposure. In logistic regression analysis controlling for age, gender, HAZ, WAZ, hemoglobin, and residential altitude, higher air pollution exposure was significantly associated with pneumonia hospitalization (OR = 3.68, 95% CI 1.09-12.44; P = 0.036; Table 3).

There were no significant differences in age, gender, or nutritional parameters (HAZ, WAZ, MUAC, hemoglobin) between previously ill and healthy children. However, SpO₂ was significantly lower in previously ill children compared to healthy children ($93.6 \pm 3.0\%$ vs. $94.5 \pm 3.0\%$; P < 0.001). In chi-square and regression analyses, neighborhood SES factors were not associated with any nutritional, environmental, or illness characteristics or outcomes.

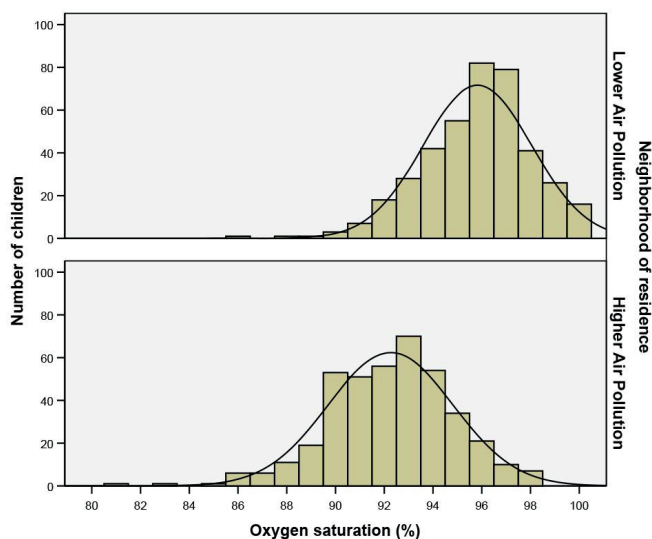


Figure 3. Pulse-oximetry oxygen saturation (SpO_2) values by residential ambient air pollution exposure.

SpO_2 are significantly lower in the high air pollution neighborhood, compared to the lower air pollution neighborhood, $P < 0.001$

Table 3. Hospitalization for pneumonia: Logistic regression analysis controlling for cofactors.

Cofactors	All children (N = 792)			Not anemic (N = 331)			Anemic (N = 461)		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Age	1.11	1.03-1.19	0.004	1.06	0.94-1.18	0.36	1.14	1.04-1.26	0.007
Gender	1.02	0.43-2.44	0.96	0.60	0.13-2.90	0.52	1.35	0.46-4.02	0.59
HAZ	1.48	0.76-2.89	0.25	1.50	0.46-4.85	0.45	1.54	0.66-3.57	0.32
WAZ	0.86	0.48-1.54	0.60	0.81	0.30-2.22	0.69	0.83	0.40-1.73	0.63
Hemoglobin	0.92	0.65-1.31	0.65	0.63	0.20-1.97	0.43	0.76	0.38-1.54	0.45
Air pollution exposure*	3.68	1.09-12.4	0.036	1.04	0.08-13.9	0.98	6.82	1.45-32.0	0.015
Altitude	1.00	0.99-1.01	0.21	0.99	0.98-1.01	0.25	1.01	1.002-1.02	0.017

Table 4. Medical consultations for respiratory illness: Logistic regression analysis controlling for cofactors.

Cofactors	All children (N = 792)			Not anemic (N = 331)			Anemic (N = 461)		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Age	1.01	0.99-1.03	0.23	1.02	0.99-1.05	0.26	1.01	0.98-1.04	0.49
Gender	1.04	0.78-1.38	0.81	0.86	0.52-1.28	0.38	1.24	0.85-1.80	0.26
HAZ	0.97	0.79-1.20	0.78	0.86	0.61-1.20	0.60	1.08	0.82-1.42	0.58
WAZ	0.94	0.78-1.14	0.53	1.09	0.80-1.48	0.60	0.84	0.65-1.08	0.63
Hemoglobin	1.08	0.96-1.20	0.22	1.01	0.71-1.42	0.97	1.11	0.88-1.39	0.38
Air pollution exposure*	2.93	1.92-4.47	< 0.001	3.28	1.72-6.24	< 0.001	2.66	1.50-4.70	0.001
Altitude	1.004	1.002-1.007	0.001	1.004	1.001-1.008	0.022	1.004	1.001-1.007	0.017

Modification of the effect of air pollution exposure by anemia

Fifteen of the 22 hospitalized children were anemic. The presence of anemia increased the OR for pneumonia hospitalization in children with high air pollution exposure from 3.68 to 6.82 (Table 3). In the absence of anemia, higher air pollution exposure was not predictive of pneumonia hospitalization (OR 1.04; 95% CI 0.08- 13.93). In contrast, anemia status did not change the influence of air pollution exposure on ARI events that required consultation with a physician (Table 4).

DISCUSSION

Our most important result is that anemic children exposed to relatively high levels of ambient air pollution are at increased risk of pneumonia hospitalization compared to children exposed to lower levels of air pollution. In addition, we found that children (both anemic and non-anemic) who were exposed to higher levels of air pollution were significantly more likely to have received a physician consultation for acute respiratory illness compared to children exposed to lower levels of air pollution. We also found evidence that anemia and higher air pollution exposure may interact to increase the odds of hospitalization in children with pneumonia. The strongest evidence of the potential interaction effect is that anemia increased the OR for the association between higher air pollution exposure and pneumonia hospitalization from OR (3.68, 95% CI 1.09-12.44; $P = 0.036$) for all children to OR 6.82 (95% CI 1.45-32.00; $P = 0.015$) for anemic children. In non-anemic children, the OR for hospitalization with pneumonia in children exposed to higher levels of air pollution dropped from 3.68 (noted above) to 1.04 (95% CI 0.08-13.9; $P = 0.98$). These results suggest a possible interaction between anemia and ambient air pollution exposure as risk factors. This result is important as these risk factors have not been examined concurrently before, however, our study did not have the statistical power to formally assess the potential interaction between pneumonia hospitalization and anemia status. Larger studies specifically designed to examine this finding are required.

The central pathophysiological deficit in pneumonia is poor tissue oxygenation (23). Anemia and low hemoglobin oxygen saturation independently decrease oxygen delivery. Hypoxia increases mortality during pneumonia two to five-fold (23,24). The association between anemia and pneumonia has been recognized since at least 1925 (25). Anemic children have less oxygen carrying capacity and suffer greater hypoxia during pneumonia than normal children (26). Oxygen saturation was significantly lower ($P < 0.001$) in children with high air pollution exposure. It is biologically plausible that anemia, and the lower hemoglobin oxygen saturation of higher air pollution exposure children, could additively or

multiplicatively decrease tissue oxygenation during pneumonia, especially at high altitude, resulting in more severe disease and more hospitalizations.

Reasons for differential oxygen saturations in normal populations are limited to only two possibilities: either marked differences in altitude, which was not the case in our study (127 m difference), or dyshemoglobinemia (26). Carbon monoxide (CO) and NO_x (particularly NO), produced by vehicular engine combustion, reduce oxygen saturation through formation of the dyshemoglobins carboxyhemoglobin and methemoglobin (27). Vehicular engine combustion is the major source of air pollution in Quito (12, 16, 17).

Previously, Estrella et al. (12) documented elevated carboxyhemoglobin levels in primary school children in Quito. Carboxyhemoglobin levels were $2.52\% \pm 1.12$ in a northern Quito school adjacent to our lower pollution neighborhood, and $5.09\% \pm 1.7$ ($P < 0.05$) in a central city school, adjacent to our higher air pollution neighborhood. Carboxyhemoglobin levels above 2.5% are abnormal; 43% of children living in northern Quito and 92% of the children living in central Quito exceeded this threshold (12). Rare causes of dyshemoglobinemia (anesthetic or dapsone use, high-nitrate water ingestion), are unlikely alternative explanations since few children in this study would have undergone anesthesia or dapsone treatment, and all children shared a low-nitrate municipal water supply. Therefore, the most likely explanation for the marked SpO₂ difference between the higher and lower air pollution exposure children is their differential exposure to common, combustion-related, air pollutants that bind to hemoglobin.

One potential limitation of our O₂ saturation data was that we used non-invasive pulse oximetry rather than directly measuring carboxyhemoglobin and methemoglobin levels, as the latter would have required venous blood sampling, potentially decreasing study participation. However, oximetry underestimates carboxyhemoglobin-related oxygen saturation deficits (26,27), suggesting the deficits we found are conservatively estimated.

This study supports interaction between ambient air pollution levels, anemia, and hospitalization for pneumonia because (1) the OR increased in the setting of both risk factors, and (2) a biologically plausible pathophysiological mechanism for synergy can be identified. However, our study was designed for a straightforward comparison and sample size was too small to conclusively test for significant interaction in either tabular analysis using the Breslow-Day approach or by using an interaction term in the logistic regression model. Mauderly and Samet (2009) noted the difficulty of establishing synergism in air pollution studies because sample sizes of people with impaired health are generally too low for rigorous statistical testing (28). This study, however, is informative for the design of larger studies looking specifically for evidence of this interaction.

The study children resided in impoverished neighborhoods (Table 1) which were carefully chosen to minimize differential nutritional status secondary to family socioeconomic status. We hypothesize that the incrementally worse nutritional status (HAZ, WAZ, and anemia) of

the higher exposure children (Table 2) was due to their incrementally higher incidence of respiratory infections related to higher air pollution (Tables 3 and 4). Higher rates of infection are known to worsen nutritional status, including anemia, in children (1,2,10,11,29).

It could be argued that the increased pneumonia hospitalization of the higher air pollution exposure children was due to their greater malnutrition, and not their air pollution exposure. However, in the entire study population, children with and without a history of respiratory illness did not differ by age, gender, or any nutritional parameters (HAZ, WAZ, MUAC, hemoglobin). The only significant difference between ill and healthy children was the lower oxygen saturation level seen in children with a pneumonia hospitalization or physician consultation ($93.6 \pm 3.0\%$ vs. $94.5 \pm 3.0\%$; $P < 0.001$). We believe this difference in SpO₂ represents air pollution-related dyshemoglobinemia.

Data from a retrospective study must be interpreted cautiously. First, although the site populations did not differ by SES metrics, we did not collect household SES data that potentially could have provided additional insight into the role(s) of various socioeconomic factors. Second, families had to recall health information regarding their children over the previous 12 months. Although we inquired about events that were likely to be remembered (indeed, specific diagnoses were recalled for all (100%) hospitalizations and 495 of 512 (97%) physician consultations), we could not independently verify that all recalled diagnoses were accurate. Third, we did not correct for some potential confounding variables such as biofuel smoke in indoor air or cigarette smoking, however, ~ 98% of households in both sites used gas and not biofuels (Table 1), and therefore it is unlikely that biofuel smoke introduced a major source of bias. Also, in a tobacco-use survey (Harris et al, unpublished data), 30% of adults in LP and JR stated that they smoked cigarettes but 96% of users smoked ≤ 5 cigarettes per day because of cost. Furthermore, Estrella et al (12) found no difference in carboxyhemoglobinemia in children from households with and without tobacco use. These factors suggest that smoking was not a major source of bias in our study. We used data for 2005 from CORPAIRE, which monitors ambient air pollution using accepted methods (17,30) and we assumed that living in a more polluted neighborhood was linked to higher personal exposure to ambient air pollution, a widely accepted assumption (6). Lastly, it is possible that unknown confounding factors we did not measure accounted for the higher rates of pneumonia, rather than the combination of air pollution and anemia.

In summary, we found evidence of possible synergy between a common manifestation of malnutrition-anemia-and ambient air pollution as risk factors for pneumonia hospitalization in a high-altitude setting. Their effects on oxygen delivery could plausibly mediate this association. We suspect air pollution-related dyshemoglobinemia reduces oxygen saturation, while anemia decreases oxygen carrying capacity. Our study suggests that both improving nutrition, and controlling air pollution, could most rapidly decrease hospitalizations for

pneumonia, the leading cause of death in children. Further study is needed to confirm our finding of synergy between air pollution and anemia.

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Chapter 5

Effects of air pollution on lung innate lymphoid cells: review of in vitro and in vivo experimental studies

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ABSTRACT

Outdoor air pollution is associated with respiratory infections and allergies, yet the role of innate lymphoid cells (ILCs) in pathogen containment and airway hyperresponsiveness relevant to effects of air pollutants on ILCs is poorly understood. We conducted a systematic review to evaluate the available evidence on the effect of outdoor air pollutants on the lung type 1 (ILC1) and type 2 ILCs (ILC2) subsets. We searched five electronic databases (up to Dec 2018) for studies on the effect of carbon monoxide (CO), sulfur dioxide (SO₂), nitrogen dioxide (NO₂), diesel exhaust particles (DEP), ozone (O₃), and particulate matter (PM) on respiratory ILCs. Of 2209 identified citations, 22 full-text papers were assessed for eligibility, and 12 articles describing experimental studies performed in murine strains (9) and on human blood cells (3) were finally selected. Overall, these studies showed that exposure to PM, DEP, and high doses of O₃ resulted in a reduction of interferon gamma (IFN-γ) production and cytotoxicity of ILC1. These pollutants and carbon nanotubes stimulate lung ILC2s, produce high levels of interleukin (IL)-5 and IL-13, and induce airway hyperresponsiveness. These findings highlight potential mechanisms by which human ILCs react to air pollution that increase the susceptibility to infections and allergies.

Keywords: lung innate lymphoid cells; ILC; air pollutants; airway hyperresponsiveness

1. INTRODUCTION

Air pollution exposure is associated with an array of respiratory problems, particularly in children because their lungs and immune system are still maturing [1,2,3,4]. Development of lung infections and exacerbation of allergic airway diseases are more frequently found in people living in highly polluted areas [5,6,7,8]. In fact, we found that Ecuadorian children highly exposed to CO, NO₂, fine particulate matter with diameters no greater than 2.5µm (PM_{2.5}) or volcanic ashes presented increased susceptibility to respiratory infections [7], high rates of hospitalization for pneumonia [8], and elevated rate of emergency room visits due to acute upper and lower respiratory infections and asthma-related conditions [9].

PM, DEP, O₃, and other chemicals and polluting compounds have been shown to have deleterious effects on the respiratory function of humans [3,5,6,7,8,10,11,12,13]. Exposure to these outdoor environmental pollutants can result in acute airway inflammation [14,15], increased mucosal secretions [16], oxidative lung damage [17,18,19], and loss of antibacterial functions [20,21,22,23,24]. These conditions may be mediated by a harmful effect of the pollutants on lung immune cells.

The effects of specific air pollutants on certain *classic* lung innate immune cells including alveolar macrophages (AM), polymorphonuclear (PMN), and dendritic cells (DC) have been recently gaining the attention [25,26,27,28]. For instance, it has been reported that particulate matter alters the anti-mycobacterial function of human respiratory epithelium [29] and that DEP impairs antibacterial immunity by suppressing the nucleolar factor NF-κβ pathway in human blood monocytes [30]. Also, concentrated urban particles have been shown to hamper bacterial clearance by AMs and PMNs in mice [31], whereas DEP induces activation of inflammatory signaling molecules and cytokine synthesis in AMs [32]. However, not much is known about the effects of air pollutants on newer sets of immune cells, so-called innate lymphoid cells (ILCs), which play an essential role in lymphoid tissue formation, tissue remodeling, tissue homeostasis [33,34], inflammation, and regulation of host responses to infection [34,35]. These cells are involved in the initiation, modulation, and resolution of lung diseases [36].

ILCs are derived from a bone marrow common lymphoid progenitor [37], have a lymphoid morphology, and lack both antigen-specific receptors and myeloid phenotypic markers. They populate barrier surfaces, including skin, intestine, lung, and some mucosal-associated lymphoid tissues [36]. Three major groups of ILCs have been defined based on the transcription factors needed for their development and the cytokines they produce [37,38,39]. Figure 1 summarizes the current understanding of ILC types, functions and activation pathways. The ILC1 group includes classical natural killer (NK) and non-NK cells, which are cytotoxic and produce interferon gamma (IFN-γ) [40,41]. The ILC2 group expresses interleukin (IL) IL-5 and IL-13 [41]. The type 3 (ILC3) group comprises of LTi cells, NKp46⁺ ILC3,

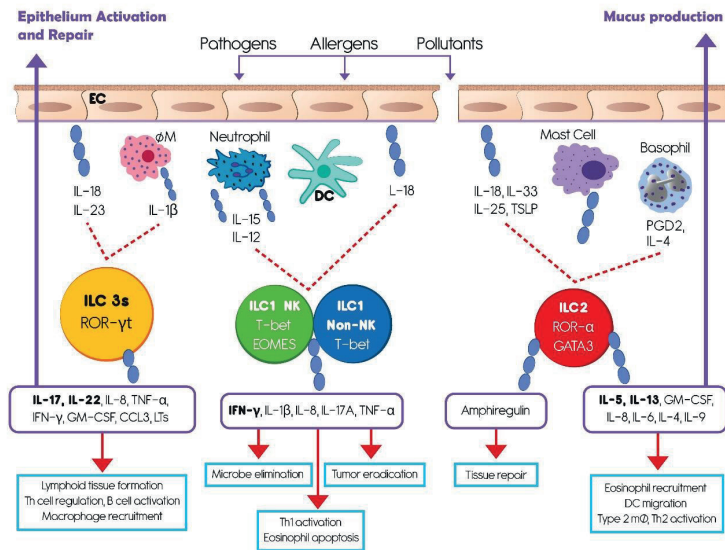


Figure 1. Summarizes the understanding of innate lymphoid cell (ILC) types, activation pathways and functions. Innate lymphoid cells are derived from a common lymphoid progenitor, have a lymphoid morphology, and lack antigen-specific receptors. Based upon the transcription factors needed for their development and the cytokines they produce, ILCs are divided in three groups which mainly populate barrier surfaces. ILC1 includes classical natural killer (NK) and non-NK cells and depends on the transcription factor T-bet. ILC2 depends on the transcription factors GATA3 and RORα. ILC3 requires the transcription factor ROR-γt and comprises a heterogeneous subset of cells. After external antigen contact, respiratory epithelial cells and classical innate immune cells produce several cytokines which stimulate different ILCs groups. IL-12, IL-15, IL-18 prime ILC1s to produce IFN-γ and other cytokines involved in microbe elimination, Th1 activation, and tumor eradication. ILC2s are activated by IL-4, prostaglandin D2 (PGD2), IL-33 and IL-25 to produce amphiregulin involved in tissue repair, IL-5 to recruit eosinophils, and IL-13 to stimulate mucus production by epithelial cells. ILC3s are primed by IL-18, IL-23, and IL-1β to produce principally IL-17 and IL-22 which participate in lymphoid tissue formation, Th cell regulation, B cell activation, and epithelium activation and repair. EC, epithelial cell; MΦ, macrophage; DC, dendritic cell; PGD2, prostaglandin D2; TSLP, Thymic Stromal Lymphopoietin.

and NKp46⁺ ILC3, which produce IL-17 and/or IL-22 [41]. Due to their cytokine production, ILCs 1, 2, and 3 resemble the adaptive T helper (Th) 1, Th2, and Th17 cells respectively [42]. In addition, human ILCs are highly heterogeneous among patients, tissues, and health conditions because they exhibit diversity in the expression of their surface markers [43,44].

In this paper, we systematically appraised all available studies to date that examined the effect of PM, O₃, and DEP on lung ILC subsets in *in vivo* and *in vitro* models. While the role of ILCs in inflammation and the effects of air pollutants on classic innate immune cells are recognized, the effects of air pollutants on the lung ILCs remains scarce. Defining such

relationships may help to understand how air pollutants affect respiratory infections and allergies. These investigations may also provide insights on potential strategies for improving diagnosis and treatment for these diseases.

2. MATERIALS AND METHODS

The systematic review was conducted using the general principles of the PRISMA-P (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) [45] aimed to facilitate the development and reporting of systematic review protocols. The study protocol is available online [46].

2.1. Search Strategy

For the literature search, no limits were set on study designs, language or year of publication. We searched five electronic databases: Embase (via Embase.com), Medline (via Ovid), Web of Science, Cochrane Central, and Google Scholar from their inception until May 2018 for studies that measured the effect of air pollution exposure on number, viability, or function of any of the ILCs. The search strategy was constructed in cooperation with a medical information specialist (Wichor M Bramer, Medical Library, Erasmus MC) and combined terms related to air pollution (e.g., “air pollution”, “CO”, “DEP”, “PM2.5”, or “O3”) with those related to ILCs (e.g., “innate lymphoid cells”, “ILC1”, “ILC2”, “ILC3”, or “NK”), respiratory health (e.g., “asthma”, “respiratory infections”), or immunity (e.g., “innate immunity” or “cytokines”). We additionally checked references cited in selected articles and hand searched the PubMed engine database to retrieve additional articles. The full search strategy is provided in the Supplementary material, Resource S1: Search terms, and Figure 2.

2.2. Screening and Eligibility Criteria

Article titles and abstracts were initially screened for eligibility by two authors (B.E., M.C.). We included all studies based on cohorts, cross-sectional, experimental, in vitro, or in vivo designs in either animals or humans, that examined the effect of air pollutants exposure (measured by either pollution measurements or serologic markers of exposure) on lung ILCs and/or cytokines. We excluded systematic reviews, comments, consensus reports, editorials, guidelines, and protocols.

2.3. Study Selection

First, we read the abstract of the retrieved references and selected those that included any of the specified respiratory health (e.g., asthma, airway hyperreactivity, and respiratory infection), as well as ILCs cell number and viability, cytokine production and activity, infection susceptibility and allergen-induced response, and airway hyperresponsiveness.

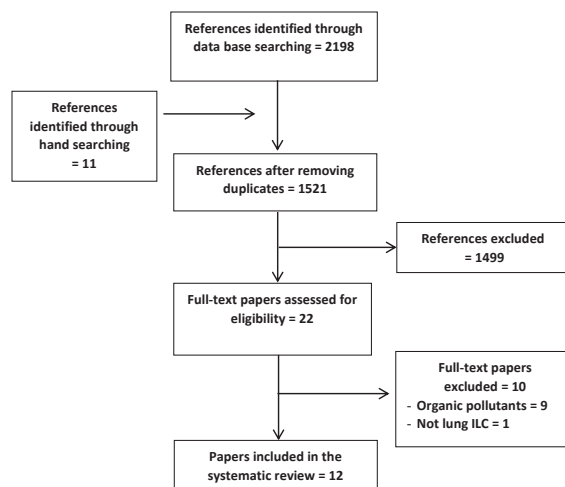


Figure 2. Flow diagram of study selection.

Second, we read the full paper of the selected abstracts to confirm if they fulfilled all selection criteria. Disagreements were resolved by consensus and in consultation with a third independent reviewer (Josje Schoufour, Department of Epidemiology, Erasmus MC).

2.4. Data Extraction

Extracted data from each article were registered in a predesigned form to record study design, analysis unit, and type of exposure, route and doses of exposure, experimentation arm/groups, findings, and conclusions (Supplementary Material Resource S2: Methodological details of the studies).

2.5. Quality Assessment of the Evidence

The quality of each study was assessed in terms of reproducibility of experimental methods and results, using a modified version of the Animal Research Reporting in vivo Experiments (ARRIVE) guidelines [47], and an adapted scale for in vitro experiments in human cells (Supplementary Material, Resource S3: Modified ARRIVE guidelines, and Resource S4: Adapted scale from ARRIVE guidelines for experimental studies in human cells).

2.6. Synthesis of the Evidence

The diversity in study designs, models, doses, and ways of assessing exposure and outcomes, did not allow us to carry out comparative quantitative analysis. Instead, we

provided a qualitative overview of the extracted data and characterized the studies, exposures, outcomes, and the main findings.

3. RESULTS

The search of electronic databases and hand searching provided 2209 citations. After removing duplicates, 1521 unique titles remained. Of these, 1499 studies were excluded based on the initial screening criteria. For the remaining 22 references, full-text papers were retrieved and further assessed for eligibility. From these, ten studies were not considered for the purpose of this review because one study did not measure lung ILCs and nine did not include the inorganic pollutants as the study objective. These studies were designed to induce allergic airway inflammation and observe how ILCs interact with the adaptive immune system, particularly Th2. (Supplemental Material, Resource S5) (For flow diagram see Figure 2). Out of the 12 remaining articles selected for the final evaluation, six studies investigated the effects of different pollutants on NK cells and six studies examined the effects on various other ILCs (Table 1). There were nine studies based on in vivo and in vitro murine experimental models [48,49,50,51,52,53,54,55,56], and three studies based on ex vivo and in vitro human cell models [57,58,59].

Regarding the quality of the experimental procedures, experimental animals, and experimental outcomes, all murine model studies met at least 75% of requirements stipulated by the modified ARRIVE guidelines. Experimental procedures and experimental outcomes of the human cell studies met at least 89% of requirements stipulated by the adapted scale (Supplementary Material, Resource S3: Modified ARRIVE guidelines, and Resource S4: Adapted scale from ARRIVE guidelines for experimental studies in human cells). These percentages ensure the reliability of the in vitro experimental procedures.

Table 1 lists the 12 selected studies and their main findings, and Table 2 integrates the main findings per cell type, focusing first on ILC1-NK cells and then summarizing the effects of air pollutants on ILC2 cells. Overall, the studies show the diversity in study designs, models, doses, and ways of assessing exposure and outcomes. They were:

- (a) 3 studies on the effects of O₃ on ILC2 (all in mice) [51,52,55],
- (b) 3 studies on the effects of O₃ on NK cells (1 in mice and 2 in humans) [54,58,59],
- (c) 1 study on the effects of carbon nanotubes on ILC2 (in mice) [48],
- (d) 1 study on the effects of DEPs on ILC2 (in mice) [49],
- (e) 2 studies on the effects of DEPs on NK cells (1 in mice and 1 in humans) [50,57],
- (f) 1 study on the effects of PM_{2.5} on ILC2 (in mice) [56],
- (g) 1 study on the effects of PM_{2.5} on NK cells (in rats) [53].

Table 1. Characteristics and main findings of the studies.

Authors, Year	Type of Exposure (Doses; Method of Administration)	Outcome	Summary of Findings/Observed Effects of Exposure on the Outcome
<i>Type of Cell: Mice ILCs</i>			
Beamer, et al. 2013 [48]	Multi-walled carbon nanotubes (50 µg; oropharyngeal)	IL-33 function on ILC2	<ul style="list-style-type: none"> • Epithelial cells (type II pneumocytes) in the lavage fluid induce secretion of IL-33 • Elevated levels of IL-33 induce recruitment of ILCs in the airways • ILCs acting in response to IL-33 stimulate AHR and eosinophil recruitment through the release of IL-13
<i>Type of Cell: Mice ILC2</i>			
De Grove, et al. 2016 [49]	DEP (25 mg on days 1, 8, and 15; intranasal)	Function and cytokine production	<ul style="list-style-type: none"> • DEP alone has little effect but enhances the effects of house dust mite (HDM) exposure • Marked increase in epithelium-derived cytokines IL-25 and IL-33 • Increased numbers of DCs, neutrophils, ILC2s, CD41 T cells, CD81 T cells, and eosinophils. • ILC2s marginally contribute to DEP-enhanced allergic airway inflammation • Dysregulation of ILC2s and Th2 cells attenuated DEP-enhanced allergic airway inflammation. • A crucial role for the adaptive immune system on concomitant DEP plus HDM exposure
Mathews, et al. 2017 [51]	O ₃ (2 ppm for 3 hours; inhaled)	IL-33 action on ILC2 and γδ T	<ul style="list-style-type: none"> • Interaction between Obesity and O₃ • Increased lung IL-13+ innate lymphoid cells type 2 (ILC2) and IL-13+ γδ T cells in obese mice • Increased ST2+γδ T cells, indicating that these cells can be targets of IL-33, • O₃ induced type 2 cytokine expression in ILC2s and γδ T cells in obese mice • Little or no effect of O₃ on IL-33 in lean mice. • ILC2s and γδ T appear to contribute to the effects of IL-33
Yang, et al. 2016 [52]	O ₃ (3 ppm for 2 hours on day 16; inhaled)	IL5 and IL13 RNA expression	<ul style="list-style-type: none"> • O₃ exposure increased airway levels of IL-33, a potent activator of lung ILC2s • Lung-resident ILC2s were the predominant early source of the Th2 cytokines IL-5 and IL-13 in O₃-exposed mice • No ILC2 influx or proliferation within 12 hours after O₃ exposure • ILC2s from the lungs: greater increased activation of IL5 and IL13 mRNA 12 hours after O₃
Kumagai, et al. 2017 [55]	O ₃ (0.8 ppm on day 1 or for 9 consecutive weekdays; inhaled)	ILC2 in airway inflammation, mucus cell metaplasia, and Type 2 immunity	<ul style="list-style-type: none"> • O₃ induced pulmonary eosinophilic inflammation in ILC sufficient mice • O₃ induced mucus cell metaplasia in proximal airway epithelium • O₃ increased mRNA transcripts of type 2 immunity in lung

Table 1. Characteristics and main findings of the studies (continued).

Lu, et al. 2018 [56]	PM _{2.5} (25 mL/kg of a suspension of 15 g/L on days 1, 8, 15, and 21; intranasal)	ILC2-related transcription factors	<ul style="list-style-type: none"> Increased expression of RORα and GATA3 transcription factors, which are vital factor for ILC2. Increased IL33-levels which activates ILC2s
Type of Cell: Rats NK			
Burleson, et al. 1989 [54]	O ₃ (1.0 ppm for 23.5 hours /day on 1, 3, 7, or 10 consecutive days; ambient)	Number and function of NK, and function of adherent cells	<ul style="list-style-type: none"> O₃ induced suppression of pulmonary NK activity Cell/products involved in NK activation mediate the immunosuppression O₃ decreased number but not viability of NK
Zhao, et al. 2014 [53]	PM _{2.5} (1, 5, or 10mg/kg body weight; intratracheal)	Number and bacterial response	<ul style="list-style-type: none"> PM_{2.5} increases susceptibility to respiratory infection by <i>S. aureus</i>. PM_{2.5} decreases the number of NK cells in the lung and suppress AM phagocytosis, which provides a potential mechanism to explain that association between ambient air pollution and pulmonary bacterial infections
Type of Cell: Mice NK			
Finkelman, et al. 2004 [50]	DEP (2 mg once; injected i.p.)	INF gamma production	<ul style="list-style-type: none"> DEP potently inhibits IFN-γ production by NK and NKT cells, which is rapid in onset, long lasting, and dose-related DEP induces an inhibitory effect on steady-state INF-γ mRNA levels and may also suppress INF-γ production through posttranscriptional mechanisms
Type of Cell: Human NK			
Müller, et al. 2013 [57]	DEP (10 µg/mL; direct exposure of cell)	Function and cytokine release	<ul style="list-style-type: none"> DEP reduced expression of the cytotoxic NK cell surface marker CD16, gene and protein expression of granzyme B and perforin, and the ability to kill target cells
Kucuksezer, et al. 2014 [58]	O ₃ (1, 5, 10, and 50 mg/mL cRPMI; direct exposure of cell)	Number, function	<ul style="list-style-type: none"> O₃ increased number of CD16 cell and cytotoxicity of NK
Müller, et al. 2013 [59]	O ₃ (0.4 ppm; direct exposure of cell)	Effect of O ₃ exposed epithelial cells on natural killer cells function, cytokine release.	<ul style="list-style-type: none"> O₃ reduced markers of activation, INF-γ production, and cytotoxic function. O₃ upregulated ligands for NK in epithelial cells.

Table 2. Effects of air pollutants on NK cells and ILC2: integration of findings.

NK cell features ^a						
Exposure	PM _{2.5}	DEP		O ₃		CN
Model	Rats	Mice	Human	Rats	Human	Mice
Number	↓NK BALF ↓Influx into airways	↓NK in spleen		↓ % lung lymphocytes	Low doses: ↑ number	
Cytokine		↓IFN-γ	↑IL-1β ↑ IL-8 ↑ TNF-α no changes in INF- γ		↓ IFN-γ	
Activity	↑ Susceptibility to respiratory infection by <i>S. aureus</i>		↓ Cytotoxicity ↓ CD16 expression ↓ Granzyme B levels ↓ Perforin levels	↓ Pulmonary NK activity	↓ Cytotoxicity ↓ Granzyme B levels ↓ Markers of cytotoxicity Low doses: ↑ cytotoxicity	

ILC2 cell features ^b						
Exposure	PM _{2.5}	DEP		O ₃		CN
Model	Rats	Mice	Human	Mice	Human	Mice
Number		↑ in alveolar space but not in lungs		No effect in lean mice ↑IL-5+ and IL-13+ ILC2s in BAL in obese mice		↑ in lung
Cytokine		↑IL-5 ↑IL-13		↑IL-5 ↑IL-13		↑IL-13
Air way hyperresponsiveness (AHR)	Enhances AHR: ↑ RORα and GATA3 transcription factors related to ILC2	Enhances AHR: Accumulation of ILC2s and Th2 cells and type 2 cytokine production		Induces AHR: ↑ expression of lung mRNA transcripts associated with type 2 immunity		Induces AHR: ↑ IL-13 from ILC2

3.1. Effects of Air Pollutants on ILC1-NK Cells

NK cells play a fundamental role in the immunity against intracellular infections and tumor immune surveillance (Figure 1). Overall, the summary showed that exposure to air pollutants results in decreased or impaired NK cell number, and modified cell function and cytokine release (Table 2).

3.1.1. Cell Number and Viability

Studies in murine strains demonstrated that PM_{2.5} exposure decreases significantly both the absolute NK cell number in broncho-alveolar lavage fluid (BALF) and the NK cell influx into the airway lumen at 24 h post *S. aureus* infection [53]. Similarly, DEP exposure decreased the number of NK cells in spleen in mice [50]. Concerning O₃ exposure, this pollutant decreases the percentage of lung lymphocytes in mice, although without effect on their viability [54]. In contrast, in human cells, 3–5 days of low dose (1mg/mL) O₃ exposure significantly increased the total NK cell population defined as CD3-CD16+/56+ without significant changes among the expression levels of other surface molecules [58].

3.1.2. Cytokine Production

NK cells stimulated by IL-12 or IL-18, secreted from dendritic cells and macrophages, produce several cytokines, principally IFN- γ , IL-1 β , IL-8, IL-17A, and tumor necrosis factor alpha (TNF- α), which are involved in lung inflammatory processes and infection resistance (Figure 2). DEP exposure caused strong, rapid in onset, long-lasting, and dose-related suppression of IFN- γ production in murine NK following lipopolysaccharide (LPS) stimulation; this suppression was due to the inhibition of the IL-12, and IL-18 response to LPS by accessory cells as well as by a direct inhibitory effect on IFN- γ mRNA levels, partly through post-transcriptional mechanisms [50]. In NK cells from healthy non-smoking non-asthmatic volunteers, DEP produced a modest increase of IL-1 β , IL-8, and TNF- α release with no changes in IFN- γ [57]. O₃ exposure also reduces the expression of IFN- γ on human NK cells by affecting the direct cell-cell interactions between epithelial and NK cells, and it is dependent on UL16 binding protein 3 (ULBP3) and major histocompatibility complex class I chain-related protein A and B (MICA/B) on epithelial cells [59].

3.1.3. Activity

Cytotoxic activity of the NK cell is achieved through the release of granzyme B and perforin once the activating receptors have recognized their ligands (Figure 1). Activating receptors include NKp46, NKp44, CD16, CD69, and NKG2D. The murine experiments with the preceding PM_{2.5} exposures have demonstrated that: (a) exposure triggers a significant increase in bacterial load in the lung of rats infected by *S. aureus*; and (b) adoptive NK cell transfer to the lung of those rats markedly reduces the bacterial load to a level comparable to control rats that were infected with *S. aureus*, but not exposed to PM_{2.5}. A potential mechanism explaining these observations is that alveolar macrophages, cultured with NK cells, have a high rate of phagocytosis of *S. aureus*, and suggests that interactions between NK cells and lung macrophages facilitate better control of bacterial infection by innate phagocytic cells [53]. In vitro and ex vivo experiments in human blood cells showed that exposure to DEP alone significantly reduces the cytotoxic potential of NK cells as compared to controls. At

the same time, exposure to DEP, in the context of stimulation with the viral mimetic polyI:C, decreases the expression of CD16, granzyme B, and perforin, and suppresses the ability of NK cells to kill target cells without affecting the percent of NKG2D+ and NKp46+ cells [57]. In murine strains, continuous exposure to 1.0 ppm O₃ for 1, 5, and 7 days had a significant immunosuppressive effect on pulmonary NK cell activity compared to controls, but this effect was reversed after 10 days of continuous O₃ inhalation [54]. Furthermore, it has been demonstrated that the effect on pulmonary NK cells involved several cell types and/or their products that stimulate NK cells [54].

Similarly, studies on the interrelation between O₃-exposed human epithelial cells and NK cells showed that direct exposure to O₃ reduces, although non significantly, the expression of NK cell receptors (NKG2D and NKp46), the intracellular levels of granzyme B, and cytotoxicity function [59]. Yet, in another study, low doses of O₃ exposure (1 mg/mL and 5 mg/mL) induced an increase in human NK-cell cytotoxicity without a significant difference between doses [58].

3.2. Effects of Air Pollutants on ILC2

ILC2s play critical roles in immune protection, tissue repair, brown fat biogenesis, and in the regulation of the inflammatory process (Figure 1). Air pollutants stimulate or inhibit ILC2s as shown in experiments in mouse models (Table 2).

3.2.1. Cell Number and Viability

ILC2s (nuocytes) represent approximately 1% of the total number of cells in the whole lung lavage in mice [48]. Exposure to DEP plus house dust mite (HDM) increased the number of cytokine-expressing ILC2s along with Th2 T cells in the alveolar space of mice, but not in the lung tissue itself [49]. O₃ exposure did not affect the total number of pulmonary ILC2s in non-obese mice [52,55], suggesting no influx or proliferation within 12 h after O₃ exposure in such mice [52]. However, in obese mice O₃ increased the number of IL-5+ and IL-13+ ILC2s [51]. Conversely, exposure to carbon in a multi-walled carbon nanotube experiment in two strains of mice resulted in a significant increase of ILC2 numbers [48].

3.2.2. Cytokine Production and Activity

Exposure to DEP plus HDM also increased IL-5 and IL-13 levels in the BALF ILC2s compared with DEP alone and saline exposed groups, but this increase was also seen in Th2 cells, which was the principal source of those cytokines [49]. Experimental studies in mice showed that O₃ [51,52] and multi-walled carbon nanotube [48] exposure induced the production of IL-33 by lung tissue which in turn activated several cells in the lung including Th2 T cells and ILC2 cells to produce IL-13 [48,51,52] and IL-5 [51,52]. In fact, it was reported that 12 h of O₃ exposure significantly increased the transcription of IL-5 and IL-13 mRNA with the

consequent increase of IL-5 and IL-13 production [52]. Additionally, it was confirmed that ILC2s were the principal source of IL-5 and IL-13 since O₃ exposure of CD4⁺ Thy1⁺ Th cells isolated from the lungs did not induce those cytokines 12 h after exposure [52], and that bronchoalveolar lavage type 2 cytokines were not significantly reduced in O₃-exposed obese mice when CD4⁺ T cells were depleted by treating mice with a depleting anti-CD4 antibody [51].

3.2.3. Allergen-Induced Response and Airway Hyperresponsiveness (AHR)

IL-33, IL-5 and IL-13 are critical for AHR since they induce granulocyte infiltration and changes in airway epithelia (Figure 1). In an ovalbumin murine asthma model, exposure to PM_{2.5} exacerbated the asthma symptoms by significantly increasing the expression of ROR α and GATA3 levels in peripheral blood mononuclear cell, transcription factors related to ILC2, suggesting that ILC2s play a crucial role in serious asthma induced by PM_{2.5} [56].

AHR was significantly enhanced by DEP exposure in HDM exposed mice due to accumulation of ILC2s and Th2 cells and type 2 cytokine production (IL5 and IL-13); however, the contribution of ILC2 after DEP exposure was marginal since ILC2 deficient mice exposed to DEP showed AHR depending on Th2 cells activation [49].

ILC-sufficient mice exposed for 9 days to ozone showed significantly greater BALF eosinophils, mucous cell metaplasia with more mucins in the proximal airway epithelium, and increased expression of lung mRNA transcripts associated with type 2 immunity than air-exposed mice [55]. Additionally, mice treated with anti-Thy1.2 antibodies, which significantly reduce a total number of both ILCs and T cells, dramatically lost the O₃-induced influx of eosinophils and mucous cells metaplasia [55]. Importantly, intra-tracheal transfer of ILC2s to mice treated with anti-Thy1.2 showed that O₃-exposed mice stimulated with methacholine present dramatically enhanced AHR compared to air-exposed mice [52].

Concerning carbon nanotube exposure, this regimen also induced AHR in mice through IL-13 action. The mechanism was via activation of epithelial cells to produce IL-33, which in turn recruits and stimulates ILC2 to produce IL-13 [48].

3.3. Effects of Air Pollutants on ILC3

No studies were identified that explored the effects of air pollutants on ILC3 numbers, viability, or cytokine production.

4. DISCUSSION

In this systematic review, we showed that PM, DEP, O₃, and carbon nanotubes affect ILCs in the respiratory system in two ways. The pollutants generally inhibit ILC1 (NK cell) cytotoxicity

and cytokine (IFN- γ) production, thereby increasing the susceptibility to infections and allergies. The pollutants also stimulate ILC2 to produce IL-5 and IL-13, thereby increasing airway hyperresponsiveness.

With regard to mechanism of action, the reviewed studies suggest that exposure to particulate contaminants (DEP and PM_{2.5}) and high doses of O₃ impair lung NK cell activity directly by decreasing expression of the activating receptors CD16 and CD69, granzyme B and perforin levels, and also indirectly by dampening the activity of neighboring cells implicated in NK cell activation. In addition, these same pollutants decrease IFN- γ secretion of NK cells, an important cytokine responsible for stimulating macrophages [60,61,62] and for inducing adaptive Th1 differentiation and function [63,64,65]. These alterations of NK cells jointly lessen the ability of the organism to fight against intracellular pathogens, viruses in particular, and may thereby result in increased susceptibility to respiratory infections.

With regard to ILC2 cells, experimental studies in murine strains indicate that DEP, O₃, and multi-wall carbon nanotubes activate ILC2 to produce IL-5 and IL-13, via a larger production of IL-33. As IL-5 and IL-13 are also produced by Th2 cells and are responsible for IgE class switching on B cells and the recruitment and activation of eosinophils [66,67,68,69], ILC2s stimulated by air pollutants likely act synergistically with Th2 cells to mount allergic respiratory processes, such as rhinitis and asthmatic episodes associated to air pollutants.

This review contributes to our understanding of the role that ILCs play in the effect of environmental air pollutant exposure on respiratory infections and allergic disease. Our findings may be useful to identify potential intervention strategies, targeting the key molecules which enhance pulmonary NK cell function to protect against infections or to control an excessive activation of ILC2, thus decreasing the production of allergy-related cytokines and minimizing airway allergic inflammation. With respect to the development of preventive strategies, a comprehensive solution against harmful effects of air pollution on the respiratory health should require the implementation of and compliance with a broad range of policies to improve air quality [70].

We excluded nine studies that addressed the crosstalk between ILCs, particularly ILC2, and adaptive type 2 immune response during a natural airborne allergen-induced allergic airway inflammation, but not included any of the inorganic pollutants. The general conclusion is that both type of cells work together in a bidirectional way to maintain airway hyperreactivity. In the supplementary material we provided the list of the excluded studies.

This is the first comprehensive systematic review that builds a general picture of how ILCs are affected by different air pollutants and how respiratory health is affected. Since most of the evidence included in this review is based on *in vitro* and *in vivo* studies, thus the extrapolation of our findings to general population may be difficult. The experimental models could also underestimate or overestimate the effects of contaminants on the ILCs because a) the animal studies use inbred strains, which may differ in immune responsiveness

to the heterogeneous responses found in the general population; b) the doses and duration of the exposure used in the experimental conditions may not represent real-life exposures; and c) the relationship between a dose of exposure and number of exposed cells may not accurately represent reality. Therefore, it would be important that future studies examine the effects of air pollution on human ILCs, for example in sputum samples and, preferably, in combination with reliable exposure measurements. These studies should help to understand the role and mechanisms underlying the participation of these cells in infections and airway hyperreactivity associated with air pollutants exposure.

5. CONCLUSIONS

This systematic review of available studies on air pollution and ILCs shows that air pollution impairs the function of ILCs, increasing the susceptibility to infections and allergies. Exposure to key air pollutants, including PM, DEP, and O₃ stimulate lung ILC2s to produce high levels of IL-5 and IL-13 and generally inhibit the cytotoxicity and IFN- γ production by ILC1-NK cells. These processes most likely play an essential role in the airway hyperresponsiveness and increased susceptibility to respiratory infections triggered by exposure to air pollution. Our findings also highlight substantial gaps in knowledge and the need to better understand the mechanisms by which human immune systems react to air pollution.

SUPPLEMENTARY MATERIALS

The following are available online at <https://www.mdpi.com/1660-4601/16/13/2347/s1>. Resource S1: Search terms, Resource S2: Methodological details of the studies, Resource S3: Modified ARRIVE guidelines, Resource S4: Adapted scale for in vitro experiments in human cells, Resource S5: List of the excluded studies.

AUTHOR CONTRIBUTIONS

Conceiving and designing the study, searching process, study selection, quality assessment, data extraction, data analysis, manuscript preparation: B.E.; conceiving and designing the study, searching process and study selection: M.C.; contribution to the text and writing, review and editing: E.N.N., P.D.K., T.V.; supervision and editing: H.A.D.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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SUPPLEMENTARY MATERIAL

Resource S1. Search terms

Five databases were searched in cooperation with a medical information specialist to identify relevant studies: Embase.com, Medline Ovid, Web of science, the Cochrane Library, Google scholar)

Embase.com 1118

('air pollution'/de OR 'air pollutant'/exp OR 'air pollution indicator'/de OR 'environmental exposure'/de OR 'exhaust gas'/de OR acetylene/de OR benzene/de OR '1, 3 butadiene'/de OR 'carbon monoxide'/de OR dust/de OR ethane/de OR ethylbenzene/de OR ethylene/de OR 'airborne particle'/de OR 'nitrogen dioxide'/de OR 'particulate matter'/de OR toluene/de OR xylene/de OR 'polycyclic aromatic hydrocarbon'/exp OR combustion/de OR 'black carbon'/de OR 'volatile organic compound'/de OR 'tobacco use'/exp OR 'smoking and smoking related phenomena'/exp OR 'carbon nanotube'/de OR ((air NEAR/3 (clean*)) OR ((environment* OR personal) NEAR/6 (expos* OR toxican*)) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon NEXT/1 monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* NEAR/3 matter*) OR (nitro* NEXT/1 dioxide*) OR pm1 OR 'pm2 5' OR pm10 OR 'pm 1' OR 'pm 2 5' OR 'pm 10' OR soot OR toluene* OR xylene* OR ufp* OR 'black carbon' OR (polycyclic NEAR/3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile NEAR/3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex OR smoke OR smoking OR tobacco OR (carbon NEAR/3 nanotube*)):ab,ti) AND ('lymphoid cell'/exp OR 'cytokine'/exp OR 'cytokine response'/exp OR (((lymphoid) NEAR/3 cell*) OR lymphocyte* OR ilc OR ilcs OR (natural* NEAR/3 killer*) OR cytokine* OR interferon* OR ifn OR interleukin*):ab,ti) AND (asthma/exp OR 'respiratory tract infection'/exp OR 'respiratory tract inflammation'/exp OR (asthma* OR (airway* NEAR/6 (hyperreactiv* OR allerg* OR inflammat* OR immun*)) OR ((respiratory* OR airway*) NEAR/3 infect*)):ab,ti) AND ('innate immunity'/de OR (innate* OR non-specific* OR nonspecific* OR in-born OR inborn):ab,ti) NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Editorial]/lim)

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("air pollution"/ OR exp "Air Pollutants"/ OR "environmental exposure"/ OR "Vehicle Emissions"/ OR acetylene/ OR benzene/ OR "Benzene Derivatives"/ OR "butadienes"/ OR "carbon monoxide"/ OR dust/ OR ethane/ OR ethylenes/ OR "nitrogen dioxide"/ OR "particulate matter"/ OR toluene/ OR "Polycyclic Hydrocarbons, Aromatic"/ OR ((air ADJ3 (clean*)) OR ((environment* OR personal) ADJ6 (expos* OR toxican*)) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene*

OR (carbon ADJ monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* ADJ3 matter*) OR (nitro* ADJ dioxide*) OR pm1 OR "pm2 5" OR pm10 OR "pm 1" OR "pm 2 5" OR "pm 10" OR soot OR toluene* OR xylene* OR ufp* OR "black carbon" OR (polycyclic ADJ3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile ADJ3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex OR smoke OR smoking OR tobacco OR (carbon ADJ3 nanotube*).ab,ti.) AND (exp "Lymphocytes"/ OR exp "Cytokines"/ OR (((lymphoid) ADJ3 cell*) OR lymphocyte* OR ilc OR ilcs OR (natural* ADJ3 killer*) OR cytokine* OR interferon* OR ifn OR interleukin*).ab,ti.) AND (asthma/ OR "respiratory tract infection"/ OR "respiratory tract inflammation"/ OR (asthma* OR (airway* ADJ6 (hyperreactiv* OR allerg* OR inflammat* OR immun*)) OR ((respiratory* OR airway*) ADJ3 infect*).ab,ti.) AND ("Immunity, Innate"/ OR (innate* OR non-specific* OR nonspecific* OR in-born OR inborn).ab,ti.) NOT (letter OR news OR comment OR editorial OR congresses OR abstracts).pt.

Cochrane 3

((air NEAR/3 (clean*)) OR ((environment* OR personal) NEAR/6 (expos* OR toxican*)) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon NEXT/1 monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* NEAR/3 matter*) OR (nitro* NEXT/1 dioxide*) OR pm1 OR 'pm2 5' OR pm10 OR 'pm 1' OR 'pm 2 5' OR 'pm 10' OR soot OR toluene* OR xylene* OR ufp* OR 'black carbon' OR (polycyclic NEAR/3 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile NEAR/3 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex OR smoke OR smoking OR tobacco OR (carbon NEAR/3 nanotube*)):ab,ti) AND (((lymphoid) NEAR/3 cell*) OR lymphocyte* OR ilc OR ilcs OR (natural* NEAR/3 killer*) OR cytokine* OR interferon* OR ifn OR interleukin*):ab,ti) AND ((asthma* OR (airway* NEAR/6 (hyperreactiv* OR allerg* OR inflammat* OR immun*)) OR ((respiratory* OR airway*) NEAR/3 infect*)):ab,ti) AND ((innate* OR non-specific* OR nonspecific* OR in-born OR inborn):ab,ti)

Web of science 481

TS=(((air NEAR/2 (clean*)) OR ((environment* OR personal) NEAR/5 (expos* OR toxican*)) OR pollut* OR microenvironment* OR exhaust* OR emission* OR acetylene* OR benzene* OR butadiene* OR (carbon NEAR/1 monoxide*) OR co OR carbonmonoxide* OR Coarse OR dust OR ethane OR ethylbenzene* OR ethylene* OR ethene* OR particle* OR (particul* NEAR/2 matter*) OR (nitro* NEAR/1 dioxide*) OR pm1 OR "pm2 5" OR pm10 OR "pm 1" OR "pm 2 5" OR "pm 10" OR soot OR toluene* OR xylene* OR ufp* OR "black carbon" OR (polycyclic NEAR/2 (hydrocarbon* OR carbon*)) OR pah OR pahs OR combust* OR (volatile NEAR/2 compound*) OR VOCs OR voc OR tVOCs OR tvoc OR btex OR smoke

OR smoking OR tobacco OR (carbon NEAR/2 nanotube*)) AND (((lymphoid) NEAR/2 cell*) OR lymphocyte* OR ilc OR ilcs OR (natural* NEAR/2 killer*) OR cytokine* OR interferon* OR ifn OR interleukin*)) AND ((asthma* OR (airway* NEAR/5 (hyperreactiv* OR allerg* OR inflammat* OR immun*)) OR ((respiratory* OR airway*) NEAR/2 infect*)) AND ((innate* OR non-specific* OR nonspecific* OR in-born OR inborn))) AND DT=(article)

Google scholar

Pollution|pollutant|pollutants|exhaust|"particulate matter"|pah|pahs|combustion|
"black carbon"|vac "lymphoid cell|cells"|lymphocyte|lymphocytes|cytokine|cytokines|
interferon| interferons|asthma innate|"non-specific"|nonspecific|inborn

Resource S2. Methodological details of the studies

Author (year) Reference	Specie/ Analysis Unit	Exposure/ Dose/ route	Experimentation arm/groups	Outcomes	Findings	Conclusions
Breaner C.A. et al. 2013	Mice	Multi-walled carbon nanotubes, Dose: 50 µg, Route: Oropharyngeal	1. MWCNT C57BL/6 mice 2. Controls (IL-13-/- and IL-33-/- mice)	<ul style="list-style-type: none"> Assessment of pulmonary inflammation 	<p>MWCNT:</p> <ul style="list-style-type: none"> Elevated levels of IL-33 in the lavage fluid Recruitment of ILCs in the airways. ILCs acting in response to IL-33 stimulate AHR and eosinophil recruitment through the release of IL-13. <p>MWCNT:</p> <ul style="list-style-type: none"> Induce secretion of IL-33 by epithelial cells (type II pneumocytes) twenty-four hours after exposure FA21 MWCNT and mIL-33 result in elevated levels of eotaxin, IL-5, and IL-6. 	MWCNT induce epithelial damage that results in release of IL-33, which in turn promotes innate lymphoid cell recruitment and the development of IL-13-dependent inflammatory response
	<i>in vivo</i> <i>in vitro</i>			<ul style="list-style-type: none"> Cytokine/chemokine (Eotaxin, IL-5, IL-6, and IL-33) 		
Mathews J. et al. 2016	Mice	O ₃ Dose: 2 ppm for 3 hrs Route: inhaled	1. Ozone db/db ((lacking leptin) obese 2. Ozone WT (C57BL/6j) 3. AIR db/db obese 4. TCR6-/- vs WT	<ul style="list-style-type: none"> IL-33 levels BAL 	<p>Ozone in Obese mice vs. lean WT mice</p> <ul style="list-style-type: none"> Increase of BAL IL-33, neutrophils, and airway responsiveness. Increased lung IL-13+ innate lymphoid cells type 2 (ILC2) and IL-13+ γδ T cells. Increased ST2+γδ T cells, indicating that these cells can be targets of IL-33, Equal decrease of serum IL-33 and was approximately 50% lower after O3 than air in both WT and obese mice 	IL-33 contributes to augmented responses to ozone in obese mice. Obesity and ozone also interacted to promote type 2 cytokine production in γδ T cells and ILC2 in the lungs, which may contribute to the observed effects of IL-33.
	Groups <i>in vitro</i>			<ul style="list-style-type: none"> IL-33 dependent BAL cytokines and chemokines 	<ul style="list-style-type: none"> Increase in BAL concentrations of IL-5, IL-13, and IL-9. Increases in BAL CXCL1, IL-6, IL-2, eotaxin (CCL11), CSF3, IL-1α, IL-10, IL-12 (p40), CXCL10, LIF, RANTES, CXCL9 and CCL4 anti-ST2 treated group: concentrations of IL-5, IL-13, IL-6, CXCL1 and CCL4 were significantly reduced versus isotype group. BAL IL-9 was not significantly reduce 	
				<ul style="list-style-type: none"> Pulmonary resistance (RL) 	<ul style="list-style-type: none"> Increased baseline RL > increased methacholine-induced changes in RL > increased coefficients of lung tissue damping (G) and elastance (H) 	

Author (year) Reference	Species/ Analysis Unit	Exposure/ Dose/ route	Experimentation arm/groups	Outcomes	Findings	Conclusions
De Grove K.C, et al. 2016	Mice	DEP, HDM	C57BL/6 WT 1,2,3,4 : DEP, HDM, DEP+HDM, Saline	• Airway inflammation	DEP+HDM vs. other 3 groups in wild mice • Marked increase in epithelium-derived cytokines IL-25 and IL-33 • Increased numbers of DCs, neutrophils, ILC2s, CD41 T cells, CD81 T cells, and eosinophils. • All ILC2s expressed ST2, resembling natural ILC2s • BALF: increased IL-5 and IL-13 levels • MLNs: markedly higher IL-4, IL-5 and IL-13 levels • Serum: significantly increased HDM-specific IgG1 titers • Presence of AHR	Dysregulation of ILC2s and TH2 cells attenuates DEP-enhanced allergic airway inflammation. In addition, a crucial role for the adaptive immune system was shown on concomitant DEP+HDM exposure.
	Groups	Dose: DEP 25 mg HDM 1 mg	GATA3^{+/flox} (Gata-3-haploinsufficient) 1,2,3,4 : DEP, HDM, DEP+HDM, Saline	• Type 2 cytokine production		
	<i>In vivo</i>	DEP+HDM				
	<i>ex vivo</i>	Route: Intranasal instillation days 1, 8, and 15	RORα^{fl/y}ILR7^{Cre} (ILC2-deficient) 1, 2: HDM, DEP+HDM			
			RORα^{fl/y}ILR7^{+/+} (lack adaptive immune system) 1, 2: HDM, DEP+HDM			
			Rag2^{-/-} 1,2,3,4 : DEP, HDM, DEP+HDM, Saline			

Author (year) Reference	Specie/ Analysis Unit	Exposure/ Dose/ route	Experimentation arm/groups	Outcomes	Findings	Conclusions
Yang Qi, et al. 2016	Mice	O ₃	1. O ₃ BALB/c 2. O ₃ C57BL/6 3. Air BALB/c 4. Air C57BL/6 5. O ₃ BALB/c 6. O ₃ BALB/c treated with anti-Thy1.2 mAb	<ul style="list-style-type: none"> BAL (Bronchoalveolar lavage) cell counts 	<ul style="list-style-type: none"> O₃ BALB/c vs other groups Enhanced A fumigatus-induced eosinophilia in BAL (doubled the numbers of eosinophils) 	O ₃ exposure increased airway levels of IL-33, a potent activator of ILC2s.
	Groups	3 ppm		<ul style="list-style-type: none"> ILC2s and RNA extraction from removed lungs 	<ul style="list-style-type: none"> Activates lung-resident ILC2 Increased IL-5 by lung ILC2s in response to IL-33. Induced IL-33 mRNA activation and increased protein expression in the lung tissue in both strains. No ILC2 influx or proliferation within 12 hours after O₃ exposure. ILC2s from the lungs: greater increased activation of IL5 and IL13 mRNA 12 hours after O₃ 	Lung-resident ILC2s were the predominant early source of the Th2 cytokines IL-5 and IL-13 in O ₃ -exposed mice.
	<i>ex vivo</i> <i>in vitro</i>	Route: Inhaled 2 hours on day 16 Aspergillus fumigatus mixed with alum for Allergen sensitization Dose: 20 mg	O ₃ BALB/c treated with anti-CD4 mAb	<ul style="list-style-type: none"> BAL IL-4 and IL-5 expression 	<ul style="list-style-type: none"> ILC2s O₃ did not induce IL-5 or IL-13 production by CD41Thy11 TH cells isolated from the lungs 12 hours after exposure LC2s mediate O₃-induced airway inflammation and AHR 	
		Route: Intraperitoneal injections on days 0 and 7 Intranasal on day 13				

Kumagai K, et al. 2017	Mice	O ₃		1. O3 C57BL/6 (ILC-sufficient Mice) 2. AirC57BL/6 3. O3 Rag2 ^{-/-} (deficient Mice) 4. Air Rag2 ^{-/-} 5. O3 Rag2 ^{-/-} IL2rg ^{-/-} (depleted of all lymphoid cells including ILCs) 6. Air Rag2 ^{-/-} IL2rg ^{-/-}	<ul style="list-style-type: none"> • Mucous Cell Metaplasia 	<p>O3 C57BL/6 vs other groups:</p> <ul style="list-style-type: none"> • Mucous cell metaplasia • Greater volume densities of mucosubstances in airway epithelium lining this proximal large-diameter <ul style="list-style-type: none"> • Increased expression of genes involved in type 2 immune responses 	<p>Murine ILCs, but not T or B cells, play a</p> <p>crucial role in ozone-induced mucous cell metaplasia, eosinophilic inflammation, and type 2 immunity in the lungs of mice.</p>
	Groups	Dose: 0.8 ppm Route: Inhaled 1 day or 9 consecutive weekday (4 hr/day).			<ul style="list-style-type: none"> • Gene expression in type2 immune response 		
	<i>in vivo</i> <i>ex vivo</i>				<ul style="list-style-type: none"> • Number ILCs 	<ul style="list-style-type: none"> • As small number of ILCs were present in ozone-exposed Rag2^{-/-} / Rag2^{-/-} Air- and ozone exposed Rag2^{-/-} / mice had statistically similar numbers of lung ILC2s 	

Author (year) Reference	Species/ Analysis Unit	Exposure/ Dose/ route	Experimentation arm/groups	Outcomes	Findings	Conclusions
Burleson GR, et al. 1989	Fischer-344 rats	O ₃	1. O ₃ day 1, 2. O ₃ day 5 3. O ₃ day 7 4. O ₃ day 10. 5. Control: Clean air	NK cytotoxicity	Continuous exposure to 1.0 ppm ozone vs. clean air: • 1, 5 or 7 days: immunosuppressive effect on pulmonary NK activity. Ozone-suppressed pulmonary NK activity returned to control levels • 10 days: The suppressed pulmonary NK activity returned to normal levels in the continued presence of ozone inhalation. Suppression of NK activity by pollutant exposure may affect the ability of the host to defend against viral and neoplastic disease.	Ozone-induced suppression of pulmonary NK activity may be due to a direct effect on cells mediating NK activity. Ozone-suppressed pulmonary NK activity returned to normal levels in the continued presence of ozone inhalation. Suppression of NK activity by pollutant exposure may affect the ability of the host to defend against viral and neoplastic disease.
	Groups	Dose: Continuous: 1.0 ppm 23.5 hours /day/ 1,3,7,0,10 consecutive days. Doses response: 0.5 ppm or 0.1ppm Rout: Ambient		Dose response studies	• NK activity was suppressed at 0.5 ppm ozone, but not at 0.1 ppm ozone, following 23.5 hours of exposure.	
	<i>in vivo</i> <i>in vitro</i>			Total viable cell number, percent viability, and cell type	• No differences in percent viability of cells from whole-lung homogenate were observed for air- or ozone-exposed groups at 1, 5, 7 or 10 days of exposure • A decreased percentage of lymphocytes due to ozone exposure on day 5	
				Function of adherent cells	• Removal of adherent cells resulted in an enhanced NK activity in the non-adherent cell population for both air- and ozone-exposed animals compared to the percent lysis observed before removal of the adherent cells. • Removal of adherent cells did not restore the ozone-suppressed NK activity to control levels	
Lu X, et al. 2018	Mice	PM2.5	1. Control 2. OVA 3. OVA + PM2.5 4. OVA + PM2.5 + LX44	ILC2-related transcription factors	OVA + PM _{2.5} vs other groups	PM2.5-induced inflammation plays a key role in the progression of asthma mice.
	Groups	Dose: suspension: 15 g/L PM2.5 intranasal			PM _{2.5} : Increased expression of ROR α and GATA3 transcription factors.	
	<i>in vivo</i> <i>in vitro</i>					

Author (year) Reference	Specie/ Analysis Unit	Exposure/ Dose/ route	Experimentation arm/groups	Outcomes	Findings	Conclusions
Finkelman F, et al. 2004	Mice Groups <i>In vivo</i> <i>In vitro</i>	DEP Dose: 10 mg/mL	1. DEP 2. Saline	Cytokine production	DEP vs. Saline • DEP stimulate production of IL-6 • IFN- γ levels were considerably decreased, in some experiments decrease was 75% when mice were injected with DEP on 3 successive days • Injection of 2 mg of DEP 2 h before LPS administration completely suppressed the LPS-induced IFN- γ response and partially suppressed LPS-induced IL-10 production, but had no effect on LPS induction of IL-6 or TNF production • DEP suppressed the LPS-induced increase in IFN- γ mRNA (although not as completely as it suppressed IFN- γ secretion).	DEP potently inhibits IFN- γ production by NK and NKT cells, which is rapid in onset, long lasting, and dose related. DEP induces an inhibitory effect on steady state IFN- γ mRNA levels, and may also suppress IFN- γ production through posttranscriptional mechanisms.
				TLRL4-induced IFN- γ production,		
				NK and NKT	• DEP suppressed IFN- γ production individual NK and NKT cells following LPS stimulation • DEP failed to suppress anti-CD3 mAb-induced production of IFN- γ , or other cytokines, which are NKT cell derived.	
Zhao H, et al. 2014	Rats Groups <i>In vivo</i> <i>In vitro</i>	PM _{2.5} Dose: 1, 5, or 10mg/kg body weight Route: Intratracheal instillation <i>S. aureus</i>	1. PM _{2.5} + <i>S. aureus</i> 2. Control PBS+ <i>S. aureus</i> 3.	Number NK	PM _{2.5} vs Control • PM _{2.5} :decreased NK cell response to subsequent <i>S. aureus</i> infection in the airway lumen • PM _{2.5} : decreased absolute NK cell number in BALF	• PM _{2.5} increases susceptibility to respiratory infection by <i>S. aureus</i> . • PM _{2.5} decreases the number of NK cells in the lung and suppress AM phagocytosis which provide a potential mechanism to explain that associate ambient air pollution and pulmonary bacterial infections.
				Host defense restoration with adoptive transfer NK	• Prior PM _{2.5} : increase in bacterial burden in the lung • Adoptive NK cell transfer to the lung of previously PM _{2.5} -exposed rats markedly reduced the bacterial burden	
				Potential mechanism by which NK cells contribute to host defense in pulmonary staphylococcal infection	• PM _{2.5} : AMs co-cultured with NK cells demonstrated a higher rate of phagocytosis of <i>S. aureus</i>	

Author (year) Reference	Specie/ Analysis Unit	Exposure/ Dose/ route	Experimentation arm/groups	Outcomes	Findings	Conclusions
Müller L, et al. 2013a	Human <i>ex vivo</i> NK cells isolated from peripheral blood	DEP and pi:C Route: direct exposure of cell	1. DEP 2. pi:C 3. DEP+pi:C	Cytokine release	<ul style="list-style-type: none"> pi:C significantly increased release of all cytokines (IL-1β, IL-2, IL-4, IL-8, IL-10, IL-12p70, IFN-γ, or TNF-α) tested other than IL-5 and IL-13 DEP: little impact on cytokine release, other than a modest increase of IL-1β, IL-8, and TNF-α. DEP+pi:C: induced increases were blunted for most cytokines, though this change was not statistically significant except for IL-4. 	DEP reduced expression of the cytotoxic NK cell surface marker CD16, gene and protein expression of granzyme B and perforin, and the ability to kill target cells.
				Granzyme B and perforin expression	<ul style="list-style-type: none"> pi:C: increased the expression of granzyme B and perforin compared to the vehicle control. DEP: did not affect the RNA level of either granzyme B or perforin. 	
				NK cell phenotype	<ul style="list-style-type: none"> pi:C+DEP: expression of CD16, was reduced but the percentage of CD16- NK cells was increased DEP alone showed no effects on the NKp46 expression on NK cells 	
Kucuksezer UC, et al. 2014	Human PBMC from peripheral blood cell <i>ex vivo</i>	O ₃ Dose: 1, 5, 10, and 50 mg/mL cRPMI Route: direct exposure of cell	1. O ₃ 2. unstimulated	NK number	<ul style="list-style-type: none"> O₃ vs. unstimulated 	O ₃ increased number of C16 cell and cytotoxicity.
				CD107a expression on NK cells Function	<ul style="list-style-type: none"> O₃ 1 mg/mL: increased total CD3- CD16+/56+ NK on both 3rd and 5th days of cell culture. No significant changes were observed among the expression levels of other surface molecules. O₃ 1 and 5 mg/mL: induced increase in NK-cell cytotoxicity K562 cells induced a strong increase in the levels of CD107a expression Ozone exposure did not induce an increase of CD107a beyond K562-stimulated levels 	

Author (year) Reference	Species/ Analysis Unit	Exposure/ Dose/ route	Experimentation arm/groups	Outcomes	Findings	Conclusions
Müller L, et al. 2013b	Human NK from peripheral blood cell <i>ex vivo</i>	O ₃ Dose: 0.4 ppm Route: Direct exposure of cell	1. O ₃ 2. Filtered air	Granzyme B expression	O ₃ vs. Filtered air • Intracellular granzyme B levels were lower in NK cells cocultured with NECs, albeit not statistically significant • O ₃ decreased the expression of IFN- γ and enhanced, albeit not statistically significant, the expression of IL-4 in NK cells • O ₃ -induced upregulation of ULBP3 and MICA/B on NECs mediates the suppression of IFN- γ . O ₃ alters the expression of NK cell ligands on NECs. O ₃ : induced a reduction in NK cell cytotoxic function.	O ₃ reduced markers of activation, INF- γ production, and cytotoxic function. O ₃ upregulated ligands for NK in epithelial cells.
				Cytokine production		
				Cell ligands to NK		
				Cytotoxicity		

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Resource S3. Modified ARRIVE guidelines, and adapted scale from ARRIVE guidelines for experimental studies in human cells

Modified ARRIVE: Average score for 9 studies carried out in animals

			FULFILLMENT %
TITLE	1	Provide as accurate and concise a description of the content of the article as possible.	100
ABSTRACT	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	94
INTRODUCTION			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale.	100
		b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.	50
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	100
METHODS			
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	88
Study design	6	For each experiment, give brief details of the study design including the number of experimental and control groups.	50
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example:	
		a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s).	94
		b. When (e.g. time of day).	100
		c. Where (e.g. home cage, laboratory, water maze).	100
In vitro procedures	7a	a. Describe in detail all the in vitro procedures	100
		b. Describe in detail all the reagents, cells, kits including manufacturer used (manufacturer)	100
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range).	88
		b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.	100
Housing and husbandry	9	Provide details of:	
		a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish).	77

		b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment).	50
Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group.	50
		b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used.	
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done.	50
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).	100
Statistical methods	13	a. Provide details of the statistical methods used for each analysis.	72
		b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron).	16
		c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.	100
RESULTS			
Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated).	0
Numbers analysed	15	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%) (Schulz et al., 2010).	0
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).	66,5
DISCUSSION			
Interpretation/scientific implications	18	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.	100
Generalisability/translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.	70
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.	80
TOTAL			74,9

Resource S4. Adapted scale for *in vitro* experiments in human cells (From ARRIVE guidelines).

Average score for 3 studies carried out in human cells

			FULFILLMENT %
TITLE	1	Provide as accurate and concise a description of the content of the article as possible.	100
ABSTRACT	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	100
INTRODUCTION			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale.	100
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	100
METHODS			
Ethical statement	5	Indicate the nature of the ethical review permissions, written informed consent	100
Study design	6	For each experiment, give brief details of the study design including the number of experimental and control groups.	75
In vitro procedures	7	a. Describe in detail all the in vitro procedures	100
		b. Describe in detail all the reagents, cells, kits including manufacturer used (manufacturer)	100
Sample size	10	a. Specify the total number of subjects	100
		b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used.	0
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).	100
Statistical methods	13	a. Provide details of the statistical methods used for each analysis.	75
		b. Describe any methods used to assess whether the data met the assumptions of the statistical approach.	100
RESULTS			
Baseline data	14	For each experimental group, report relevant characteristics and health status of human subjects	0
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).	100
DISCUSSION			
Interpretation/scientific implications	18	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.	100
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.	75
TOTAL			89.1

Resource 5S. List of the excluded studies

Christianson CA, Goplen NP, Zafar I, Irvin C, Good JT, Jr., Rollins DR, et al. Persistence of asthma requires multiple feedback circuits involving type 2 innate lymphoid cells and IL-33. *J Allergy Clin Immunol.* 2015;136(1):59-68 e14. DOI: 10.1016/j.jaci.2014.11.037.

Everaere L, Ait-Yahia S, Molendi-Coste O, Vorng H, Quemener S, LeVu P, et al. Innate lymphoid cells contribute to allergic airway disease exacerbation by obesity. *J Allergy Clin Immunol.* 2016;138(5):1309-18 e1. DOI:10.1016/j.jaci.2016.03.019.

Farhadi N, Lambert L, Triulzi C, Openshaw PJ, Guerra N, Culley FJ. Natural killer cell NKG2D and granzyme B are critical for allergic pulmonary inflammation. *J Allergy Clin Immunol.* 2014;133:827-35. DOI: 10.1016/j.jaci.2013.09.048.

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Iijima K, Kobayashi T, Hara K, Kephart GM, Ziegler SF, McKenzie AN, et al. IL-33 and thymic stromal lymphopoietin mediate immune pathology in response to chronic airborne allergen exposure. *J Immunol.* 2014;193(4):1549-59. DOI:10.4049/jimmunol.1302984.

Kamijo S, Takeda H, Tokura T, Suzuki M, Inui K, Hara M, et al. IL-33-mediated innate response and adaptive immune cells contribute to maximum responses of protease allergen-induced allergic airway inflammation. *J Immunol.* 2013;190(9):4489-99. DOI:10.4049/jimmunol.1201212.

Klein Wolterink RG, Kleinjan A, van Nimwegen M, Bergen I, de Bruijn M, Levani Y, et al. Pulmonary innate lymphoid cells are major producers of IL-5 and IL-13 in murine models of allergic asthma. *Eur J Immunol.* 2012;42(5):1106-16. DOI:10.1002/eji.201142018.

Klein Jan A, Klein Wolterink RG, Levani Y, de Bruijn MJ, Hoogsteden HC, van Nimwegen M, et al. Enforced expression of GATA3 in T cells and group 2 innate lymphoid cells increases susceptibility to allergic airway inflammation in mice. *J Immunol.* 2014;192(4):1385-94. DOI:10.4049/jimmunol.1301888.

Li BW, de Bruijn MJ, Tindemans I, Lukkes M, KleinJan A, Hoogsteden HC, et al. T cells are necessary for ILC2 activation in house dust mite-induced allergic airway inflammation in mice. *Eur J Immunol.* 2016;46(6):1392-403. DOI:10.1002/eji.201546119.

Chapter 6

Air pollution control and the occurrence of acute respiratory illness in school children of Quito, Ecuador

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ABSTRACT

Outdoor air pollution is associated with respiratory infections and allergies, yet the role of innate lymphoid cells (ILCs) in pathogen containment and airway hyperresponsiveness relevant to effects of air pollutants on ILCs is poorly understood. We conducted a systematic review to evaluate the available evidence on the effect of outdoor air pollutants on the lung type 1 (ILC1) and type 2 ILCs (ILC2) subsets. We searched five electronic databases (up to Dec 2018) for studies on the effect of carbon monoxide (CO), sulfur dioxide (SO₂), nitrogen dioxide (NO₂), diesel exhaust particles (DEP), ozone (O₃), and particulate matter (PM) on respiratory ILCs. Of 2209 identified citations, 22 full-text papers were assessed for eligibility, and 12 articles describing experimental studies performed in murine strains (9) and on human blood cells (3) were finally selected. Overall, these studies showed that exposure to PM, DEP, and high doses of O₃ resulted in a reduction of interferon gamma (IFN- γ) production and cytotoxicity of ILC1. These pollutants and carbon nanotubes stimulate lung ILC2s, produce high levels of interleukin (IL)-5 and IL-13, and induce airway hyperresponsiveness. These findings highlight potential mechanisms by which human ILCs react to air pollution that increase the susceptibility to infections and allergies.

Keywords: lung innate lymphoid cells; ILC; air pollutants; airway hyperresponsiveness

INTRODUCTION

Traffic-related air pollution has been associated with harm to respiratory health worldwide (1–11). Children are highly prone to harmful effects of air pollutants on lung function (12–14) due to the small size of their airways and immaturity of defense mechanisms (12, 15). Reduction of several pollutants, especially particulate matter ($PM_{2.5}$ and PM_{10}), nitrogen dioxide (NO_2), and ozone (O_3) has been associated with improvement of lung function (16, 17) plus the reduction of respiratory symptoms, bronchitis, allergic disorders in children with and without asthma, and in the number of daily asthma events (18–22). In contrast, some studies have demonstrated that the reduced exposure to traffic-related air pollutants has little effect on lower respiratory symptoms (23) and did not affect the prevalence of respiratory/allergic symptoms in school children (24). The reasons for such discrepancies are not clear, but might be attributed to effects of several confounding factors such as exposure misclassification, temporal, and spatial trends in exposure, health, socioeconomic status, and smoking (25), as well as population susceptibility, life style changes, or the use of statistical methods that bypass the link-by-link approach of classical accountability in evaluating the regulatory impacts (26).

In several Latin American countries, including Brazil (6, 27), Mexico (2, 28), Chile (29, 30), Colombia (31), and Ecuador (32–34) researchers have demonstrated associations between respiratory problems and urban air pollution. These countries have created programs for air pollution management and control. Studies are lacking on the effects of such programs on respiratory health in children.

The city of Quito is the most polluted city in Ecuador, due to the number of cars contributing to air pollution and to the mountain range that impedes airflow to reduce contamination (35, 36). From January through April of 2000, we studied 616 children, aged 6–12 years, attending schools located in areas with different traffic intensities—moderate in the North and high in the Center. We found that 90% of school-aged children in the high-traffic area of Quito, and 43% of children in the city area with moderate traffic had blood concentrations of COHb higher than the safety level of 2.5% (33). Trained pediatricians identified ARI episodes in about 70% of children in the high-traffic area and 30% of children in the moderate-traffic area. Children with COHb > 2.5% were 3.25 (95% CI 1.65, 6.38) times more likely to present with ARIs than children with COHb ≤ 2.5% (33). Our study served as the foundation for city-wide public policies: creation of the Metropolitan Atmospheric Monitoring Network Quito (REMMAQ), plus vehicular emissions control in Quito with technical inspection of vehicles in 2002 (34). By 2007, the annual average concentrations of sulfur dioxide (SO_2), CO, and PM_{10} decreased to acceptable levels. CO was reduced by 35% (from 1.29 to 0.83 $\mu g/m^3$) (37). Such drastic reduction in air pollution seemed likely to lead to improvements in the respiratory health of city residents.

Was the reduction of traffic-related pollutant levels, specifically in CO exposure between 2000 and 2007, associated with both the incidence of ARI and COHb levels in school-aged children? We conducted a 15-week prospective study of 730 children attending elementary schools in the North and Center areas of Quito and compared the findings with results of the 2000 study of ARI and the COHb levels in children attending schools in the same locations (33).

METHODS

Study design and participants

The design and methods followed in the 2007 study, described in this section, were similar to those in the 2000 study (33) with the additional analysis of associations of ARI incidence and ambient CO measurements. In both studies, a pediatrician visited each child in the school twice weekly to examine the child's respiratory signs and symptoms and to determine the presence of upper acute respiratory illnesses and lower acute respiratory illnesses.

Out of 736 recruited children attending public elementary schools in the two areas of Quito, 6 (<1%) children withdrew from their school for various reasons (change of address or presence of varicella). Between February and June 2007, we measured the incidence of ARI and COHb levels in remaining 730 children. To ensure comparability between the studies, we conducted the 2007 study in the winter season (see [https:// nutrition.tufts.edu/ sites/ default/ files/ documents/ ENaumova- SupplementalMaterialCOHbARIPaper_09-24-18.pdf](https://nutrition.tufts.edu/sites/default/files/documents/ENaumova-SupplementalMaterialCOHbARIPaper_09-24-18.pdf)) (1) and in two schools located in the immediate vicinity of the previously studied schools (Center and North areas). The selected schools had similar characteristics: building, number of children, and socioeconomic status of the children.

During the screening period, we delivered detailed information about the study to teachers and parents of each child. We excluded three children due to presence of asthma ($n = 1$), congenital cardiopathy ($n = 1$), and major chest deformity ($n = 1$). A total of 736 children met the inclusion criteria: 6–11 years of age (age was confirmed by birth certificate), formal written consent freely signed by parents, and child assent.

Carboxyhemoglobin status

Before starting the follow-up period, we obtained a 5-mL venous blood sample from each child using a plastic syringe and placing the sample in a collecting tube with Ethylenediaminetetraacetic acid (EDTA). These tubes were refrigerated until transported for the analysis to a laboratory at Universidad Católica del Ecuador. (This laboratory also measured COHb in the 2000 study.) We measured COHb levels by spectrometry (38) within

24 h, and expressed results as a percentage of plasma hemoglobin. COHb concentration of 2.5% was considered the reference value, the level below which no symptoms would be found (39).

Acute respiratory illness measurements

Trained practitioners visited each child weekly at her/his school by to monitor respiratory symptoms and signs, and to determine the presence of acute upper and lower respiratory illnesses. Children who presented with respiratory illness were treated, but not necessarily removed from school, and followed until the resolution of the episode. A new case could be identified after 2 weeks free of respiratory illness. Acute upper respiratory illness was defined as the presence of two or more of the following signs/symptoms: cough, nasal secretion, fever $> 37.5^{\circ}\text{C}$ (axillary temperature), inflammation of pharynx, and anterior cervical lymphadenitis. Presence of otitis (local pain, aural pus, and eardrum congestion) was also considered as acute upper respiratory illness. Acute lower respiratory illness was defined by tachypnea (respiratory rate > 20) and/or lower respiratory tract secretions (alveolar or bronchoalveolar) assessed by thoracic auscultation, plus one or more of the following: fever, cough, and chest retractions (40).

Anthropometric measurements

We measured each child's weight and height by standard procedures (41) using instruments calibrated by the Ecuadorian Institute of Normalization. Weight was measured with a DETECTO balance (New York), that included a height gage graduated in cm. Weight was recorded to the nearest 0.1 kg. Height was recorded to the nearest 0.1 cm.

Nutritional status

Weight-for-age Z-score (WAZ) and height-for-age Z-score (HAZ) were determined for each child using Nutstat software (Epi Info(TM) CDC, 2004). Children having a WAZ < -2 SD were classified as underweight. Children having a HAZ < -2 SD were classified as stunted.

Exposure to pollution

Household survey

To determine the indoor CO contamination, we sent a survey to the parents of study children in the first 3 weeks of the study. It asked about the type of fuel used for cooking (kerosene, gas, alcohol, firewood), and the presence of smokers. Ninety-four % of the surveys were completed.

Air quality measurements

In 2004, 4 years after the 2000 study was completed, Quito started city-wide routine air quality monitoring. To provide CO proxies for both time intervals, we obtained from two

monitoring stations located in the Center and North areas all available monthly records for CO levels during the period 2004–2007. Using monthly records, we interpolated CO average values from 2004 for COHb measurements in 2000 by direct assignment of the closest available monthly measurements in 2004 (4.5 mg/m³ for CO level). We used these interpolated values in our analysis. For 2007, we obtained daily records of environmental CO, SO₂, and PM_{2.5} levels, collected now as part of the automatic network of passive monitoring maintained by Quito Air Corporation (CORPAIRE). We supplemented the analysis with daily records for ambient temperature and precipitation from the National Institute of Meteorology and Hydrology (INAMHI), Quito, Ecuador (42).

Statistical analysis

We compared descriptive statistics about children from the 2000 and 2007 studies. Continuous variables were described as the mean and standard deviation; categorical variables as absolute frequency and percentages. We compared the distributions of the studied parameters with *t* Student and chi-squared tests for continuous and categorical variables, respectively.

We calculated the incidence rates (episodes/1000 child-weeks (CW) for the follow-up period) of acute respiratory illness and the annual frequency of ARI episodes/ child in both 2007 and 2000 study groups. We estimated the average concentrations for the selected environmental air pollutants measured during the 15-week study.

The relationships between COHb levels and environmental CO were assessed by several regression models, using the CO values from the day of and the day prior to the COHb measurement. The models were gradually adjusted for individual characteristics (age, sex, underweight, stunting), variables for the household sources of indoor air pollution (indoor firewood use and smoking), and meteorological characteristics (see [https:// nutrition.tufts.edu/ sites/ default/ files/ documents/ ENaumova-SupplementalMaterialCOHbARIpaper_09-24-18.pdf](https://nutrition.tufts.edu/sites/default/files/documents/ENaumova-SupplementalMaterialCOHbARIpaper_09-24-18.pdf)) (2). To evaluate the association of COHb, as a marker for CO exposure, with the presence of respiratory illness, we used two logistic regression models. In the first model, the explanatory variable, COHb, was used as a continuous variable (concentrations) and in the second as a binary variable (0, if COHb ≤ 2.5%; and 1, if COHb > 2.5%). We expressed the results in Adjusted Odds Ratios (AOR) with 95% confidence intervals.

To allow for multiple episodes of ARI in a child, we applied the Generalized Estimating Equation (GEE) for Poisson regression models to evaluate the association between COHb level and the incidence of ARI (ARI/1000 child-weeks). We applied the same model to examine the relationship between ambient CO concentrations and ARI incidence. We also explored the relationship between ARI incidence and COHb levels and between ARI incidence and ambient CO concentrations, using the classic Poisson regression

model (see [https:// nutrition.tufts.edu/ sites/ default/ files/ documents/ ENaumova-SupplementalMaterialCOHbARIPaper_09-24-18.pdf](https://nutrition.tufts.edu/sites/default/files/documents/ENaumova-SupplementalMaterialCOHbARIPaper_09-24-18.pdf)) (3). The results are expressed in rate ratios with 95% confidence intervals.

Logistic and GEE regression models were adjusted for age, sex, nutritional status (underweight and stunted), fire wood use for cooking, and presence of smokers in the households. Regression coefficients for study parameters were considered statistically significant if the corresponding two-sided *p* value was equal to or below 0.05.

Finally, we constructed a Poisson regression model for ARI occurrence for 2007 with CO, SO₂, and PM_{2.5} concentration values (same day of COHb measurements) and adjusted for individual covariates.

We entered and managed data using the ACCESS program (version 11.5614.5602, 2003). We analyzed these data using the SPSS program (Version 22.0, Lead Technologies Inc., SPSS Inc., Chicago, Illinois, USA). The complete database contained information from both 2007 and 2000 study.

Ethics approval and consent to participate

We obtained ethical approval for the study from the Ethical Committee of the Corporación Ecuatoriana de Biotecnología (CEB). We obtained voluntary formal written consent from parents, plus assent from each child.

RESULTS

General characteristics of participants

In 2000, 616 children aged 6–12 years were studied (33). Thirty-one percent (196/616) of the children had COHb measured, and 87.5% completed the survey. There were a total of 7337 weekly visits. In 2007, we enrolled 730 children aged 6–12 years in the study. All children had COHb measured and 98% completed the survey. A total of 10,683 child-weeks of observation were accumulated in the 2007 study. As compared to 2000, average age, the proportion of females, stunted, and underweight children were significantly higher in the 2007 study. Between the two study years, there were no significant differences in the percentage of home smokers and the use of firewood in households (Table 1).

Table 1: Baseline characteristics of children in 2000 and 2007 studies.

Study parameters	2000 (n = 616)	2007 (n = 730)	p value
Baseline characteristics			
Age (years)	8.64 ± 1.01	9.39 ± 1.53	< 0.0001
Females (%)	242 (39.4)	388 (53.2)	< 0.0001
Weight (kg)	27.38 ± 5.6	28.05 ± 7.71	0.074
Underweight ¹	17 (2.8)	95 (13.1)	< 0.0001
Height (cm)	127.5 ± 8.15	126.34 ± 12.42	0.067
Stunted ²	56 (9.1)	233 (31.9)	< 0.0001
BMI (kg/m ²) ³	16.74 ± 2.42	17.25 ± 2.42	< 0.0001
Survey			
Completed surveys	539 (87.5)	718 (98.4)	< 0.0001
Households with kerosene use	11 (2.17)	0 (0.0)	
Households with fire wood use	5 (0.9)	2 (0.2)	0.111
Households with smokers	128 (25.2)	159 (22.2)	0.22

Data are mean ±SD or n (%).

¹ Underweight was defined as weight for age Z score < -2SD.

² Stunted was defined as Height for age Z score < -2SD.

³ BMI - Body Mass Index

Environmental contaminants

In the study of year 2000, there was no systematic monitoring of contaminants by areas in the city of Quito. Ambient CO levels were steadily declining over the period, based on city-wide routine monitoring, from 4.5 mg/m³ in 2004 to 0.78 mg/m³ in 2007 (Fig. 1a). During the study period in 2007, the maximum daily concentration of consecutive 8-h moving averages for CO and O₃ and the maximum daily concentration of consecutive 24-h moving averages for PM_{2.5}, NO₂, and SO₂ in north and center monitoring stations were within desirable and acceptable limits, as permitted by Ecuadorian Air Quality Standards (NECA) (37, 43) (Fig. 1b).

ARI incidence and carboxyhemoglobin levels

While there was no difference in the percentage of children who presented with ARI in 2007 and 2000, the number of ARI episodes and the annual frequency of ARI per child were significantly lower in 2007 as compared to 2000 (Table 2). Similarly, the incidence rate of ARI in 2007 was significantly lower than in 2000 (43.52 per 1000 CW vs. 83.14 per 1000 CW), equivalent to a 48% lower rate of ARI (RR 0.52; 95% CI 0.45–0.62, $p \leq 0.0001$).

In 2007, the average level of COHb was below 2.5%, in contrast to 2000, when it exceeded that safety level. Furthermore, in 2007, the fraction of children with COHb > 2.5% was significantly lower as compared to the 2000 study (4.9% vs. 64.9%) (Table 2).

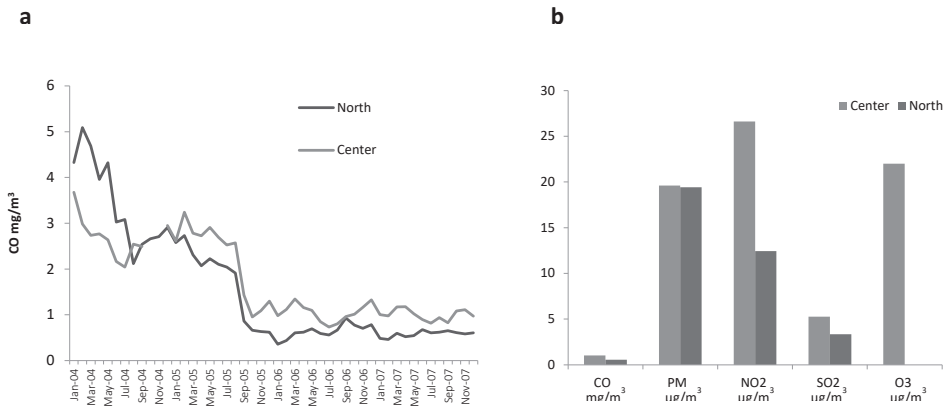


Fig. 1 Air quality in Quito (2004–2007).

a Declining trend in the outdoor CO ambient concentration (mg/m³) measured at the North and Center stations; **b** Maximum daily concentration of consecutive 8-h moving averages for CO and O₃, and maximum daily concentration of consecutive 24-h moving averages for PM_{2.5}, NO₂, and SO₂ in Center and North monitoring stations in 2007

Table 2: Incidence of acute respiratory illness and exposure measurements for children in 2000 and 2007 studies.

Study parameters	2000 (n = 616)	2007 (n = 730)	p value
Outcome			
Children with ARI	308 (50.0)	357 (48.9)	0.689
ARI episodes	610	465	< 0.0001
Annual frequency of ARI/child	4.35	2.33	< 0.0001
Incidence rate of ARI/1000 child weeks	83.14	43.52	< 0.0001
Blood tests (n)	196	730	
COHb levels (%) ¹	3.88 ± 1.93	1.90 ± 0.39	< 0.001
COHb > 2.5% ²	136 (69.4)	36 (4.9)	< 0.001

Data are mean ±SD or n (%)

ARI - Acute Respiratory Illness

¹ COHb: Carboxyhemoglobin as percentage of CO fixed to hemoglobin

² COHb over the safe level of 2.5%

ARI incidence, carboxyhemoglobin, and CO

In 2007, neither ambient CO concentrations (Table 3) nor levels of COHb (Table 4) were associated with the risk of having ARI as compared to 2000 study. In 2007, having COHb levels > 2.5% was not associated with ARI (adjusted OR 1.29; 95% CI 0.65–2.53, $p = 0.468$), in contrast to 2000 when AOR was 5.44 (95% CI 2.38–12.42, $p < 0.0001$) (Table 5). Similarly, in 2007, levels of COHb were not associated with presence of ARI (adjusted OR 1.30; 95% CI 0.89–1.91, $p = 0.187$), in contrast to 2000 when AOR was 1.57 (95% CI 1.28–1.93, $p < 0.0001$).

Table 3. Risk factors associated with acute respiratory illness incidence in 2000 and 2007 studies and overall.

	All		2000		2007	
	RR (95% CI)	<i>p</i> value	RR (95% CI)	<i>p</i> value	RR (95% CI)	<i>p</i> value
CO (mg/m ³) ¹	1.23 (1.13,1.34)	<0.0001	10.65(4.66,24.37)	<0.0001	1.12 (0.91,1.35)	0.290
Age (year)	0.93 (0.86,0.97)	0.017	1.06(0.848,1.13)	0.585	0.95 (0.89,1.00)	0.097
Female	1.04 (0.87,1.36)	0.657	1.10 (0.72,1.67)	0.641	1.07 (0.89,1.28)	0.446
Underweight ²	1.02 (0.79,1.33)	0.832	0.59(0.215,1.66)	0.325	1.06 (0.81,1.39)	0.654
Stunted ³	1.21 (0.98,1.50)	0.066	1.30 (0.68,2.51)	0.422	1.18(0.97,1.45)	0.096
Indoor fire wood use	1.46 (0.42,5.05)	0.544	1.89(0.86,4.13)	0.110	0.66(0.16,2.72)	0.566
Indoor smokers	1.07 (0.88,1.30)	0.466	1.04 (0.64,1.58)	0.950	1.13(0.92,1.39)	0.234

Rate Ratio (RR) - results from the Log-linear Poisson Generalized Estimating Equation model

¹ CO, Carbon monoxide as continuous variable on the day of COHb measurement

² Underweight was defined as weight for age Z score <-2SD

³ Stunted was defined as height for age Z score <-2SD

Table 4. Risk factors associated with acute respiratory illness incidence in 2000 and 2007 studies and overall.

	All		2000		2007	
	RR (95% CI)	<i>p</i> value	RR (95% CI)	<i>p</i> value	RR (95% CI)	<i>p</i> value
COHb levels ¹	1.26 (1.17,1.34)	<0.0001	1.24 (1.10,1.38)	<0.0001	1.13(0.94,1.36)	0.165
Age (year)	0.94 (0.89,1.00)	0.059	0.89 (0.72,1.09)	0.285	0.95 (0.89,1.04)	0.129
Female	1.06 (0.89,1.26)	0.472	1.48 (0.66,1.66)	0.842	1.07(0.89,1.29)	0.434
Underweight ²	1.48 (0.80,1.36)	0.729	0.75(0.22,2.04)	0.578	1.08 (0.82,1.42)	0.570
Stunted ³	1.17 (0.95,1.45)	1.200	1.33 (0.67,2.64)	0.403	1.18(0.96,1.45)	0.099
Indoor fire wood use	1.72 (0.48,6.18)	0.402	3.13(0.72,12.72)	0.110	0.65(0.16,2.54)	0.528
Indoor smokers	1.23 (0.92,1.36)	0.245	1.02 (0.64,1.75)	0.799	1.13(0.92,1.39)	0.241

Rate Ratio (RR) - results from the Log-linear Poisson Generalized Estimating Equation model

¹ COHb, as continuous variable

² Underweight was defined as weight for age Z score <-2SD

³ Stunted was defined as height for age Z score <-2SD

Table 5: Risk factors associated with the presence of acute respiratory illness in children in 2000 and 2007 studies.

	2000		2007	
	AOR (95% CI)	p value	AOR (95% CI)	p value
COHb >2.5% ¹	5.44 (2.38–12.42)	<0.0001	1.29 (0.65–2.53)	0.468
Age/year	0.91 (0.61–1.34)	0.626	0.95 (0.86–1.05)	0.278
Female	1.76 (0.82–3.79)	0.146	1.13(0.84–1.52)	0.431
Underweight ²	2.53(0.37–17.17)	0.342	0.92 (0.57–1.49)	0.743
Stunted ³	1.94 (0.62–6.06)	0.254	1.21(0.85–1.73)	0.278
Indoor fire wood use	1.30 (0.07–25.76)	0.865	0.83(0.05–13.52)	0.865
Indoor smokers	0.85 (0.36–1.96)	0.696	1.14(0.79–1.64)	0.484

Adjusted odds ratios (AOR) result from binary logistic regression model

¹COHb, Carboxyhemoglobin over the safe level of 2.5%, as binary variable

²Underweight was defined as weight for age Z score <–2SD

³Stunted was defined as Height for age Z score < –2SD

ARI incidence in center and north areas of Quito

In 2007, the number of episodes of ARI, the percentage of children with ARI, and the annual frequency of ARI per child were comparable across the schools in the North and Central areas. The percentage of children with COHb > 2.5% was significantly lower in the Center school. The percentage of underweight children was significantly higher in the Center area school. While most characteristics of the study children in two schools were comparable, children at the Center area school were, on average, 1 year older as compared to those from the North area and there were more girls (Table 6).

In the Center area of Quito in 2007 as compared to 2000, there were significantly lower values for the percentage of children with ARI, the number of acute respiratory illnesses, annual rate of respiratory illness, COHb levels, and percentage of children with COHb > 2.5%. The children in 2007 were also significantly older, with more females, stunted children, and underweight children than those in the 2000 study (Table 6). The North area of Quito had a significantly higher percent of children with ARI, more ARI episodes, and a higher annual ARI rate, yet significantly lower values for COHb levels. The percentage of children with COHb > 2.5% observed in 2007 was low compared to 2000. Again, children in 2007 were significantly older and the percentage of stunted and underweight children was higher as compared to 2000.

For indoor CO contamination factors, the use of firewood as fuel was comparable across the time and locations, while the use of kerosene declined (Table 6). For the 2007 study, in a multi-pollutant model, daily CO and SO₂ levels were significantly associated with number of episodes of ARI (see [https:// nutrition.tufts.edu/ sites/ default/ files/ documents/ ENaumova- SupplementalMaterialCOHbARIpaper_09-24-18.pdf](https://nutrition.tufts.edu/sites/default/files/documents/ENaumova-SupplementalMaterialCOHbARIpaper_09-24-18.pdf)) (Table S7).

Table 6: Incidence of ARI and exposure measurements for children at Center and North in 2000 and 2007 studies.

Variables	Center 2000 (n = 313)	Center 2007 (n = 359)	North 2000 (n = 303)	North 2007 (n = 371)	p value ¹
Outcome					
Children with ARI	219 (70.0)	169 (47.0)	90 (29.7)	189(50.9)	a, b, c, d, e
No. of ARI episodes	496	224	114	241	a, b, c, d, e
Annual rate of ARI	6.89	2.25	1.63	2.41	a, b, c
Baseline characteristics					
Age (years)	8.36 ± 1.08	9.56 ± 1.56	8.94 ± 0.84	9.23 ± 1.49	a, b, c, d, e, f
Females	92 (29.5)	204 (56.8)	150 (49.5)	184 (49.6)	a, b, c
Weight (kg)	27.0 ± 5.97	28 ± 8.12	27.75 ± 5.21	28.04 ± 7.30	
Underweight ²	10 (3.2)	61 (17.0)	7 (2.31)	34 (9.16)	a, c, d, e, f
Height (cm)	126.46 ± 8.5	126.79 ± 11.7	128.57 ± 7.65	125.89 ± 13.1	d
Stunted ³	27 (8.26)	116 (32.31)	29 (9.57)	117 (31.53)	a, c, d, e
BMI (kg/m ²)	16.75 ± 2.3	17.11 ± 2.5	16.7 ± 2.4	17.3 ± 2.3	c, d
Survey					
Completed surveys	238 (76)	348 (96.93)	301 (99.34)	370 (99.73)	a, b, c
Households with kerosene use	7 (2.94)	0 (0.0)	4 (1.68)	0 (0.00)	a, c
Households with fire wood use	1 (0.42)	1 (0.29)	4 (1.34)	1 (0.27)	
Households with smokers	37 (15.14)	74 (21.26)	91 (30.54)	85(22.97)	b, d
Blood tests					
COHb levels (%) ⁴	5.09 ± 1.7	1.88 ± 0.29	2.55 ± 1.20	1.91 ± 0.46	a, b, c, d, e
COHb > 2.5% ⁵	97 (91.50)	12 (3.34)	39 (43.33)	24 (6.47)	a, b, c, d, e

ARI Acute respiratory illness

¹ p-value < 0.05: a (Center 2000 vs. Center2007), b (Center 2000 vs. North 2000), c (Center 2000 vs. North 2007), d (North 2000 vs. North 2007), e (North 2000 vs. Center 2007), f (North 2007 vs. Center 2007).

² Underweight was defined as weight for age Z score < -2SD

³ Stunted was defined as Height for age Z score < -2SD

⁴ COHb, as continuous variable

⁵ COHb, Carboxyhemoglobin over the safe level of 2.5%

DISCUSSION

Five years of vehicle emissions control effectively and significantly decreased mean COHb levels, percent of children with COHb above the safety level > 2.5%, and incidence of ARI. The strongest evidence of the relation between declining of CO air pollution and respiratory health is that the RR for the association COHb > 2.5% and incidence of ARI decreased by 67.5% in this period. In 2007, the average value of COHb in blood of study children was below the safety level of 2.5%, in contrast to 2000 study when the average exceeded the safety level. Furthermore, the percentage of children with COHb > 2.5% decreased by 92%, and the annual frequency of ARI/ child declined 46% compared to the year 2000 study. We suggest that reduced ambient levels of CO resulted in reduced COHb concentration and increased number

of children with the safe COHb level. Did this environment lead to decreased susceptibility to ARIs in children residing in areas with high micronutrient and oxygen deficiency?

This evidence is consistent with other limited trials that have demonstrated benefits in child respiratory health from air pollution reduction policies, and other actions implemented to improve air quality. Although those studies were not specific for CO reduction, overall declining trends in several air pollutants were associated with a decrease of medical visits for asthma and lower respiratory infections (44), decrease of bronchitis symptoms (19), improvements in lung function (16–18), less pulmonary inflammation (45), and reduction in asthma prevalence (20). School bus retrofits—used to reduce tailpipe and engine emissions—are associated with large reductions in bronchitis, asthma, and pneumonia incidence among children (46).

It is important to mention that some studies did not show definite effects of air pollution regulation on respiratory illnesses in children (23, 24). We believe that changes might better be attributed to the difference in pollutant exposure between subjects, the type of the policy that was implemented, or differences in study designs. Some studies on air pollution and health suggest that the variability in the relationship between decreases in pollution and respiratory symptoms may be due to unexplored heterogeneity between and within communities (18), measurement errors associated with instrument precision, spatial variability of pollutants (47, 48), and the lack of direct measurements of how emissions are changing and/or how they are responding to specific regulations (26).

Although lessening of both outdoor CO and COHb levels were seen in both study areas in 2007, the large reduction (67%) in the annual rate of IRA/child was detected only in the school located at the Center. This reduction was related to a marked decrease in the average of COHb levels in those children (63% reduction seen in Table 6). Other risk factors for acute respiratory infection, such as underweight and stunting (49–52) were not related to ARI (Tables 3 and 4). Potentially, this could indicate that ambient CO levels affect children independently of the nutritional status.

In the North area, the annual rate of ARI/child was 32% higher than in 2000 despite a 25% reduction of COHb levels observed in 2007 (Table 6). It is possible that other factors, such as immune status or other outdoor pollutants, which were not evaluated in this study, explain this finding. In fact, in the 2004–2007 period, the CO levels recorded in the North area were within desirable limits, and the pollutants of greatest concern were O₃ and PM_{2.5} because they increased annually albeit within acceptable levels. Our hypothesis that the increase of PM in the north of Quito in 2007 may explain the rise in the incidence of respiratory illness becomes more plausible because of other factors that could influence the presence of such illness. Those factors that were not associated with the increase in ARIs include male sex, younger age, presence of underweight, or stunting.

Even if overall air quality showed improvement from January to April 2007, $PM_{2.5}$ levels had an increasing trend in the North of Quito. During February, levels were above the maximum levels allowed by the national standard ($19.67 \mu g/m^3$) (37). Such increases reflect a significant increase in the car fleet with which the city of Quito has experimented across the years (7% per year or $\sim 30,000$ vehicles per year) (35).

It is worth mentioning that in the peripheral areas of Quito, such as in the north, the residential population has grown as has the number of commuters, with a resulting increase in heavy vehicular circulation, especially diesel buses (43). Diesel combustion is known to generate approximately ten times more nanoparticles than the combustion of gasoline (53). Even low levels of PM cause asthma and other respiratory diseases (46). Diesel exhaust particles induce alveolar macrophages to produce nitric oxide which can combine with superoxide anions to produce peroxynitrite, a potent oxidizing compound that alters body cells, any somatic cells (54). As indicated by a multi-pollutant model in 2007, CO and SO_2 levels were associated with the number of episodes of ARI. The high rate of respiratory illness in 2007 in neighborhoods charged with nanoparticles, suggests that control of CO emission and other pollutants, like SO_2 , were not sufficient to prevent illnesses associated with chronic respiratory inflammation.

Due to the differences in study designs and measurements of both ambient pollutants and respiratory outcomes, direct comparisons of our findings with studies addressing the benefit for respiratory health of short- or long-term air pollution reduction are not completely feasible. Nevertheless, our findings support those studies, demonstrating that improvements in respiratory health can be expected when there are significant reductions in environmental pollution, even for the short periods.

The 48% reduction in the incidence of ARI ($RR = 0.52$, 95% CI 0.45, 0.62, $p < 0.0001$) that we found after 5 years of vehicle emission control might be comparable to the 37–40% drop in pneumonia cases due to reduced emissions of diesel-related toxics particulates from school buses reported by Beatty and Shimshack in 2011 (46). Similarly, the reduced prevalence of common cold ($OR = 0.78$; 95% CI 0.68, 0.89) in children aged 6–15 years related to the decline in PM_{10} levels that was found by Bayer et al. in 2015 (21); and with the decreased prevalence of respiratory symptoms (bronchitis, cough, and phlegm) in adolescents with asthma due to decreases of NO_2 , $PM_{2.5}$, PM_{10} , and O_3 that Gilliland et al. demonstrated in 2017 (18). Those studies were carried out in developed countries where children are less exposed to environmental risks than children in developing countries, such as Ecuador. Our findings are needed if we are to alert developing countries to the importance of having healthy environments to prevent respiratory problems.

Ecuador has been listed as a country with a high level of air pollution, but there are others whose populations are also at risk: Peru, Colombia, Venezuela, Mexico, Honduras, Chile, and Guatemala comprise the top Latin American countries with the worst air pollution (55). Our

findings suggest that policies intended to abate vehicle air pollution may be appropriate in other parts of the developing world with similar geographic conditions and where the impact of air pollution on respiratory health is severe. In addition, such control strategies are likely to be sustainable, as Quito has already demonstrated feasibility in a developing country.

Taken together, these findings show a strong relationship between CO, COHb, and ARI, explained by biologically plausible mechanisms. They indicate a benefit of the lower concentration of environmental CO on the respiratory health of children achieved by vehicle exhaust control. CO, one of the principal polluting gases from vehicular emissions (56), affects respiratory mucosae from the nose to the alveoli, causing respiratory infections—from colds to bronchospasm and pneumonia (4, 57); and these infections increase hospitalizations (4, 57–59). When CO enters the blood stream it reacts with hemoglobin to form COHb. This compound does not allow an adequate supply of oxygen to reach tissues and organs of the human body in a dose-dependent manner (60, 61). Thus, an increase in ambient CO could lead to a potential increase in an individual's COHb level indicative of a condition that poses a risk for respiratory illness.

The major strengths of our study include (a) the representative sample size for Quito city, making the results relevant to other Andean countries, (b) the objective measure of CO exposure through COHb levels, (c) the direct detection of ARI cases by experienced pediatricians who examined each child every week, and (d) the inclusion of factors important for the development of acute respiratory illness. Furthermore, we compared our findings with the results of a 2000 study to determine whether a policy change had a long-term effect on a similar population. These strong points are responsible for robust analyses and trustworthy results.

The study has several limitations. Because CO monitoring was not available in 2000, it was not possible to evaluate directly the change of CO concentrations. For the analysis, we used conservative estimates based on the observed trend in measurements collected after 2004, when routine monitoring started. We compensated for the lack of measurements in 2000 with a more detailed analysis of CO lagged by 1 day with data available for 2007. We chose the lag of 1 day because during exposure to a fixed CO concentration, COHb levels increase rapidly over the first 2 h, and then begin to plateau at around 3 h, reaching an equilibrium steady state at 4–6 h. To maintain COHb below 2.5%, CO exposure cannot exceed 10 ppm (62). In blood samples collected in tubes containing EDTA or heparin and stored at room temperature or at 4 °C, COHb concentrations were stable for at least 5 days (38). The analysis of the CO concentration from the same day and a day prior COHb measurements reached similar conclusions.

We reconstructed the time series of CO measurements and demonstrated a trend in CO levels over time in two city areas. We have demonstrated that the ambient CO levels were strongly associated with the individual's COHb concentration (see https://nutrition.tufts.edu/sites/default/files/documents/ENaumova-SupplementalMaterialCOHbARIPaper_09-24-18.pdf)

(2–3). Reduction of COHb levels observed in 2007 might indicate the effect of the reduced outdoor CO level. In addition, CORPAIRE reported that the annual average concentrations of CO was reduced by 35% in the period 2004–2007 (from 1.29 to 0.83 $\mu\text{g}/\text{m}^3$), while the traffic density of light vehicles increased by approximately 47% (250,000 vs. 368,000 vehicles) (35).

Although we selected children from the same schools studied in 2000, the 2007 population had slightly different mean ages and nutritional status. Our multivariate models demonstrated that these variables were not associated with ARI occurrence. Unfortunately, we did not consider other factors that could have influenced ARI occurrence, such as physical activity, the use of transportation, or socioeconomic status. We did not evaluate what changes in lifestyle or immune status of each child that could have influenced the respiratory health status of the children.

While Quito's population increased from $\sim 1,820,000$ (63) to $\sim 2,120,000$ (64) during the period 2000–2007, there was no evidence that other factors affected the health outcomes, such as substantial changes in healthcare, or whether children stayed home when sick. Perhaps the same number of children developed ARIs, but less frequently or there was a dramatic improvement in indoor air quality.

In September 2006, the National Congress approved a law to regulate the use and consumption of tobacco and its derivatives; however, the law was never enforced. In July 2011, the National Congress banned smoking in public places, but not within households (65).

Over the study period, no specific emission control measures were implemented and it is unlikely that other sources of CO (industry, smoking) were reduced. As expected, the vehicle fleet increased in the city and in the northern area there were more buses fueled by diesel than in the downtown area. This might have increased ARI episodes in the North school area.

In light of these findings, we believe that broad policies should be implemented to improve air quality throughout our city to reduce the health problems due to environmental contaminants. Sustainable community-wide campaigns to raise awareness of the need for systematic controls of public and private motor vehicles can be useful preventive health actions. Our findings suggest that local pollution policies, such as control of exhaust emission from gasoline engine vehicles, and the gradual removal of old-fashioned carburetor vehicles from the circulation can contribute to the reduction of respiratory illnesses in children. These policies are likely to decrease the risk of school absenteeism and to reduce health care costs (45, 46). A comprehensive policy analysis may further demonstrate the benefits of the improved catalytic converters, the reduction of diesel emissions, and the use of biofuel in rapidly growing Latin American cities.

CONCLUSIONS

Our findings show that a substantial decline in ambient carbon monoxide level that resulted from a city-wide 5-year vehicular emission control program is associated with reduction of both incidence of respiratory illnesses and carboxyhemoglobin levels in school-aged children. Our study, along with others, supports the value of implementing preventive policies, and the need for sustained long-term programs in countries with poor air quality.

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SUPPLEMENTAL MATERIAL

Weather description

While the general climate of Ecuador is hot and subtropical, the climate of Quito, the national capital, is defined by its mountainous location. The city is situated 2850m above the sea level in the Andean Region. While the monthly temperatures are stable throughout the year averaging $\sim 15^{\circ}\text{C}$, the season are defined by precipitation. The dry (summer) season generally extends from June to September with the rest of the months reserved for rainy (winter) season (Table S1).

Table S1. Monthly average meteorological characteristics for 2000 and 2007.

Month	2000		2007	
	Monthly Precip sum	Monthly Average Shade air temperature ($^{\circ}\text{C}$)	Monthly Precip sum	Monthly Average Shade air temperature ($^{\circ}\text{C}$)
Jan	177.3	13.6	62.2	16.0
Feb	165.8	13.4	67.5	15.4
Mar	149.5	13.9	177.2	15.0
Apr	187.6	14.1	188.1	14.8
May	123.8	14.1	101.0	15.3
Jun	66.4	14.6	22.2	15.3
July	22.1	14.6	12.5	15.6
Aug	9.7	15.3	33.4	15.2
Sep	67.3	14.2	3.0	16.1
Oct	43.6	15.5	160.1	14.5
Nov	16.7	14.6	197.4	14.9
Dec	0.0	13.8	0.0	14.0

Data from: National Institute of Meteorology and Hydrology (INAMHI). Anuario Climatológico 2007. Quito, Ecuador. Available at www.serviciometeorologico.gob.ec/wp-content/uploads/anuarios/.../Am%202007.pdf

Model building for the relationship between ambient CO and COHb levels

The relationship between COHb levels and air quality on the day of and the day prior the blood draw was assessed by linear regression model. We included in the model individual characteristics (age, sex, underweight, stunting) and variables for the household sources of indoor air pollution (indoor firewood use and smoking). We developed the model in a sequential manner, starting with the simple analysis of relationship between $\log(\text{COHb})$ and CO (Table S2). We then gradually increased the model complexity by adding the individual covariates (Table S3), and two meteorological characteristics (Table S4). Table S4 is limited to

the ambient measurements for the day prior to COHb measurements. Please note, that in absence of actual measurements for air quality in 2000 we use interpolated values of 4.5mg/m³ for daily CO level (Tables S2-S4).

Table S2. Regression models for log (COHb) measurement and CO ambient concentration for 2000 and 2007.

	Beta	SE	p-value
The day of COHb measurements			
Constant	0.506	0.017	<0.0001
CO (mg/m ³)	0.145	0.007	<0.0001
The day prior to COHb measurements			
Constant	0.511	0.017	<0.0001
CO (mg/m ³)	0.144	0.007	<0.0001

Table S3. Adjusted regression models for log(COHb) measurement and CO ambient concentration for 2000 and 2007.

	Beta	SE	p-value
The day of COHb measurements			
Constant	0.787	0.076	<0.0001
CO (mg/m ³)	0.127	0.008	<0.0001
Age (year)	-0.024	0.008	0.002
Female	-0.037	0.021	0.082
Underweight ¹	-0.009	0.036	0.800
Stunted ²	-0.025	0.026	0.346
Indoor fire wood use	0.016	0.157	0.920
Indoor smokers	-0.058	0.026	0.027
R ² = 0.288			
The day prior to COHb measurements			
Constant	0.787	0.076	<0.0001
CO (mg/m ³)	0.127	0.008	<0.0001
Age (year)	-0.023	0.008	0.002
Female	-0.039	0.021	0.067
Underweight ¹	-0.004	0.036	0.919
Stunted ²	-0.031	0.026	0.232
Indoor fire wood use	0.007	0.156	0.964
Indoor smokers	-0.063	0.008	<0.0001

R² = 0.292

¹ Underweight was defined as weight for age Z score < -2SD

² Stunted was defined as height for age Z score < -2SD

Table S4. Adjusted regression adjusted Model for log(COHb) measurement and CO ambient concentration for 2000 and 2007.

	Beta	SE	p-value
The day prior to COHb measurements			
Constant	-5.590	1.224	<0.0001
CO (mg/m ³)	0.049	0.026	0.058
Age (year)	-0.021	0.007	0.005
Female	-0.021	0.021	0.317
Underweight ¹	0.009	0.035	0.788
Stunted ²	-0.028	0.025	0.276
Indoor fire wood use	0.002	0.150	0.988
Indoor smokers	-0.057	0.002	0.023
Ambient Temperature (°C)	0.358	0.710	<0.0001
Precipitation (mm)	0.011	0.002	<0.0001

R² = 0.345

¹ Underweight was defined as weight for age Z score < -2SD

² Stunted was defined as height for age Z score < -2SD

We then run a regression model for only 2007, which included actual measurements for available air pollutant (except for NO and O₃ due to incomplete records) as well as for temperature and precipitation for the day prior to COHb measurements (Table S5). In contrast to model presented in Table S4, where the effect of CO is driven by the substantial decline of CO concentration, the relationship between COHb measurements and ambient CO levels were not significant.

Table S5. Adjusted regression model for log (COHb) measurement and ambient concentration for CO, SO₂ and PM_{2.5} measured on a day prior the COHb measurements for the 2007 study.

	Beta	SE	p value
Constant	1.670	0.734	0.002
CO (mg/m ³)	-0.016	0.021	0.451
SO ₂ (μ/m ³)	-0.003	0.004	0.396
PM _{2.5} (μ/m ³)	0.002	0.002	0.173
Age (year)	0.003	0.005	0.577
Female	0.018	0.014	0.190
Underweight ¹	-0.003	0.022	0.904
Stunted ²	-0.013	0.016	0.404
Indoor fire wood use	0.158	0.126	0.212
Indoor smokers	-0.012	0.017	0.472
Ambient Temperature (°C)	-0.037	0.046	0.425

R² = 0.012

¹ Underweight was defined as weight for age Z score < -2SD 2 Stunted was defined as height for age Z score < -2SD

Model building for the relationship between ambient CO and COHb levels and respiratory illness

We constructed a Poisson regression model for ARI occurrence for 2000 and 2007 with COHb level and CO concentration values (same day) and adjusted for individual covariates. Only COHb levels are significant associated with number of episodes of ARI (Table S6).

Table S6. Adjusted Poisson regression model for ARI (number of episodes) and COHb concentration for 2000 and 2007.

	Exp (Beta)	95 CI	p value
The day of COHb measurements			
Constant	0.645	0.344-1.208	0.170
COHb (%)	1.215	1.125-1.314	< 0.0001
CO (mg/m ³)	0.992	0.923-1.065	0.821
Age/year	0.947	0.892-1.005	0.073
Female	1.060	0.900-1.249	0.488
Underweight ¹	1.060	0.813-1.382	0.669
Stunted ²	1.188	0.976-1.447	0.086
Indoor fire wood use	1.648	0.678-4.003	0.270
Indoor smokers	1.102	0.908-1.337	0.326

¹ Underweight was defined as weight for age Z score < -2SD

² Stunted was defined as height for age Z score < -2SD

Model building for the relationship between ambient CO, SO₂ and PM_{2.5} levels and respiratory illness in 2007.

We constructed a Poisson regression model for ARI occurrence for 2007 with CO, SO₂ and PM_{2.5} concentration values (same day of COHb measurements) and adjusted for individual covariates. CO, SO₂ levels are significant associated with number of episodes of ARI (Table S7).

Table S7. Adjusted Poisson regression model for ARI (number of episodes) and air pollutants in 2007.

	RR (95% CI)	P-value
CO (mg/m ³) ¹	1.56 (1.17–2.08)	0.002
SO ₂ (µg/m ³) ¹	1.10 (1.04–1.16)	>0.0001
PM _{2.5} (µg/m ³) ¹	0.98 (0.95–1.01)	0.060
Age/year	0.91(0.85–0.09)	0.007
Female	1.06 (0.89–1.27)	0.469
Underweight ²	1.20 (0.98–1.47)	0.472
Stunted ³	1.17 (0.96–1.44)	0.066
Indoor fire wood use	0.62 (0.14–2.76)	0.539
Indoor smokers	1.14 (0.93–1.39)	0.186

Rate Ratio (RR) - results from the Log-linear Poisson Generalized Estimating Equation model

¹ Continuous variable on the day of COHb measurement

² Underweight was defined as weight for age Z score <-2SD

³ Stunted was defined as height for age Z score < -2SD

Chapter 7

General conclusions

GENERAL CONCLUSIONS

The body of work presented in this thesis on air pollution exposure and respiratory health in Ecuadorian children shows the following:

With regard to carbon monoxide exposure we found that:

1. Children attending primary schools in high traffic polluted areas present acute respiratory infection episodes more frequently than children in less polluted areas.
2. Carboxyhemoglobin (a biomarker of carbon monoxide exposure) above the safe level of 2.5% is associated with a 3-fold increase in the annual rate of ARI incidence in children as compared to COHb < 2.5%.
3. The average concentrations of blood carboxyhemoglobin in the studied population of children living in Quito exceeded a safe level of 2.5% mostly due to elevated COHb in children from the area with high traffic volume.
4. Hematocrit (a measure of a compensatory oxygen-delivery function) level did not affect the observed relationship between individual COHb concentration and ARI occurrence.

These data imply that exposure to a high level of CO, the primary reason for increased COHb concentration, may lead to increased susceptibility to ARIs.

We assume the following hypothetical immunological mechanisms are present depending on a hypoxic environment caused by COHb:

1. A disruption of cytoskeleton integrity and apoptosis of alveolar epithelium could permit the entrance of microorganisms.
2. Inhibitory effect on the recruitment and differentiation of AMs as well as low phagocytic activity of AMs and immature DC would allow the survival of microorganisms.
3. Upregulation of monocyte/macrophage and mature DC pro-inflammatory cytokines and chemokines would aggravate the inflammatory process and delay the recovery of respiratory tissue and further lead to recurrent infections.

With regard to volcanic ash exposure we found that:

1. Volcanic ash exposure increased the rate of emergency room visits due to acute respiratory infections and asthma in children less than 15 years of age.
2. The rate of asthma and asthma-related diagnoses was two times higher during the period of fumarolic activity of Pichincha.
3. Children under 4 years were more affected by volcanic ash exposure than older children.

These data imply that there is a strong relationship between ash exposure and respiratory health in children.

We assume the following hypothetical immunological mechanisms:

1. At the beginning of the volcanic ash exposure, there is a first wave of ramifications with a transient acute irritant effect on the mucous membrane of the upper respiratory tract which can explain the increased number of asthma cases.
2. Later, when volcanic ash reaches the alveoli it may impair macrophage-mediated removal of particles and bacterial killing which could explain the development of respiratory infections.
3. Furthermore, a functional activation of the NLRP3 inflammasome by volcanic can occur in macrophages which aggravates the inflammatory process.
4. Finally, youngest children appear to have been affected the most, due to a) the anatomic and functional characteristics of their respiratory system which permit faster access of the pollutants to the lung, and b) the immaturity of their immune system.

These hypotheses suggest that volcanic ash exposure, while not seriously compromising lung cell function, may be able to impair innate immunity responses in exposed individuals.

With regard to PM and NO_x exposure we found that:

1. Children under 36 months of age exposed to higher levels of PM and NO_x had a higher risk of pneumonia-related hospitalizations, total respiratory illnesses and anemia than children exposed to lower levels of air pollution.
2. Anemic children under 36 months of age exposed to higher levels of pollution presented a risk of pneumonia-related hospitalization six times higher compared to non-anemic children in the same polluted neighborhood.

These data imply that anemic children exposed to relatively high levels of PM and NO_x present increased susceptibility to lower respiratory infections, including pneumonia, and that a possible synergy exists between a common manifestation of nutritional anemia and ambient air pollution as risk factors for pneumonia-related hospitalization in a high-altitude setting.

We assume the following hypothetical immunological mechanisms:

1. Loss of integrity of the respiratory epithelia allows greater exposure to pathogens.
2. Impairment of NK cells cytotoxicity could increase susceptibility to infections.

3. Excessive production of proinflammatory cytokines by NK, AMs, DC could threaten lung gas exchange, especially in anemic children, and aggravate any respiratory episode.
4. Hypoxia due to anemia and methemoglobin formation could alter the function of some innate cells and delay recovery.

With regard to the effects of air pollution on lung ILCs we found that:

1. Exposure to key air pollution, including PM, DEP, and O₃ stimulates lung ILC2s to produce high levels of IL-5 and IL-13.
2. Exposure PM, DEP, and O₃ inhibits in general the cytotoxicity and IFN- γ production by lung ILC1 (NK) cells (apart of low doses of O₃).

We assume the following hypothetical participation of ILCs on respiratory problems associated with exposure to pollutants:

1. ILC2s would be involved in airway inflammation through production of IL-5 which recruits and activates eosinophils in the respiratory mucosa, causing inflammation through their cytotoxic proteins.
2. ILC2s through production of IL-13 could stimulate airway smooth muscle contraction by modulating Ca²⁺ responses and stimulating innate immune cells to secrete chitinase.
3. ILC1 NK cells would aggravate inflammatory process by secreting proinflammatory cytokines (IL-1 β , IL-8 and TNF- α) which in turn elicit innate immune cells to produce inflammatory proteins.
4. ILC1 NK cells exposed to air pollutants could be involved in increased susceptibility to respiratory infections since they present low cytotoxicity capacity and reduced production of IFN- γ , two key mechanism to fight against pathogens .

With regard to air pollution policy control we found that:

1. Local air pollution control policies, such as the control of exhaust emissions by gasoline-engine vehicles, and the gradual removal of outdated carburetor-vehicles from the circulation significantly reduces the frequency of respiratory illnesses in children.
2. A decline in ambient carbon monoxide levels resulting from vehicular emission control programs is associated with a reduction of carboxyhemoglobin levels in school-age children.
3. Reduction of carboxyhemoglobin levels results in a reduction of respiratory illness.

In conclusion, these data taken together indicate that exposure to high levels of outdoor CO, PM, NO₂ and volcanic ashes is associated with increased susceptibility to respiratory illness, and supports the benefit of a policy aimed at lowering the concentration of environmental pollutants to decreased susceptibility to ARIs in children residing in areas with high micronutrient and oxygen deficiencies.

Chapter 8

Discussion

DISCUSSION

Environmental pollution is a critical growing public health problem especially in developing countries (1). According to the WHO, 570,000 children under the age of five die every year as a result of respiratory infections caused by indoor and outdoor air pollution and exposure to environmental tobacco smoke (2). Therefore, there is a continuing demand for improving air quality to avoid those deaths. The studies presented in this thesis support the relationship between air pollution and respiratory infection in children (3-5), focus the effect of the main air pollutants (PM, O₃, and DEP) on the lung immune system, and emphasize the importance of establishing policies to reduce environmental pollution (6). Overall, these findings show that children exposed to CO, PM, NO_x from traffic exhaust fumes or volcanic ash are at risk of developing respiratory illness and that this risk could be exacerbated by the child's nutritional and health (anemia) status. Besides, the results provide a general picture of how immune cells, and particular ILCs, are involved in the airway hyperresponsiveness and in the increased susceptibility to respiratory infections when exposed to different contaminants. Our results also demonstrate that the decline in traffic-air pollution due to vehicle emissions control is associated with a reduction in respiratory illness incidence in school-aged children.

Interpretation of the results

1. Carbon monoxide exposure and respiratory infections

The toxic effects of CO in different human tissues are due principally to its ability to bind to the heme complex within hemoglobin capturing oxygen and forming COHb, a compound that prevents hemoglobin from delivering oxygen throughout the body (7) causing a hypoxemic status on the tissues. The lowest level of COHb, at which adverse effects are observed, ranges from 2.9 to 3%, and a concentration of < 2.5% is considered safe (8). Possibly, long-term CO exposure could damage the lung tissue, resulting in respiratory problems.

Although CO is one of the main environmental pollutants, few studies have been conducted regarding its effects on respiratory infections. For example, it has been reported that higher levels of CO increase the risk of hospital admissions for total respiratory conditions (9) and pneumonia (10, 11). Also, the prevalence of bronchitis symptoms among children with asthma increase as the concentration of CO increases (12). Our results confirm the existing literature that exposure to CO is associated with an increased risk of respiratory events in children (Chapter 2). However, it is important to note that our study measured individual concentrations of COHb, as a quantifiable proxy for CO exposure and likely render more accurate results as compared to the aforementioned studies that used environmental measurements of several pollutants, including CO, and examined associations with respiratory outcomes in a cross-sectional manner.

There are no studies yet on the plausible immunological mechanisms for CO to cause respiratory infections. Hypothetically, the mechanism could be attributed to the hypoxic status caused by high concentrations of CO. Hypoxemia impairs the cytoskeleton and induces apoptosis of the epithelial cells (13) causing broad uncovered respiratory surfaces through which microorganisms can enter. After crossing the epithelial barrier, pathogens should be eliminated by phagocytic cells; however, this does not happen because a) hypoxia impedes optimal recruitment of alveolar macrophages (14, 15) while permitting high migration levels of immature DCs towards the secondary lymphoid organs (16) and b) the few remaining alveolar macrophages (MAs) and immature dendritic cells (DCs) exhibit a reduced phagocytic activity (14, 15, 17). Besides, hypoxic MAs and DCs which are unable to phagocytize, secrete proinflammatory cytokines and chemokines that would aggravate the inflammatory process and delay the recovery of respiratory tissues leading to recurrent infections in those children.

Consequently, children with COHb above the safe level of 2.5% would have a higher hypoxemic status that puts them at an increased risk of infection. Precisely, we found that low COHb concentrations (< 2.5%) were associated with a low rate of ARI (0.6 episodes per child per 12 weeks) while a greater exposure to CO was related to a higher frequency of acute respiratory episodes. Furthermore, our study revealed that with one percent increase in COHb above the safety level, children are 1.15 (95% CI, 1.03–1.28) times more likely to have an additional case of ARI, meaning that minor increases in COHb concentration would lead to a greater risk of respiratory illness. In light of this evidence, cities with growing urbanization rates, and a subsequent increase in their number of circulating vehicles, should build up their public health efforts to control exhaust emissions to lower the risk of respiratory infections.

2. PM and NOx exposure and respiratory infections

Several pieces of scientific evidence show that PM and NOx have harmful respiratory effects in children in both developed and developing countries. Exposure to PM has been associated with an increase in respiratory symptoms (18) as well as an increased risk of upper and lower respiratory infections and pneumonia (19-21). A higher risk of emergency room visits, hospital admissions, health care utilization for respiratory infections has also been reported (22-28). At the same time, some studies have shown no significant effects of PM on the risk of pneumonia (29, 30) or on hospitalization due to respiratory infection (9, 22). It is likely that the effects depend on the PM concentration, air temperature, weather, wind, age and sex of subjects or even on the type of respiratory pathogen. A relationship between NOx and increased respiratory symptoms (12), respiratory infections (29, 30), and risk of emergency room visits or hospital admissions for respiratory infections (10, 31) has also been found. Our study in young children living in areas with different air pollution conditions adds to the evidence that exposure to higher outdoor air pollution (PM and NOx),

is associated with higher numbers of total respiratory illnesses and increased respiratory-related hospitalizations (chapter 4).

Few studies have examined the mechanisms by which PM can contribute to an increase in acute respiratory infections, but the most likely explanation lies in the adverse impact that high concentrations of these pollutants have on the innate immune cells. First, PM exposure would provoke a loss of integrity of the respiratory epithelia because it induces oxidative stress (32) and impairs the mitochondrial structure and DNA biogenesis (33) resulting in activation of multiple cell death pathways allowing greater exposure to pathogens. In addition, PM reduces the production of antimicrobial peptides by epithelial cells, which limit the capacity to clear or remove pathogens from the respiratory tract (32). Second, pathogens that go beyond the epithelial barrier can easily proliferate because the PM exposed macrophages are unable to display their phagocytic activity (34) and anti-microbicidal function (35). The intracellular pathogens can also survive since PM suppresses the ability of ILC1s (NK) to secrete perforin and granzyme B to kill infected cells (36) (chapter 5). Third, a sustained inflammatory state would be generated because epithelial, dendritic, and NK cells are elicited by PM to secrete IL-6, TNF- α , IL-6, IL-8 and IL-12 among others (36-39), with the consequent PMN infiltration of the lung where they are permanently activated and produce superoxide radicals that may contribute to lung tissue injury (40). Finally, PM stimulated DCs may potentiate T-lymphocyte cytotoxic responses by increasing the damage to the airways and the severity of the infection (41).

NOx exposure also contributes to causing lung tissue injury through a) formation of reactive oxygen and nitrogen species causing the death of epithelial cells (42), b) increasing the inflammatory process by attracting PMN cells (43), and c) causing a hypoxic state by binding to hemoglobin and forming methemoglobin.

Taken together, PM and NOx create an imbalance of inflammatory mediators and suppress the microbial ability of innate cells. These effects possibly are more pronounced in young children, which might explain why the study children (< 36 months of age) living in highly polluted areas were at three times more risk of respiratory illness including severe pneumonia and required four times more hospitalizations compared to children exposed to a lower level of pollution (chapter 4). Therefore, strategies for ambient air quality control are needed to improve the respiratory health conditions of Ecuadorian children living in areas of poor air quality and of others in similar situation.

3. The triad of anemia, air pollution, and respiratory infections

Several studies have demonstrated the relationship between anemia and respiratory infections (44-48), others have shown that air pollution causes anemia (49-51), or respiratory infections (3, 10, 11, 20, 21, 26, 29, 31, 52), and a few others have revealed that bacterial infections can decrease iron levels and cause anemia (53, 54). However, in the reviewed

scientific literature there are no studies displaying the relationship between anemia, air pollution, and respiratory infections in a population group, except for our study showing that anemic children under 36 months of age exposed to higher PM and NOx pollution presented six times more risk of pneumonia-related hospitalization compared to non-anemic children in the same polluted areas (presented in chapter 4).

Anemia and air pollution separately account for several cases of respiratory infections, but both factors acting together likely involve a higher risk for infections. There seems to be an intricate synergism among air pollution, anemia (iron deficiency), and infection. These interlaced effects could have as a common denominator for an impaired immune system (Figure1).

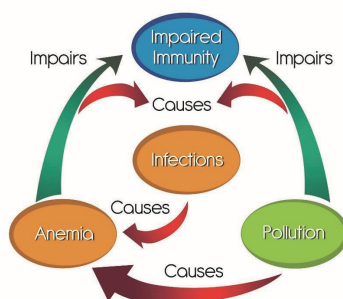


Figure 1. This figure shows a diagram of the triad “anemia, respiratory infections, and immune system”.

Anemia and pollution impair the immune system and cause infections. Infection reduces iron in the body causing transitory anemia. Air pollution causes anemia by reducing red blood cells and hemoglobin.

Anemia and respiratory infections: Iron-deficiency anemia is the most common micronutrient disorder worldwide. Women of childbearing age and children under 5 years of age are the most vulnerable groups. In Ecuador 26% of preschool children suffer from iron-deficiency anemia (Hb levels <11g/dL), and the percentage is higher in children under 1 year of age (62%) (55). Consumption of iron-poor diets is the main cause for anemia, especially in the Andean region where Quito is located (55).

Iron deficiency has been related to an impairment of cell-mediated immunity (56) and bactericidal activity of phagocytic cells (57, 58). These deleterious effects can be explained by the critical role that iron has on proliferation, differentiation and activation of monocytes, macrophages, and T lymphocytes (59) as well as on the modulation of cytokines production and the oxidative process of the innate immune cells (57). Clinical and experimental studies have shown that iron deficiency decreases serum IL-6 (58) and IL-2 levels (60); reduces phagocytic activity of monocytes, and oxidative burst activity of neutrophils and monocytes

(58, 61); lowers the levels of CD4-T cells and decreases CD4:CD8 ratio,(56); but does not alter humoral immunity (56, 62).

An impaired immunity related to iron deficiency possibly makes children more vulnerable to both viral and intracellular bacterial infections. In fact, some studies have reported that anemic children were more susceptible to lower respiratory tract infections compared to non-anemic children (47, 48, 63, 64). This evidence supports in part our findings on the higher risk of pneumonia hospitalization in anemic children living in Quito, and leads us to think that nutritional programs to diminish the rates of anemia on the population could also be beneficial to lower the risk of respiratory infections in those children.

Air pollution as a cause of anemia and respiratory infections: Some studies have shown that long-term exposure to air pollution is associated with presence of anemia, and that ambient PM and NO₂ are the main pollutants implicated in this association (50, 51). It has been shown that high concentrations of air pollutants alter iron metabolism in the body resulting in iron-deficient anemia, with low values of hematocrit, hemoglobin, and red blood cell count (RBC) without changes in RBC indices (42, 49, 51, 65). One of the main proposed mechanisms explaining these findings is the inflammatory status triggered by PM and NO₂ exposure, which in turn, decreases erythropoietin secretion and induces erythropoietin resistance in the bone marrow (42). Additionally, studies show that NO₂ can produce RBC membrane peroxidation, (65), formation of methemoglobin (42, 66).

The possible mechanisms by which PM and NO₂ impair immunity and cause respiratory infections have been described earlier in this chapter, and in Chapters 1 and 5.

In conclusion, ambient air pollution and anemia appear to synergistically increase respiratory infections due to an impairment of the immune system. On the one hand, PM and NO₂ induce innate immune cells (EC, NK, AMs, and DC) to produce excessive proinflammatory cytokines which could threaten lung tissue integrity leaving it exposed to respiratory pathogens, and may also cause anemia. On the other hand, hypoxia due to anemia and methemoglobin would alter cell-mediated immunity and the bactericidal activity of phagocytic cells, worsening the infectious episode.

This evidence supports our finding that children living in highly polluted areas of Quito were 1.5 times more likely to be anemic and had four times more pneumonia-related hospitalization rates compared to children living in less polluted areas (chapter 4). Optimal control of air pollution as well as the prevention of anemia in young children should be implemented in developing countries to decrease hospitalizations for pneumonia, the leading cause of death in children. Definitely, additional studies are needed to confirm our findings of synergy between air pollution and anemia.

4. Volcanic eruptions and respiratory infections

Volcanic eruptions and respiratory problems have been well established. The magnitude and severity of the respiratory problems depend on numerous factors including the intensity of the eruption, the physical and chemical characteristics of the emissions, the weather conditions, and the characteristics of the affected people(67-70). Children, the elderly, and people with previous respiratory problems are at higher risk of presenting respiratory illnesses. It is known that the range of respiratory affections can vary from mild (i.e. throat irritation), to severe such as asthma or respiratory infections, but neither the behavior of these problems during the phases of a volcanic eruption nor the involved immunological mechanisms have been explored in depth.

In chapter 3, we described the variation in pediatric ER visits for respiratory conditions before, during, and after volcanic ash falls in Quito, Ecuador, due to Guagua Pichincha volcano eruption which occurred in April of 2000. We showed that during the eruption, the rate of emergency room visits due to acute respiratory infections and asthma in children under 15 years of age increased (Figure 2). We also posited that the rate of asthma and asthma-related diagnoses was two times higher during the period of fumarolic activity of the volcano (Figure 2); and that children under 5 years were more affected by volcanic ash exposure. These data are in accordance with some other studies showing an increase of ER/hospital visits for cardiorespiratory causes (71), acute respiratory diseases (72), and asthma(68)in adults and children during a volcanic eruption.

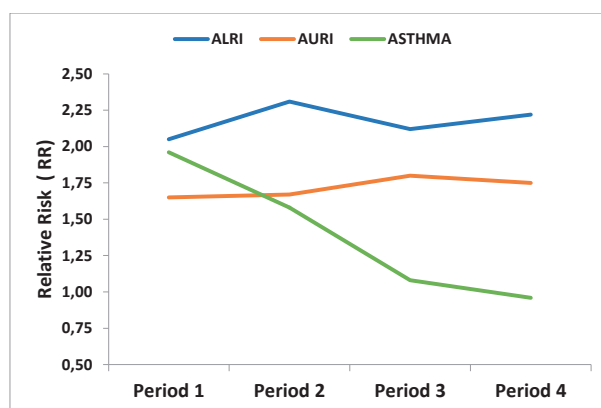


Figure 2. Relative risks associated with Guagua Pichincha's volcanic activity for three respiratory diagnostic groups.

ALRI -Acute lower respiratory infection; AURI-Acute upper respiratory infection; Period 1- Fumarolic; Period 2-one week after 1; Period 3-two weeks after 1; Period 4-three weeks after 1.

In order to explain this behavior from an immunological standpoint, we assume the following hypothetical immunological mechanisms elicited by the gases and particles of the volcanic emission.

During the fumarolic period, a transient acute irritant effect on the epithelial membrane of the upper respiratory tract takes place due to volcanic gases, especially SO₂ reacting with the epithelial fluid to produce bisulfite and sulfite agents (73). These metabolites induce the respiratory epithelium to express two asthma related genes: mucine 5, subtypes A and C (MUC5AC) responsible for mucus secretion and airway obstruction, and the IL-13 gene, responsible for production of IL-13 (74). IL-13 is a pro- inflammatory cytokine involved in the pathogenesis of human allergic asthma by inducing contractility of airway smooth muscle cells (73), IgE switching in B cells, infiltration of inflammatory cells and mucus hypersecretion (75, 76). In addition to SO₂, particles of volcanic ash trapped by mucus can cover the airway epithelium and worsen the asthma (68). Although there are no studies in humans, it is plausible that ILC2s and their cytokines (IL-5 and IL-13) can contribute to the allergic inflammation since murine experimental studies demonstrated that particulate matter produces accumulation of ILC2s in the lungs (Chapter 5). In summary, the first contact with volcanic gases and PM <4µm may induce airway inflammation through the activation of epithelial cells (Figure 3A).

Later, when volcanic particles <4 µm reach the alveoli, macrophages try to phagocytose them without success since either toxic crystalline silica particles or excess of inert particles may impair macrophage-mediated removal of particles, and bacterial killing (68, 77). Likewise, iron-rich crystalline silica particles induce macrophages to produce large amounts of ROS (78) that can cause macrophages to die. Besides, inhalation of crystalline silica can activate the NLRP3 inflammasome in macrophages which worsens the inflammatory process (79). In conclusion, these detrimental effects of volcanic particles on macrophages and NK cells could explain the development and persistence of respiratory infections beyond the fumarolic period of a volcanic eruption (Figure 3B).

Finally, younger children could have been affected the most, due to the anatomic and functional characteristics of their respiratory system which may facilitate the access of the pollutants to the lung (80, 81) as well as the limitations given by the immaturity of their immune system (82, 83).

Our findings together with those of others studies document the damage that volcanic eruptions can produce on the respiratory health of the population, and highlight the necessity of countries surrounded by active volcanoes being prepared to protect people, especially young children, from the harmful effects of volcanic eruptions.

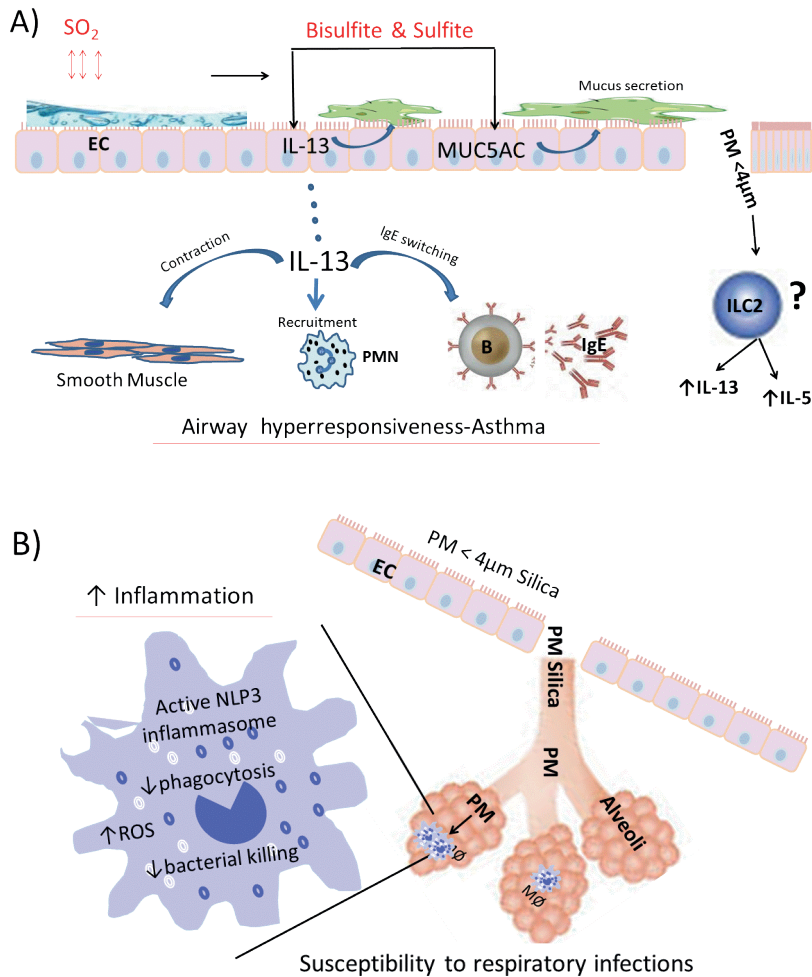


Figure 3. A) Asthma in volcanic fumarolic period. SO_2 , acting with epithelial fluids produces sulfite and bisulfite metabolites that stimulate epithelial cells (EC) to produce both mucine-5 AC (MUC5AC) responsible for mucus secretion, and IL-13 responsible for smooth muscle contraction, neutrophil (PMN) recruitment, and IgE switching in B cells. Particulate matter (PM) induces innate lymphoid cell type 2 (ILC2) to produce IL-13 and IL-5 related to allergic inflammation. **B) Respiratory infections post-fumarolic period.** Silica particulates (PM silica) reach the alveoli and induce alveolar macrophages (M ϕ) to a) activate the inflammasome system causing an inflammatory process, b) increase the production of reactive oxygen species (ROS) causing DNA damage, and c) decrease both phagocytosis and bacterial killing.

5. The participation of ILCs in respiratory illness associated with air pollution

Innate lymphoid cells (ILCs), a novel group of the innate immune system, populate barrier surfaces, including skin, intestine, lung, and some mucosal-associated lymphoid tissues (84). ILCs do not have antigen specific receptors and their activation depends on the surrounding cytokines. Once activated, ILCs perform their functions through cytokine production which varies according to the type of ILC (85-87). ILC1s include classical NK and non-NK cells that principally produce IFN- γ (86, 88). ILC2s secrete IL-5, IL-13, IL-9(89, 90), and amphiregulin (90). ILC3s produce mainly IL-17 and IL-22(91). In general, these cells coordinate the innate immune response, contribute to inflammation, modulate adaptive immunity (84), and regulate wound repair and tissue regeneration (92), but in some situations they can cause tissue injury (93).

The role of ILCs in the human lung tissue is not well studied due to the limited access to human lung samples (93, 94). Some studies have shown that the three ILC subsets are scattered within the lung tissue and probably concentrate in different places only after damage or inflammation. In the lung, ILC3s are more frequently found (60%) (93), and play an important role in antibacterial immunity, lymphoid organogenesis, recruitment of neutrophils, and protection of the epithelial barrier. ILC2s, which correspond to 30% of the ILCs (93), produce airway inflammation, bronchial hyperreactivity, increases the effects of allergic asthma (95), and also participate in airway homeostasis and lung tissue repair (96, 97). ILC1 NK cells represent only 10% of the ILCs (93), and mainly control infections by cytotoxicity, but also induce lung tissue injury(98).

Summarizing, lung ILCs are crucial for lymphoid tissue formation, respiratory tissue remodeling, airway homeostasis, they are important to fight against pulmonary infections, and contribute to inflammation. But what happens to these cells when they are subjected to air pollutants?

The systematic review presented in chapter 5, offers *in vitro* and *in vivo* experimental evidence of the deleterious effects of PM, diesel exhaust particle (DEP), and O₃ on the number, cytokine production, and activity of ILC1s (human and mice) and ILC2 (mice). With this information we propose the following hypothetical immunological mechanisms elicited by pollutants in the respiratory system:

Although there are no studies on the effect of pollutants in human ILC2s, from the studies in murine species it could be inferred that these cells would be involved in airway inflammation and hyper-responsiveness associated with exposure to pollutants (Figure 4A). Exposure to DEP, carbon nanotubes, and O₃ induces respiratory epithelial cells to secrete IL-33 (99, 100) which in turn elicits ILC2s to increase mRNA expression of IL5 and IL-13 (101) and secretion of these cytokines (99-102). On the one hand, IL-5 has several functions in the adaptive and innate responses, but preferentially recruits eosinophils from blood vessels in

the respiratory mucosa, and triggers them to degranulate cytotoxic proteins (103), and to generate ROS (104), substances responsible for airway inflammation. On the other hand, IL-13 could participate in airway hyper-responsiveness by modulating Ca^{2+} dependent responses of airway smooth muscle (105) and stimulating macrophages and epithelial cells to secrete chitinase, an enzyme that seems to induce smooth muscle contraction (106, 107).

Studies on human ILC1 NK cells showed that exposure to air pollutants results in impaired cell number, function and cytokine release, which could be related to both the establishment of airway inflammatory pathology and susceptibility to respiratory infections (Figure 4B). Once pollutants are inhaled, they stimulate epithelial cells, macrophages, and dendritic cells to secrete IL-12 (37, 108) and IL-18 (37, 109) which trigger human ILC1 NK cells to carry out several complex and diverse effects. In presence of DEP, IL-12 and IL-18 induce ILC1s NK cells to produce a modest increase of pro-inflammatory cytokines (IL-1 β , TNF- α , and IL-8) (36, 110), which in turn induce recruitment and activation of neutrophils, monocytes, and eosinophils that establish an inflammatory process in the respiratory tissue. In addition, DEP and high doses of O_3 have an immunosuppressive effect on the pulmonary NK cells' cytotoxic activity. These pollutants showed to decrease the expression of the activating marker CD16 (111), and the production of granzyme B, and perforin (36). Finally, high doses of O_3 prime human NK cells to decrease the expression and secretion of IFN- γ (112), a key cytokine that enhances the microbicidal and tumoricidal activity of macrophages (113). Accordingly, the abatement of ILC1 NK cell along with the impairment of other immune processes not mentioned here could contribute to the high incidence of lung infection associated with air pollution exposure.

As a conclusion, it seems that different air pollutants have specific, deleterious effects in the respiratory tissue through effects on innate immune cells including those ILCs subsets reviewed here. Certain pollutants, such as DEP and O_3 have opposite effects on the same cell, sometimes producing pro-inflammatory responses, while on others causing immunosuppressive effects. As a result, the first one is associated with enhancement of hyperresponsiveness, and the latter with an increased risk of infection.

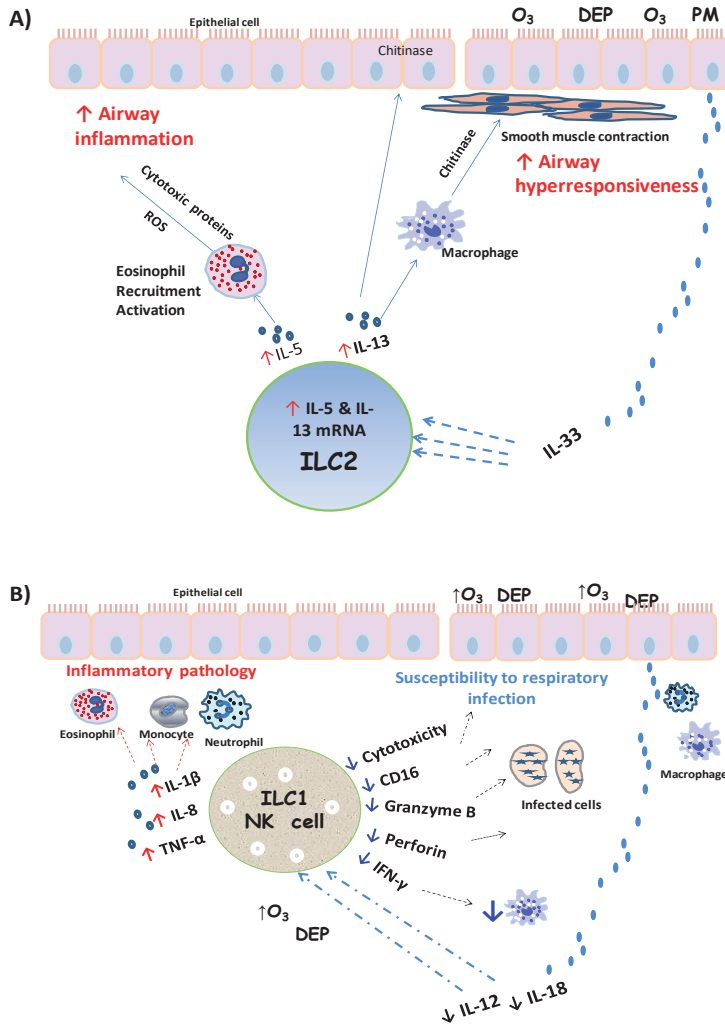


Figure 4. A) Effects of pollutants on ILC2s. Particulate matter (PM), diesel exhaust particles (DEP) and ozone (O₃), elicit epithelial cells to produce IL-33 that stimulates ILC2. ILC2s produce a) IL-5, a cytokine that recruit and activate eosinophils that degranulate cytotoxic proteins and reactive oxygen species (ROS), that result in airway inflammation, and b) IL-13, a cytokine that makes macrophages and epithelial cells produce chitinase, an enzyme related to smooth muscle contraction and airway hyperresponsiveness. B) Effects of pollutants on NK cells. DEP and high doses of O₃ decrease IL-12 and IL-18 secretion by epithelial cells. These reduced concentrations of IL-12 and IL-18 plus DEP and O₃ produce opposite effects on NK cells: a) stimulate pro inflammatory cytokines (IL-1 β , IL-8 and TNF α) secretion which induce monocytes, neutrophils and eosinophils to produce inflammatory proteins, and b) impair the capacity of killing infected cells by reducing granzyme and perforine secretion, but also reduce interferon gamma (IFN γ) secretion which alters the phagocyte capacity of macrophages.

6. The experience of a policy to reduce environmental pollution

Environmental pollution poses continuing risks to the health of human beings worldwide. For several decades, developed countries have developed strategies to limit its impact and have implemented control measures with favorable results both on the air quality and on the respiratory health of children (114-120). However, in developing countries such as those in Latin America, where pollution is a raising concern due to the rapid growth of transportation and manufacturing industries, awareness of the dangers of pollution on the large number of people exposed has not been a main concern. Few countries have applied measures for air pollution abatement (121-123), but studies of the implications of such measures on the respiratory health of children are scarce.

In Quito, the most polluted city in Ecuador, only when the problematic and the magnitude of its health consequences were demonstrated, it was possible to mobilize consciences and generate programs for the improvement and control of pollution. The study presented in chapter 2, shows a positive correlation between CO exposure (measured through COHb) and occurrence of acute respiratory infections in children attending schools located in areas with different traffic intensities in Quito in 2000. These findings were the foundation for implementing the first local policy for traffic-related pollution abatement. In 2002, the municipality of Quito issued a municipal ordinance that made passing a vehicular technical inspection mandatory. This was implemented in order to verify the compliance of vehicle safety standards including concentration of CO, CO₂, HC's and O₂ in the gases emitted from the exhaust pipe of vehicles fueled by gasoline(124). Two years later, this ordinance became the purview of the "CORPAIRE" or "Corporation for the Improvement of the Air of Quito", which assumed the vehicular technical revision process and air quality monitoring, through the Atmospheric Monitoring Network (124).

According to CORPAIRE, during the 2004-2007 period the annual average concentrations of SO₂, CO and PM₁₀ had significantly decreased. CO, specifically, presented a 35% reduction (from 1.29 to 0.83 µg/m³) (125) as a result of a tighter fume emissions control by the vehicular technical inspection process and to the gradual phasing out of outdated, heavily polluting vehicles from circulation (126). In the year 2007, we could evaluate whether the improvement of air quality had influenced the respiratory health in school children. The study presented in chapter 6 describes the magnitude of benefits from a modest five-year air pollution control policy on the incidence of acute respiratory illness in school-age children of Quito. The exposure of interest was CO, which was measured as blood COHb levels in children. The strongest evidence of the relationship between declining CO air pollution and respiratory health was the fact that the relative risk for the association COHb > 2.5% and incidence of ARI decreased by 67.5% in this period. Our results suggest that policies aimed to abate vehicle-caused air pollution may be appropriate in developing countries with similar geographic conditions and where the impact of air pollution on respiratory health

is severe. Additionally, these findings are a valuable contribution to the scientific literature on effectiveness of accountability studies as they provide a stronger basis for public health policy development.

Limitations of the study

Exposure to pollutants

A limitation of our analysis during the studies process carried out in 2000 (chapters 2 and 3) was the absence of direct measures of exposure. Regrettably, in Quito, systematic monitoring of air quality for critical air pollution was initiated only after 2000, so we could only rely on indirect proxies such as traffic intensity, limited satellite imagery records of ash emissions, and daily records of seismic activity of the Pichincha volcano to establish the timing of high exposure to pollutants. Since CO monitoring was not available in 2000, the comparative study evaluating the effect of air pollution control on respiratory illness (chapter 6) was not able to assess the actual change of CO concentrations. Therefore, for the analysis we used the most conservative estimates based on the observed trend in measurements collected after 2004, when the routine monitoring started. We compensated the lack of measurements in 2000 with a more detailed analysis of CO lagged by one day with data available for 2007.

Furthermore, in our studies carried out after 2004 (chapters 4 and 6) when the air monitoring system started in Quito, we used as reference for pollutant exposure the CO, PM and NO_x measurements from the urban monitoring stations close to the study areas. These exposure data may not faithfully represent the true exposure levels of each individual given that possible difference in inhalation and exposure mechanisms among subjects were not taken into account. It is also important to consider that urban monitoring may possibly not allow for measurements in spatial and temporal variation of the exposure. It would have been convenient to conduct personal monitoring of the exposure to make the estimates more reliable. However, in Quito there were no available measurements of biological markers of environmental pollution except for carbon monoxide. Obtaining such data would have required access to up to date laboratory facilities at a higher cost and with considerable delays. Nevertheless, there may still be a good correlation between the environmental and personal exposure estimates.

Risk estimates

A common limitation for our epidemiological studies evaluating the risk of respiratory infection due to air pollution exposure, such as volcanic emission in chapter 3 and PM and NO_x in chapter 4, is that the respiratory effects of each pollutant are difficult to separate due to a linear correlation among them. Therefore, the risks could have been under or overestimated

which puts our conclusions in caution. However, these estimates were prominent enough that the health authorities became aware of the environmental risks and decided to develop measures to reduce them.

Diagnosis of acute respiratory infection

The determination of ARI episodes in the studies presented in chapter 2, 3 and 6, was based on a clinical case definition and therefore prone to subjective and observer bias. However, to minimize the bias, ARI diagnostics were made by a small group of trained pediatricians. It would have been important to determine the infectious etiology or measure some inflammatory biomarkers such as C reactive protein (CRP), but it would have been expensive, and the extra blood sample required for these markers could have potentially decreased the number of study participants.

Innate Immunity

Immunological studies are essential to better understand the role of air pollution in the respiratory illness, but we were not able to explore such role since this involves invasive methods to obtain immune cells in children. Such methods are not allowed in our country unless there is a higher risk to benefit ratio. The lack of specific findings in pediatric patients made us extrapolate findings from adults to reconstruct the possible events that may have occurred in the respiratory immune system of children to explain the high incidence of respiratory infections.

Future Studies

“More than 1 in 4 child deaths could be prevented by cleaning up the environment” (127).

If this is obvious, why is the problem becoming more pressing especially in developing countries?

Possibly in Ecuador, the lack of public awareness about traffic-related pollution and its effect on health is one of the main problems. In the whole country, with the exception of Quito, no obvious connection has been made between the traffic-related pollution and health. Therefore, it is necessary to conduct studies exploring the effect of either individual pollutants or combined pollutants on the respiratory health in larger populations at urban and rural levels. After having the results, sustainable community-wide campaigns to raise awareness of the need for control of traffic-air pollution can be useful as a preventive health action. It is also important to carry out accountability studies, in cities that have implemented local air pollution policies, to provide meaningful information to decision makers to review the efficacy of current policies, and if necessary to develop effective new policies. Toward

a more complete understanding of the efficacy of air pollution policies, the accountability studies should incorporate assessments of air pollution impacts on relevant biomarkers of the clinical events under study so that an appropriate control of the confounding variables in the analysis would be guaranteed. These type of studies are feasible to carry out in Ecuador.

Other factor that aggravates the problem of pollution in developing countries is that the toxic effects of pollutants could be potentiated by the presence of malnutrition in children and the related immunological deficiencies. Regarding to malnutrition, specifically nutritional anemia, studies in larger population of children are required to confirm our findings of the synergy between air pollution and anemia to increase pneumonia hospitalizations. If these studies confirm the synergy, improvements in iron intake and controlling air pollution could decrease the severity of pneumonia.

Without any doubt, more studies are required to better understand the immunological mechanisms through which an individual pollutant or multipollutants could cause and exacerbate respiratory infections or asthma. These studies would need *ex vivo* assays and sophisticated lab facilities and demand close collaboration with academic research centers.

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Addendum

English Summary

Resumen: Spanish Summary

Samenvatting: Short Dutch Summary

Abbreviations

Acknowledgements

Curriculum Vitae

PhD Portfolio

Publications

ENGLISH SUMMARY

Air pollution is an acute, cumulative, and chronic threat to human health and it is a growing problem in developing countries. Respiratory infections and allergic diseases associated with environmental contamination are more frequent in children and are more serious in children with malnutrition.

This thesis presents studies that show, a) the relationship of air pollution due to vehicular and volcanic emissions with respiratory diseases in children, b) the interaction of nutritional anemia and environmental pollution in respiratory diseases, c) the alterations that different air pollutants can produce in a new group of cells of the innate immune system, and d) the beneficial effect of a vehicle emission control policy on the incidence of respiratory infections in children.

Chapter one, the introduction, places us in the context of the problem of environmental pollution and respiratory illnesses in children of Quito-Ecuador as well as on the harmful effect of pollutants on respiratory health with an emphasis on the innate immune response, and the potential solutions and measures to improve air quality. In addition, it establishes the main objectives of this thesis.

In order to know about the Quito environment, a small description is important: the city of Quito, capital of Ecuador, is situated on the equatorial line at 2828 m above sea level in the Andean Region, and it is very sensitive to pollution due to its geographical location, topography and climatology. The city sits on a long narrow valley and is surrounded by high mountains that prevent the wind from dispersing the pollutants. The altitude of the city allows increased solar radiation that transforms the pollutants into oxidants. Its topography also favors the thermal inversions where of hot air roof traps and concentrates the pollutants within the city. Because it is a fast-growing city, it possesses a large number of circulating vehicles which are responsible for ~ 82% of the environmental pollution. Additionally, it is surrounded by active volcanoes that from time to time intensify their activity with the emission of large amounts of volcanic ash. This scenario exposes the population to excessive pollution and makes it be more prone to the health problems inherent to pollution, especially respiratory diseases.

The study presented in chapter 2, analyzes the effect of the exposure to carbon monoxide (CO) in the occurrence of acute respiratory infection (ARI) in school children, using carboxyhemoglobin (COHb) as a marker of chronic exposure to CO. It is known that CO binds tightly to hemoglobin to form COHb, a compound that prevents hemoglobin from delivering oxygen throughout the body, and that the concentration of COHb $\leq 2.5\%$ is considered a safe level of oxygen supply. In the year 2000, we conducted a prospective study in 960 young children attending Quito's public elementary schools located in three areas of different traffic intensity (high, moderate, and low-traffic areas). In a random subsample of 295 children, we

determined that average COHb concentrations were significantly higher in children attending schools in areas with high and moderate traffic, compared with the low-traffic area. The percentage of children with COHb concentrations above the safe level of 2.5% were 1, 43, and 92% in low-, moderate-, and high-traffic areas, respectively. Children with COHb above the safe level were 3.25 [95% confidence interval (CI): 1.65 – 6.38] times more likely to have ARI than children with COHb $\leq 2.5\%$. Furthermore, with each percent increase in COHb above the safety level, children were 1.15 [CI 95% 1.03-1.28] times more likely to have an additional case of ARI. All association analyzes were controlled by sex, age, indoor contamination, and nutritional status. Our findings provide strong evidence of the relation between CO exposure and susceptibility to respiratory infections, and they constituted a foundation for the creation of a local policy of control of vehicular emissions.

Chapter 3 presents the variation in rates of emergency room (ER) visits for acute upper and lower respiratory infections and asthma-related conditions in children, before, during and after the ashfall in Quito due to eruptions of the Pichincha volcano in April 2000. The city of Quito is located 13 km east of the Guagua Pichincha volcano in a long narrow valley that gives incomparable scenery: a dangerous setting created by nature and enhanced by humans. In April 2000, the Guagua Pichincha volcano became active after 340 years of dormancy and emitted volcanic ashes containing silica, sulfurs, and particulate matter. This study was conducted at the Baca Ortiz Children's Hospital, Quito's largest government-subsidized facility, which provides care to approximately 307,000 children under 15 years of age residing in the Quito Metropolitan area and surrounding communities. During 2000, 22,353 children were assisted at the hospital's emergency room, and about 6% of children visited ER were treated for respiratory illnesses, mainly pneumonia. We abstracted 5169 (43% females) ER records with primary respiratory conditions treated from January 1 – December 27, 2000. The records were classified into three non-overlapping categories: acute upper respiratory infection (AURI), acute lower respiratory infection (ALRI), and asthma-related conditions. Information about Guagua Pichincha activity was collected, a timeline of exposure-related volcanic activity in January-April was created, and four period of analysis were defined: fumarolic activity (Period 1), one week (Period 2), two weeks (Period 3) and three weeks (Period 4) after the eruption. A Poisson regression model was adapted to time series of cases for AURI, ALRI, and asthma-related conditions in boys and girls for three age groups: 0–4, 5–9, and 10–15. The main findings were: a) The rate of emergency room visits due to respiratory conditions substantially increased in the three weeks after eruption for ALRI [RR = 2.22, IC 95% 1.95-2.52] and AURI [RR = 1.72, IC 95% 1.49-1.97], respectively; b) The largest impact of eruptions on respiratory distress was observed in children younger than 5 years [RR = 2.21, IC 95% 1.79-2.73]; c) The rate of asthma and asthma-related diagnosis doubled during the period of volcano fumarolic activity [RR = 1.97, IC 95% 1.19-3.24]. This study documented

significantly increased levels of respiratory disease in Quito children after a volcanic eruption using hospitalization records.

Chapter 4 relates a study evaluating the synergistic relationship of two pneumonia risk factors in children: environmental contamination and nutritional anemia. In Ecuador 25.78% of preschool children suffer iron deficiency anemia (Hb levels <11g/dL) mainly due to consumption of iron-poor diets, especially in the Andean region where Quito is located. The study was conducted in 825 children from 18 to 42 months of age, who lived in two socioeconomically similar neighborhoods but with different levels of environmental contamination ($PM_{2.5}$ 20.4 vs. 15.3 $\mu\text{g}/\text{m}^3$, and NO_2 29.5 vs. 16.6 $\mu\text{g}/\text{m}^3$). The history of medical visits and hospitalizations for respiratory problems during the year prior to the study were obtained from all the children. Anthropometric data, hemoglobin levels, and oxygen saturation (measured through oximetry) were recorded at the time of the consultation. The analyzes showed that in anemic children, the highest exposure to contamination was significantly associated with a higher number of hospitalizations for pneumonia [OR = 6.82, IC 95% 1.45-32.00], whereas in non-anemic children there was no difference in the number of hospitalizations according to exposure level. In addition, children exposed to higher levels of air pollution had significantly more hospitalizations for pneumonia [OR = 3.68, IC 95% 1.09-12.44], total respiratory disease [OR = 2.93, IC 95% 1.92-4.47], stunting [OR = 1.88, IC 95% 1.36-2.60], and anemia [OR = 1.45, IC 95% 1.09-1.93] compared to children exposed to lower levels of air pollution. Also, children exposed to higher levels of air pollution had significantly lower oxygen saturation ($92.2\% \pm 2.6\%$ vs. $95.8\% \pm 2.2\%$; $P < 0.0001$), consistent with the formation of methaemoglobin related to NO_2 exposure, as it has been described in some studies. Taking together these findings suggest that anemia may interact with environmental pollutants to increase hospitalizations for pneumonia, and that improving nutrition and reducing air pollution could reduce severe pneumonia, the leading cause of death in children.

Chapter 5 is a systematic review of 12 experimental studies on the effects of various environmental pollutants on lung innate lymphoid cells (ILCs) of murine and human species. Three major groups of ILCs have been defined on the basis of the transcription factors needed for their development and the cytokines they produce. The ILC1 group includes classical natural killer (cNK) and non-NK cells, which are cytotoxic and produce IFN gamma ($\text{IFN-}\gamma$). The ILC2 group expresses IL-5 and IL-13. The type 3 (ILC3) group comprises some cells which produce IL-17 and/or IL-22. Due to their cytokine production, ILCs 1, 2 and 3 resemble the adaptive T helper (Th) 1, Th2, and Th17 cells respectively. ILCs play an essential role in inflammation and in the regulation of host responses to infection. The experimental studies together show that lung NK cells exposed to particulate pollutants (diesel particles and $PM_{2.5}$) and high doses of O_3 present low cytotoxic capacity and reduced production of $\text{IFN-}\gamma$, an important cytokine responsible for stimulating macrophages and inducing differentiation

of Th1 cells. These same pollutants and carbon nanotubes stimulated lung ILC2s to produce high levels of IL-5 and IL-13 which are responsible for IgE class switching on B cells, and the recruitment and activation of eosinophils. In light of these findings, it can be concluded that air pollution would be related to greater susceptibility to intracellular infections due to the reduced activity of NK cells, and to the increase in respiratory allergic processes due to the action of ILC2.

Chapter 6 presents a study aimed to evaluate whether the reduction of traffic-related pollutant levels, specifically in CO exposure between 2000 and 2007, was associated with both the incidence of ARI and COHb levels in school-aged children. The study conducted in the year 2000, presented in chapter 4, was the basis for implementing the first local policy for the reduction of traffic-related pollution. In 2002, the municipality of Quito issued the municipal vehicle technical ordinance to verify compliance with vehicle safety standards, including the concentration of CO, CO₂, O₂, and hydrocarbons in the gases emitted by the exhaust pipe of gasoline vehicles. Five years after the establishment of this policy we carried out a methodological study similar to the 2000 study and compared them. Each study involved ~730 children aged 6–12 years observed for 12–15 weeks. The associations between COHb serum concentration, as an exposure proxy of carbon monoxide (CO), ambient CO, and ARI in both cohorts were examined. Compared to the 2000 study, the 2007 study found a reduction of 48 % in the ARI incidence [RR = 0.52, 95% CI 0.45–0.62] and 92% reduction in the percentage of children with COHb > 2.5%; and no association between COHb concentrations above the safe level of 2.5% and the ARI incidence ($p = 0.736$). These results show that a substantial decrease in the level of environmental CO as a result of a citywide 5-year vehicular emissions control program is associated with a reduction in the incidence of respiratory diseases and COHb levels in children in school age. This study supports the value of implementing preventive policies and the need for sustained long-term programs in countries with poor air quality.

Chapter 7 approaches the main findings of our studies on environmental pollution and delineates immunological hypotheses as an attempt to explain the effect of the main pollutants studied in the appearance of respiratory infections and allergic processes.

Finally, in chapter 8 that corresponds to the general discussion, the overall results of the studies are discussed and interpreted. At the same time, immunological hypotheses are developed to explain the possible mechanisms by which pollutants related to vehicular and volcanic emissions alter innate immunity and cause both susceptibility to respiratory infections and airway hyperreactivity related to asthma. An immunological discussion is made to explain the synergism between nutritional anemia and environmental pollution associated with respiratory infections. As final points, the inherent limitations of all the studies as a whole are presented, and future research possibilities are designed to help the understanding of the relationship between contamination and respiratory diseases and the improvement of air quality.

RESUMEN: SPANISH SUMMARY

La contaminación del aire es una amenaza aguda, acumulativa y crónica para la salud humana y es un problema creciente en los países en desarrollo. Las infecciones y los procesos alérgicos del sistema respiratorio asociados a contaminación ambiental son más frecuentes en niños y son más graves en niños con desnutrición.

Esta tesis presenta estudios que evidencian a) la relación de la contaminación del aire debida a emisiones vehiculares y volcánicas con la presencia de enfermedades respiratorias en niños, b) la interacción de la anemia nutricional y la contaminación ambiental en las enfermedades respiratorias, c) las alteraciones que diferentes contaminantes del aire pueden producir en un grupo nuevo de células del sistema inmune innato, y d) el efecto benéfico de una política de control de emisiones vehiculares sobre la incidencia de infecciones respiratorias en niños.

El primer capítulo, que corresponde a la introducción, nos sitúa en el contexto del efecto nocivo de los contaminantes en el sistema inmune innato pulmonar, del problema de la polución ambiental y las enfermedades respiratorias en niños de la ciudad de Quito, y de la eficacia de una política del control de la contaminación del aire en la reducción de enfermedades respiratorias en niños.

El capítulo 1, la introducción, nos ubica en el contexto de la polución ambiental y las infecciones respiratorias en niños de Quito-Ecuador, así como también en los efectos dañinos de los contaminantes ambientales sobre el sistema respiratorio con énfasis en la respuesta inmune y las medidas y soluciones potenciales para mejorar la calidad del aire. Adicionalmente, se establece los principales objetivos de la tesis.

Para ubicarnos en el ambiente de Quito es importante una pequeña descripción: la ciudad de Quito, capital de Ecuador, está situada en la línea ecuatorial a 2828 m sobre el nivel del mar en la Región Andina, y es muy sensible a la contaminación debido a su ubicación geográfica, topografía y climatología. La ciudad se encuentra en un valle largo y angosto y está rodeada de altas montañas que impiden que el viento disperse los contaminantes. La altitud de la ciudad permite una gran radiación solar que transforma los contaminantes en oxidantes, y su topografía favorece las inversiones térmicas donde el techo de aire caliente atrapa y concentra los contaminantes dentro de la ciudad. Por ser una ciudad de rápido crecimiento posee un gran parque automotor que se estima es responsable del 82% de la contaminación ambiental. Adicionalmente, está rodeada de volcanes activos que de tanto en tanto intensifican su actividad con la emisión de grandes cantidades de cenizas volcánicas. Este escenario hace que la población esté expuesta a mucha contaminación y más propensa a los problemas de salud inherentes a la contaminación, especialmente las enfermedades respiratorias.

El estudio presentado en el capítulo 2, analiza el efecto de la exposición a monóxido de carbono (CO) en la ocurrencia de infección respiratoria aguda (IRA) en niños escolares, utilizando la carboxihemoglobina (COHb) como marcador de exposición crónica al CO. Se sabe que el CO se une fuertemente a la hemoglobina para formar COHb, un compuesto que evita que la hemoglobina entregue oxígeno por todo el cuerpo. La concentración de COHb \leq a 2.5% se considera un nivel seguro de suministro de oxígeno. El estudio fue realizado en el año 2000 y consistió en el seguimiento durante 12 semanas a 960 niños que asistían a escuelas primarias de Quito ubicadas en tres zonas de distinta intensidad de tráfico (alta, moderada y baja) para determinar la incidencia de IRA mediante diagnóstico clínico. En una submuestra aleatoria de 295 niños, se determinó que las concentraciones promedio de COHb fueron significativamente más altas en niños que asistían a escuelas situadas en las áreas de tráfico alto y moderado, en comparación con aquellos que estaban en el área de tráfico bajo. El porcentaje de niños con concentraciones de COHb por encima del nivel seguro de 2.5%, fue de 1.43 y 92% en áreas de tráfico bajo, moderado y alto, respectivamente. Los niños con COHb por encima del nivel de seguridad fueron 3.25 [intervalo de confianza del 95% (IC): 1.65 – 6.38] veces más propensos a tener IRA que los niños con COHb \leq 2.5%. Además, se estableció que con cada incremento porcentual de COHb por encima del nivel de seguridad, los niños tuvieron 1.15 [IC: 95% 1.03-1.28] veces más probabilidades de tener un caso adicional de IRA. Todos los análisis de asociación fueron controlados por sexo, edad, contaminación intradomiciliaria y estado nutricional. Estos hallazgos proporcionaron una fuerte evidencia de la relación entre la exposición al CO y la susceptibilidad a las infecciones respiratorias y constituyeron una base para la formulación de una política local de control de emisiones vehiculares.

El capítulo 3, describe el comportamiento del número de visitas a la sala de emergencia (SE) por afecciones respiratorias en niños, antes, durante y después de la caída de cenizas en Quito debido a las erupciones del volcán Pichincha ocurridas en abril de 2000. La ciudad de Quito, está asentada a 13 km al este del volcán Guagua Pichincha en un largo y estrecho valle que le da un escenario incomparable: una cámara peligrosa creada por la naturaleza y realizada por los humanos. En abril del 2000, después de 340 años de latencia, el volcán Pichincha se activó con emisiones de vapor y cenizas que contenían sílice, azufre y material particulado. Este estudio tuvo lugar en el Hospital Pediátrico Baca Ortiz, establecimiento público de referencia más grande de Quito que brinda atención a aproximadamente 307000 niños menores de 15 años que residen en el área metropolitana de Quito y en las comunidades circundantes. Durante el año 2000, 22353 niños fueron atendidos en la SE y aproximadamente 6% de ellos fueron tratados por enfermedades respiratorias, principalmente neumonía. Se extrajeron 5169 registros por afecciones respiratorias primarias tratadas en la SE durante el período 1 de enero - 27 de diciembre de 2000. Los registros fueron clasificados en tres categorías no superpuestas: infección aguda de vías

respiratorias superiores (IRAA), infección aguda de vías respiratorias inferiores (IRAB), y afecciones relacionadas con el asma. Se recopiló información sobre la actividad del Guagua Pichincha, se creó una línea de tiempo de exposición a la actividad volcánica (enero-abril), y se definieron cuatro períodos de análisis: actividad fumarólica (Período 1), una semana (Período 2), dos semanas (Período 3) y tres semanas (Período 4) después de la erupción. Se aplicó un modelo de regresión de Poisson adaptado a series de tiempo de los casos para las tres categorías de enfermedades IRAB y IRAA y afecciones relacionadas con el asma en niños y niñas para tres grupos de edad: 0-4, 5-9 y 10-15 años. Los principales hallazgos fueron: a) la tasa de visitas a SE debido a afecciones respiratorias aumentó sustancialmente en las tres semanas posteriores a la erupción; para ALRI [Riesgo Relativo (RR) = 2.22, IC 95% 1.95-2.52] y para AURI [RR = 1.72, IC 95% 1.49-1.97], b) el mayor impacto de las erupciones en los procesos respiratorios se observó en niños menores de 5 años [RR = 2.21, IC 95% 1.79-2.73] y c) la tasa de asma y problemas relacionados con el asma se duplicaron durante el período fumarólico de la actividad volcánica (RR = 1.97, IC 95% 1.19-3.24). Con este estudio se documentan niveles significativamente más altos de enfermedad respiratoria en niños de Quito después de una erupción volcánica.

En el capítulo 4, el estudio evalúa la relación sinérgica de dos factores de riesgo de neumonía en niños: la contaminación ambiental y la anemia nutricional. En Ecuador 25.78% de niños pre escolares tienen anemia por deficiencia de hierro (Hb levels <11g/dL), debido principalmente al consumo de dietas pobres en hierro, especialmente en la región Andina donde la ciudad de Quito es localizada. El estudio fue realizado en 825 niños de 18 a 42 meses de edad, que vivían en dos barrios socioeconómicamente similares pero con distinto nivel de contaminación ambiental ($PM_{2.5}$ 20.4 vs. 15.3 $\mu\text{g}/\text{m}^3$, y NO_2 29.5 vs. 16.6 $\mu\text{g}/\text{m}^3$). De todos los niños se obtuvo el historial de visitas médicas y hospitalizaciones por problemas respiratorios durante el año previo al estudio, y los datos antropométricos, los niveles de hemoglobina y la saturación de oxígeno (medida a través de oximetría) en el momento de la consulta. Los análisis demostraron que en los niños anémicos, la mayor exposición a la contaminación se asoció significativamente con mayor número de hospitalizaciones por neumonía [Radio de Odds (OR) = 6.82, IC 95% 1.45-32.00], mientras que en los niños no anémicos no hubo diferencia del número de hospitalizaciones según nivel de exposición. Además, los niños expuestos a niveles más altos de contaminación del aire tuvieron significativamente más hospitalizaciones por neumonía [OR = 3.68, IC 95% 1.09-12.44], enfermedad respiratoria total [OR = 2.93, IC 95% 1.92-4.47], retraso en el crecimiento [OR = 1.88, IC 95% 1.36-2.60] y anemia [OR = 1.45, IC 95% 1.09-1.93] comparados con niños expuestos a más bajos de contaminación. También se encontró que los niños expuestos a alta contaminación tuvieron una saturación de oxígeno significativamente menor ($92.2\% \pm 2.6\%$ vs. $95.8\% \pm 2.2\%$; $P < 0.0001$), consistente con formación de metahemoglobina relacionada con la contaminación de NO_2 , como ha sido descrito en algunos estudios. Estos hallazgos en conjunto sugieren que

mejorar la nutrición y disminuir la contaminación del aire podrían reducir rápidamente las hospitalizaciones por neumonía, la principal causa de muerte en los niños.

En el capítulo 5 se presenta una revisión sistemática de 12 estudios experimentales sobre los efectos de varios contaminantes ambientales en las células linfoides innatas (ILCs) pulmonares de especies murinas y humanas. Se han definido tres grupos principales de ILCs en base a los factores de transcripción necesarios para su desarrollo y a las citoquinas que producen. El grupo ILC1 incluye células asesinas naturales (cNK) clásicas y no NK, que son citotóxicas y producen IFN gamma (IFN- γ). El grupo ILC2 expresa IL-5 e IL-13. El grupo de tipo 3 (ILC3) comprende células que producen IL-17 y / o IL-22. Debido a su producción de citocinas, las ILC 1, 2 y 3 se asemejan a las células adaptativas T helper (Th) 1, Th2 y Th17, respectivamente. Las ILCs juegan un papel esencial en la inflamación y en la regulación de las respuestas del huésped a la infección. Los estudios experimentales en conjunto muestran que células NK pulmonares expuestas a contaminantes particulados (partículas de diésel y PM_{2.5}) y altas dosis de O₃ presentan baja capacidad citotóxica y poca producción de gamma interferón, una citoquina importante responsable de estimular los macrófagos e inducir la diferenciación de las células Th1. La exposición a estos mismos contaminantes y a partículas de carbono estimulan a las ILC2 pulmonares para producir altos niveles de interleucina (IL) 5 e IL-13 que son responsables de la generación de inmunoglobulina E (IgE) en los linfocitos B y del reclutamiento y activación de eosinófilos. A la luz de estos hallazgos se puede concluir que la contaminación del aire estaría relacionada con mayor susceptibilidad a las infecciones intracelulares por la disminución de la actividad de las células NK, y con el incremento de procesos alérgicos respiratorios debido a la acción de ILC2.

El capítulo 6 presenta un estudio cuyo objetivo fue evaluar si la reducción de los niveles de contaminantes relacionados con el tráfico, específicamente en la exposición al CO entre 2000 y 2007, se asoció con la incidencia de los niveles de IRA y COHb en niños en edad escolar. El estudio en el año 2000, presentado en el capítulo 4, que muestra una relación positiva entre la exposición al CO y la ocurrencia de infecciones respiratorias agudas en niños escolares de Quito fue la base para implementar la primera política local para la reducción de la contaminación relacionada con el tráfico. En 2002, el municipio de Quito emitió la ordenanza municipal de revisión técnica vehicular para verificar el cumplimiento de las normas de seguridad de los vehículos, incluida la concentración de CO, CO₂, O₂ e hidrocarburos en los gases emitidos por el tubo de escape de los vehículos a gasolina. Cinco años después de la instauración de esta política realizamos un estudio metodológicamente similar al estudio del 2000 y los comparamos. Cada estudio involucró ~ 730 niños de entre 6 y 12 años de edad que fueron observados durante 12-15 semanas. Se examinaron las asociaciones entre la concentración sérica de COHb, como un proxy de exposición de monóxido de carbono, con CO ambiental y ARI en ambas cohortes. En comparación con el estudio del 2000, en el 2007 se encontró una reducción del 48% en la incidencia de IRA [RR = 0.52, IC 95% 0.45-0.62] y del

92% en el porcentaje de niños con COHb > 2.5%, pero no encontramos asociación entre las concentraciones de COHb por encima del nivel de seguridad de 2.5% y la incidencia de IRA ($p = 0.736$). Estos resultados muestran que una disminución sustancial en el nivel de monóxido de carbono ambiental como resultado de un programa de control de emisiones vehiculares de 5 años en toda la ciudad se asocia con la reducción de la incidencia de enfermedades respiratorias y niveles de COHb en niños en edad escolar. Este estudio respalda el valor de implementar políticas preventivas y la necesidad de programas sostenidos a largo plazo en países con mala calidad del aire.

En el capítulo 7 se presenta un resumen de los principales hallazgos de nuestros estudios sobre contaminación ambiental y se proponen hipótesis inmunológicas como intento de explicar el efecto de los principales contaminantes estudiados en el apareamiento de las infecciones respiratorias y en procesos alérgicos.

Finalmente, en el capítulo 8 que corresponde a la discusión general, se comentan e interpretan los resultados generales de los estudios. Seguidamente, se desarrollan hipótesis inmunológicas para explicar los posibles mecanismos por los que ciertos polutantes relacionados con las emisiones vehiculares y volcánicas alteran la inmunidad innata y causan tanto susceptibilidad a las infecciones respiratorias como hiperreactividad de las vías aéreas relacionada con el asma. También se hace una discusión inmunológica para explicar el sinergismo entre anemia nutricional y polución ambiental asociado a infecciones respiratorias. Finalmente, se presentan las limitaciones inherentes a todos los estudios en conjunto, y se bosquejan futuras posibilidades de investigación que ayuden al entendimiento de la relación contaminación-enfermedades respiratorias y al mejoramiento de la calidad del aire.

SAMENVATTING: SHORT DUTCH SUMMARY

Luchtvervuiling is een cumulatieve en chronische bedreiging voor de menselijke gezondheid en vormt een groeiend probleem in ontwikkelingslanden. Luchtweginfecties en allergische aandoeningen gerelateerd aan milieuvervuiling komen vaak voor bij kinderen en zijn ernstiger bij kinderen met ondervoeding. Dit proefschrift presenteert studies naar a) de relatie tussen luchtvervuiling als gevolg van voertuig- en vulkanische emissies en luchtwegaandoeningen bij kinderen, b) de interactie van voedingsanemie en milieuvervuiling bij luchtwegaandoeningen, c) de veranderingen die verschillende luchtverontreinigende stoffen kunnen veroorzaken in cellen van het aangeboren immuunsysteem, en d) het gunstige effect van een voertuigemissiecontrolebeleid op de incidentie van luchtweginfecties bij kinderen.

Hoofdstuk één, de inleiding, beschrijft de context van het probleem van milieuvervuiling en aandoeningen van de luchtwegen bij kinderen van Quito-Ecuador, evenals van het schadelijke effect van verontreinigende stoffen op de gezondheid van de luchtwegen, en potentiële oplossingen en maatregelen om de luchtkwaliteit te verbeteren. Bovendien worden de belangrijkste doelen van dit proefschrift beschreven.

De studie gepresenteerd in hoofdstuk 2 analyseert het effect van de blootstelling aan koolmonoxide (CO) bij het optreden van acute luchtweginfecties bij schoolkinderen, waarbij carboxy-hemoglobine (COHb) wordt gebruikt als een marker voor chronische blootstelling aan CO. In het jaar 2000 hebben we een prospectieve studie uitgevoerd bij 960 jonge kinderen op de openbare basisscholen van Quito in drie gebieden met verschillende verkeersintensiteit (gebieden met veel, matig en weinig verkeer). In een willekeurige deelsteekproef van 295 kinderen hebben we vastgesteld dat de gemiddelde COHb-concentraties significant hoger waren bij kinderen die scholen bezoeken in gebieden met veel en matig verkeer, vergeleken met de gebieden met weinig verkeer. Het percentage kinderen met COHb-concentraties boven het veilige niveau van 2.5% was respectievelijk 1.43, en 92% in gebieden met weinig, matig en veel verkeer. Kinderen met COHb boven het veilige niveau hadden 3.25 keer meer kans op luchtweginfecties dan kinderen met COHb $\leq 2.5\%$. Bovendien hadden kinderen met elke procentuele toename van COHb boven het veiligheidsniveau, 1.15 [95%CI 1.03-1.28] keer meer kans op een extra luchtweginfectie. Onze bevindingen leveren sterk bewijs voor een verband tussen blootstelling aan CO en gevoeligheid voor luchtweginfecties, en ze vormden een basis voor het opstellen van een lokaal beleid voor de beheersing van voertuigemissies.

Hoofdstuk 3 presenteert de variatie in het aantal bezoeken aan spoedeisende hulp (SH) voor acute infecties van de bovenste en onderste luchtwegen en astma-gerelateerde aandoeningen bij kinderen, vóór, tijdens en na de as-val in Quito als gevolg van uitbarstingen van de Pichincha-vulkaan in april 2000. Deze studie werd uitgevoerd in het Baca Ortiz Kinderziekenhuis. In 2000 werden 22.353 kinderen geholpen op de eerste hulp van het

ziekenhuis en ongeveer 6% van de kinderen die SH bezochten, werd behandeld voor aandoeningen aan de luchtwegen (5169, 43% vrouw), voornamelijk longontsteking. Analyses naar typen aandoeningen en tijdsreeksen van blootstelling aan vulkanische activiteit toonden aan dat: a) Het aantal bezoeken aan de eerste hulp vanwege ademhalingsaandoeningen nam aanzienlijk toe in de drie weken na uitbarsting voor zowel bovenste [RR = 2.22, 95%CI 1.95-2.52] als onderste luchtweginfecties [RR = 1.72, 95%CI 1.49-1.97]; b) Het grootste effect van uitbarstingen op ademnood werd waargenomen bij kinderen jonger dan 5 jaar [RR = 2.21, IC 95% 1.79-2.73]; c) De snelheid van astma en astma-gerelateerde diagnose verdubbelde tijdens de periode van vulkaan fumarolische activiteit [RR = 1.97, IC 95% 1.19-3.24]. Deze studie documenteerde aanzienlijk verhoogde niveaus van luchtwegaandoeningen bij Quito-kinderen na een vulkaanuitbarsting met behulp van ziekenhuisopname-records.

Hoofdstuk 4 heeft betrekking op een onderzoek naar de interactie tussen milieuvervuiling en voedingsanemie. In Ecuador lijdt 25.78% van de kleuters aan bloedarmoede door ijzertekort (Hb-waarden <11 g/dL), voornamelijk als gevolg van de consumptie van ijzerarme diëten, vooral in de Andesregio waar Quito zich bevindt. De studie werd uitgevoerd bij 825 kinderen van 18 tot 42 maanden oud, die in twee sociaaleconomisch vergelijkbare buurten woonden, maar met verschillende niveaus van milieuvervuiling (PM_{2.5} 20.4 versus 15.3 µg/m³, en NO₂ 29.5 versus 16.6 µg /m³). De geschiedenis van medische bezoeken en ziekenhuisopnames voor ademhalingsproblemen in het jaar voorafgaand aan het onderzoek werd verkregen van alle kinderen. Kinderen die werden blootgesteld aan hogere luchtvervuiling hadden meer ziekenhuisopnames voor longontsteking (OR = 3.68, IC 95% 1.09-12.44), luchtwegaandoeningen [OR = 2.93, IC 95% 1.92-4.47], stunting [OR = 1.88, IC 95% 1.36-2.60] en bloedarmoede [OR = 1.45, IC 95% 1.09-1.93] in vergelijking met kinderen die werden blootgesteld aan lagere luchtvervuiling. We vonden dat bij anemische kinderen de hoogste blootstelling aan vervuiling significant geassocieerd was met een hoger aantal ziekenhuisopnames voor longontsteking [OR = 6.82, IC 95% 1.45-32.00], terwijl er bij niet-anemische kinderen geen verband was voor luchtvervuiling. Deze bevindingen suggereren dat er interacties zijn tussen bloedarmoede en luchtvervuiling op het risico voor longontsteking, en dat zowel het verbeteren van voeding en het verminderen van luchtvervuiling ernstige longontsteking, de belangrijkste doodsoorzaak bij kinderen, kan verminderen.

Hoofdstuk 5 is een systematische review van 12 experimentele studies naar de effecten van verschillende stoffen van luchtvervuiling op lung innate lymphoid cells (ILCs) bij muizen en mensen. Drie hoofdgroepen van ILCs zijn gedefinieerd. De ILC1-groep omvat klassieke natural killer (cNK) en niet-NK-cellen, die cytotoxisch zijn en IFN-gamma (IFN-γ) produceren. De ILC2-groep brengt IL-5 en IL-13 tot expressie. De type 3 (ILC3) groep omvat enkele cellen die IL-17 en/of IL-22 produceren. Vanwege hun cytokineproductie lijken ILCs 1, 2 en 3 respectievelijk op de adaptieve T-helper (Th) 1-, Th2- en Th17-cellen. ILCs spelen een essentiële rol bij ontstekingen en bij de regulatie van gastheerreacties op infectie. De

experimentele studies tonen aan dat NK-longcellen blootgesteld aan dieseldeeltjes en PM2.5 en aan hoge doses O₃ een lage cytotoxische capaciteit en verminderde productie van gamma-interferon hebben. Dezelfde verontreinigende stoffen stimuleerden long-ILC2s om meer interleukine (IL) 5 en IL-13 te produceren. In het licht van deze bevindingen kan worden geconcludeerd dat luchtvervuiling verband houdt met een grotere gevoeligheid voor intracellulaire infecties als gevolg van de verminderde activiteit van NK-cellen en met de toename van allergisch processen als gevolg van de werking van ILC2.

Hoofdstuk 6 presenteert een evaluatiestudie naar vermindering van verkeersgerelateerde vervuiling, met name bij blootstelling aan CO tussen 2000 en 2007 en het verband met zowel de incidentie van luchtweginfecties als COHb-waarden bij schoolgaande kinderen. De studie gepresenteerd in hoofdstuk 4 was de basis voor de uitvoering van het eerste lokale beleid voor de vermindering van verkeersgerelateerde vervuiling. In 2002 heeft de gemeente Quito de technische verordening voor gemeentelijke voertuigen uitgegeven om de naleving van voertuigveiligheidsnormen te controleren, waaronder de concentratie van CO, CO₂, O₂ en koolwaterstoffen in de gassen die worden uitgestoten door de uitlaatpijp van benzinevoertuigen. Vijf jaar na de vaststelling van dit beleid hebben we een methodologische studie uitgevoerd vergelijkbaar met de 2000-studie en deze vergeleken. Bij elk onderzoek waren ~730 kinderen van 6-12 jaar gevolgd gedurende 12-15 weken. In vergelijking met de studie uit 2000 vond de studie uit 2007 een vermindering van 48% in de ARI-incidentie [RR = 0.52, 95%CI 0.45-0.62] en 92% reductie van het percentage kinderen met COHb > 2,5%. Deze resultaten tonen aan dat een substantiële daling van het niveau van CO in het milieu gepaard gaat met een vermindering van de incidentie van ademhalingsziekten en COHb-waarden bij kinderen in de schoolleeftijd. Deze studie ondersteunt de waarde van de uitvoering van preventief beleid en de noodzaak van duurzame langetermijnprogramma's in landen met een slechte luchtkwaliteit.

Hoofdstuk 7 vat de belangrijkste bevindingen van onze studies over luchtvervuiling samen. Ten slotte worden in hoofdstuk 8 de algemene resultaten van de studies besproken en geïnterpreteerd. Ook worden immunologische hypothesen beschreven die het effect van de bestudeerde stoffen in het optreden van luchtweginfecties en allergische processen zouden kunnen verklaren. Als laatste worden de beperkingen van de studies benoemd en worden aanbevelingen gedaan voor toekomstig onderzoek om de relatie tussen luchtvervuiling en aandoeningen van de luchtwegen beter te leren begrijpen.

ABBREVIATIONS

AHR	Allergen-induced response and air way hyper
AL	Adaptive lymphocytes
ALRI	Acute lower respiratory infection
AM	Alveolar macrophages
AOR	Adjusted Odds Ratios
ARI	Acute respiratory infection
ARRIVE	Animal Research Reporting <i>in vivo</i> Experiments
AURI	Acute upper respiratory infection
BAL	Bronchoalveolar lavage
BALF	Broncho-alveolar lavage fluid
BMI	Body mass index.
CEB	Corporación Ecuatoriana de Biotecnología
CI	Confidence interval
cNK	Classical natural killer
CO	Carbon monoxide
COHb	Carboxihemoglobin
CORPAIRE	Corporación Para el Mejoramiento del Aire de Quito ()
COX	Cyclooxygenase
CRP	C reactive protein
CW	Child-weeks
DC	Dendritic cells
DEP	Diesel exhaust particles
EC	Epithelial cell
EDTA	Ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency
ER	Emergency room
GAM	Generalized additive model
GEE	Generalized Estimating Equation
HAZ	height-for-age Z-score
HBD-2	human β -defensin 2
HBD-3	human β -defensin 3
HDM	House dust mite
HO.	Hydroxyl radical
HT	heavy-traffic (-school)
IFN- β	Interferon beta
IFN- γ	Interferon gamma

IL	Interleukin
ILC1	Innate lymphoid cells type 1
ILC2	Innate lymphoid cells type 2
ILC3	Innate lymphoid cells type 3
ILCs	Innate lymphoid cells
INEC	Instituto Nacional de Estadística Censos
JR	Jaime Roldos
LP	Lucha de los Pobres
LPS	Lipopolysaccharide
LT	Low-traffic area
LTi	Lymphoid tissue-inducer
MHC	Major histocompatibility complex
MICA/B	Major histocompatibility complex class I chain-related protein A and B
MMP	Matrix metalloproteinase
MØ	Macrophage
MT	moderate-traffic area of Quito (-school)
MWCNT	Carbon in a multi-walled carbon nanotube
NK	Natural killer
NKT	Natural killer T
NO ₂	Nitrogen dioxide
NO _x	Nitrogen oxides
O ₂ ⁻	Superoxide radical
O ₃	Ozone
OR	Odds ratio
OVA	Ovalbumin
PBMC	Peripheral blood mononuclear cell
PGD2	Prostaglandin D2
PM	Particulate matter
PM _{0.1}	Particulate matter < 0.1µM.
PM ₁₀	Particulate matter < 10 µM.
PM _{2.5}	Particulate matter < 2.5µm
PMN	Polymorphonuclear nuclear
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
REMMAQ	Metropolitan Atmospheric Monitoring Network of Quito
ROS	Reactive oxygen species
RR	Relative Risk
SES	Socioeconomic status
SO ₂	Sulphur dioxide

Th	T Helper
TNF- α ,	Tumor necrosis factor alpha
TSLP	Thymic Stromal Lymphopoietin
TT1	Alveolar type-1 like epithelial cells
UFPM	Ultrafine PM
ULBP3	UL16-binding proteins
VEGF	Vascular endothelial growth factor
VOC	Volatile organic compounds
WAZ	Weight-for-age Z-score
WHO	World Health Organization
μm	Micrometers

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CURRICULUM VITAE

Dr. Estrella got her Medical Doctor degree and Pediatrics specialization at the Faculty of Medical Sciences of the Universidad Central del Ecuador in Quito. She got a postdoctoral training on Immunology at National University of Colombia, and attended a 18 month-training in Advanced Coursework in Public Health at Tufts University in Boston.

As part of her PhD portfolio at Erasmus Medical Center, she attended the following courses in the last 2 years: Conceptual Foundations of Epidemiologic Study Design, Introduction to Global Public Health, Casual Inference, Markers and Prediction Research, Erasmus Summer Lectures, Causal Mediation Analysis, Basic and Translational Endocrinology, and Workshop on basic and advanced Microsoft Access 2010.

Dr. Estrella is professor of Immunology at the Medical School of the Universidad Central del Ecuador. Currently she is the Research Director at this university, and from this position she has lead a new program for training teachers in scientific research through the so called "Seed Research Proposal." In this program, she has been the methodological advisor for more than 20 studies. Dr. Estrella has also assessed around 16 theses in the Postgraduate Program on Pediatrics, and Masters in Microbiology at Central University.

Dr. Estrella has been responsible for organizing and conducting field studies in critical areas of public health with emphasis on the relationship of micronutrients, immune response, infectious diseases and growth in children and elderly people. The main reasons for this research have been to develop strategies to improve the nutritional status of children and elderly Ecuadorians and deepen the molecular mechanisms of micronutrients in the immune response of infectious diseases. Additionally, Dr. Estrella is now involved in studies on environmental health aimed to help in making decisions around environmental regulations in Quito-Ecuador. The past and present research have been sponsored and financed by national institutions (National Council of Science and Technology, National Council of Universities and Polytechnic schools, Natura Foundation, the Fund for Integrated Rural Development of the Central Bank of Ecuador, Air Corporation of Quito, Central University of Ecuador, among others) and international institutions (The National Institutes of Health of USA, Fogarty Institutes, Harvard University-ADDR, PAHO / WHO, Boston University).

Dr. Estrella has published 23 papers in peer reviewed journals and presented 14 Abstracts in international scientific meetings. She has also published several articles in national journals. For her scientific work Dr. Estrella has received awards from the Central University of Ecuador, the Ecuadorian Academy of Medicine, and the Municipality of Quito.

PHD PORTFOLIO

Tailor-made combined DSc-PhD program in Epidemiology, Infection and Immunity at Erasmus Medical Center University, Rotterdam, The Netherlands.

Name PhD student: Bertha Estrella Cahueñas, MD. Especialist in Pediatrics

Department: Epidemiology and Immunology, ErasmusMC

PhD period: September 2016 – August 2019

Promotor: Prof.dr. P.D. Katsikis

Co-promotor: Dr.ir. T. Voortman

PhD training

Course Attendance

- 2017 ESP 38- Conceptual Foundations of Epidemiologic Study Design. Erasmus NIHES
- 2017 ESP 41- Introduction to Global Public Health. Erasmus NIHES
- 2017 ESP 48- Casual Inference. Erasmus NIHES
- 2017 ESP 62- Markers and Prediction Research. Erasmus NIHES
- 2017 ESP 64- Erasmus Summer Lectures. Erasmus NIHES
- 2017 ESP 69- Causal Mediation Analysis. Erasmus NIHES
- 2017 Experimental Design and Multivariable statistical analysis. Universidad Central del Ecuador
- 2016 Course Basic and Translational Endocrinology. Erasmus MolMed
- 2016 Workshop on Microsoft Access 2010: Advanced. Erasmus MolMed
- 2016 Workshop on Microsoft Access 2010: Basic Erasmus MolMed
- 2004 MPH 222: Survey Research Methods and Data Management. Tufts University, Boston USA
- 2004 NUTR 204-01: International Nutrition Programs. Tufts University, Boston USA
- 2003 MPH 224: Infectious Disease Epidemiology. Tufts University, Boston USA
- 2003 Immunology 212: Introductory to Immunology. Tufts University, Boston USA
- 2003 MPH 206: Intermediate Biostatistics-Regression Models. Tufts University, Boston USA
- 1995 Training in Molecular Biology techniques. Universidad Nacional de Colombia
- 1990-991 Advanced course in Immunology. Universidad Nacional de Colombia

Teaching

- 2017 "Training workshop for tutors of titling, undergraduate and postgraduate thesis"
Universidad Central del Ecuador
- "Workshop: Writing a Scientific Article" Universidad Central del Ecuador
- 2017 "Workshop: Statistical Analysis in SPSS" Universidad Central del Ecuador
- "Workshop: Data base management in SPSS" Universidad Central del Ecuador
- "Workshop: Writing a Scientific Research Proposal" Universidad Central del Ecuador
- 2019 "Basic Immunology" Universidad Central del Ecuador
- 2018 "Training workshop for tutors of titling, undergraduate and postgraduate thesis"
Universidad Central del Ecuador
- 2018 "Workshop: Writing a Scientific Article" Universidad Central del Ecuador
- 2018 Workshop: Statistical Analysis in SPSS" Universidad Central del Ecuador
- 2018 "Workshop: Data base management in SPSS" Universidad Central del Ecuador
- 2018 "Workshop: Writing a Scientific Research Proposal" Universidad Central del Ecuador
- 2018 "Basic Immunology" Universidad Central del Ecuador
- 2017 "Workshop: Writing a Scientific Article" Universidad Central del Ecuador
- 2017 Workshop: Statistical Analysis in SPSS" Universidad Central del Ecuador
- 2017 "Workshop: Data base management in SPSS" Universidad Central del Ecuador
- 2017 "Workshop: Writing a Scientific Research Proposal" Universidad Central del Ecuador
- 2017 "Basic Immunology" Universidad Central del Ecuador
- 2016 "Workshop: Writing a Scientific Article" Universidad Central del Ecuador
- 2016 Workshop: Statistical Analysis in SPSS" Universidad Central del Ecuador
- 2016 "Workshop: Data base management in SPSS" Universidad Central del Ecuador
- 2016 "Workshop: Writing a Scientific Research Proposal" Universidad Central del Ecuador
- 2016 "Basic Immunology" Universidad Central del Ecuador

Honours and awards

- 2017 Universidad Central Award to the scientific publication " Vitamin D status is associated with underweight and stunting in children aged 6 -36 months residing in the Ecuadorian Andes 2017"
- 2008 Universidad Central Award to the book "The zinc on the route of our scientific researchers
- 2000 Universidad Central Award to the book "La Investigación en Medicina"

PUBLICATIONS

Publications in peer-reviewed journals

1. **Estrella B**, Naumova EN, Cepeda M, Voortman T, Katsikis PD, Drexhage HA. Effects of air pollution on lung innate lymphoid cells: review of in vitro and in vivo experimental studies. *Int J Environ Res Public Health*. 2019;16(13). pii: E2347. doi: 10.3390/ijerph16132347. Review.
2. **Estrella B**, Sempértegui F, Franco OH, Cepeda M, Naumova EN. Air pollution control and the occurrence of acute respiratory illness in school children of Quito, Ecuador. *J Public Health Policy*. 2019; 40(1):17-34. doi: 10.1057/s41271-018-0148-6
3. Mokhtar R, Holick MF, Sempértegui F, Griffiths JK, **Estrella B**, Moore LL, Fox MP, Hamer DH. Vitamin D status is associated with underweight and stunting in children aged 6 -36 months residing in the Ecuadorian Andes 2017. *Public Health Nutr*. 2018;21(11):1974-1985. doi: 10.1017/S1368980017002816
4. Jonnalagadda S, Rodríguez O, **Estrella B**, Sabin LL, Sempértegui F, Hamer DH. Etiology of severe pneumonia in Ecuadorian children. *PLoS One*. 2017 Feb 9;12(2):e0171687.
5. Sempértegui F, **Estrella B**, Rodríguez O, Gómez D, Cabezas M, Salgado G, Sabin LL, Hamer DH. Zinc as an adjunct to the treatment of severe pneumonia in Ecuadorian children: a randomized controlled trial. *Am J Clin Nutr*. 2014;99(3):497-505.
6. Harris AM, Sempertegui F, **Estrella B**, Narvaez X, Egas J, Woodin M, Durant JL, Naumova EN, Griffiths JK. Air Pollution and Anemia as Risk Factors for Pneumonia in Ecuadorian Children: A Retrospective Cohort Study. *Environ Health*. 2011;10(1):93
7. Sempértegui F, **Estrella B**, Tucker KL, Hamer DH, Narvaez X, Sempértegui M, Griffiths JK, Noel SE, Dallal GE, Selhub J, Meydani SN. Metabolic Syndrome in Elderly Living in Marginal Peri-Urban Communities in Quito, Ecuador. *Public Health Nutr*. 2011;14:758-67.
8. Egorov AI, Sempértegui F, **Estrella B**, Egas J, Naumova EN, Griffiths JK. The effect of *Helicobacter pylori* infection on growth velocity in young children from poor urban communities in Ecuador. *Int J Infect Dis*. 2010;14:e788-91.
9. Hamer DH, Sempértegui F, **Estrella B**, Tucker KL, Rodríguez A, Egas J, Dallal GE, Selhub J, Griffiths JK, Meydani SN. Micronutrient deficiencies are associated with impaired immune response and higher burden of respiratory infections in elderly Ecuadorians. *J Nutr*. 2009;139:113-9.
10. Naumova E, Yepes H, Griffiths JK, Sempértegui F, Khurana G, Jagai JS, Játiva E, **Estrella B**. Emergency room visits for respiratory conditions in children increased after Guagua Pichincha volcanic eruptions in April 2000 in Quito, Ecuador Observational study: time series analysis. *Environmental Health* 2007; 6:21 www.ehjournal.net/content/6/1/21
11. Sempértegui F, Díaz M, Mejía R, Rodríguez-Mora OG, Rentería E, Guarderas C, **Estrella B**, Recalde R, Hamer DH, Reeves PG. Low concentrations of zinc in gastric mucosa

- are associated with increased severity of *Helicobacter pylori*- Induced inflammation. *Helicobacter* 2006;12:43-48
12. Sempértegui F, **Estrella B**, Elmieh N, Jordan M, Ahmed T, Rodríguez A, Katherine L. Tucker KL, Hamer DH, Reeves PG, Meydani SN. Nutritional, immunological, and health status of the elderly population living in poor neighborhoods of Quito, Ecuador. *Brit J Nutr* 2006;96:1-10
 13. **Estrella B**, Estrella R, Oviedo J, Narváez X, Reyes MT, Gutiérrez M, Naumova EN. Acute respiratory diseases and carboxyhemoglobin status in school children of Quito, Ecuador. *Environ Health Perspect* 2005;113:607-611.
 14. Duggan CP, MacLeod WB, Krebs NF, Westcott JL, Fawzi WW, Premji ZG, Mwanakasale M, Simon JL, Yeboah-Antwi K, Hamer DH and the Zinc Against Plasmodium Study Group. Plasma zinc concentrations are depressed during the acute phase response in children with falciparum malaria. *J Nutr* 2005;135:802-7.
 15. Rodríguez A, Hamer D, Rivera J, Acosta M, Salgado G, Gordillo M, Cabezas M, Naranjo-P C, Leguisamo J, Fuenmayor G, Játiva E, Guaman G, **Estrella B**, Sempértegui F. Effects of moderate doses of vitamin A as an adjunct to the treatment of pneumonia in underweight and normal-weight children: a randomized, double-blind, placebo-controlled trial. *AJCN* 2005;82:1090-6.
 16. Hamer DH, MacLeod WB, Addo-Yobo E, Duggan CP, **Estrella B**, Fawzi WW, Konde-Lule JK, Mwanakasale V, Premji ZG, Sempértegui F, Ssengooba FP, Yeboah-Antwi K, Simon JL. Age, Temperature, and Parasitemia predict Chloroquine treatment failure and anaemia in children with uncomplicated Falciparum Malaria . *Trans Trop Med&Hyg* 2003;97:422-428
 17. Sempértegui F, **Estrella B**, Vallejo W, Tapia L, Herrera D, Moscoso F, Cerón G, Griffiths JK, Hamer DH. Selenium serum concentrations in malnourished Ecuadorian Children: a case-control study. *Internat J Vit Nutr Res* 2003; 73:181-6.
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 19. Hamer D, Sempértegui F, Yeboah-Antwi K, Premji Z, Ssengooba F, MacLeod W, Duggan C, Fawzi W, Simon J, and **the ZAP Study Group**. Effect of Zinc on the treatment of Plasmodium Falciparum malaria in children: A randomized controlled trial. *Am J Clin Nutr* 2002; 76: 405-12,
 20. Sempértegui F, **Estrella B**, Camaniero V, Betancourt V, Izurieta R, Ortiz W, Fiallo E, Troya S, Rodríguez A, Griffiths JK. The beneficial effect of weekly low-dose vitamin A supplementation on acute lower respiratory infection and diarrhea in Ecuadorian children. *Pediatrics* 1999;104:1-7.URL www.pediatrics.org/cgi/content/full,
 21. Sempértegui F, **Estrella B**, Correa E, Aguirre L, Saa B, Torres M, Navarrete F, Alarcón C, Carrión J, Rodríguez, Griffiths JK. Effects of short-term Zinc Supplementation on the

- Cellular Immunity, Respiratory Symptoms and Growth of malnourished Equadorian children. *European Journal of Clinical Nutrition* 1996;50: 42-46.
22. Sempértegui F, **Estrella B**, Egas J, Carrión P, Yerovi L, Díaz S, Lascano M, Aranha R, Ortiz W, Zabala A, Griffiths J.K. Risk of diarrheal disease in Ecuadorian day-care centers. *Pediatr Infect Dis J* 1995;14:606-612,
23. Sempértegui F, **Estrella B**, Moscoso J, Piedrahita L, Hernández D, Gaybor J, Naranjo P, Mancero O, Arias S, Bernal R, et al. Safety, Immunogenicity and Protective Effect of the SPf66 Malaria Synthetic Vaccine Against *P.falciparum* Infection in a Randomized Double-Blind Placebo Controlled Field Trial in an Endemic Area in Ecuador. *Vaccine* 1994;12:337-342.

Publications in Ecuadorian journals

1. **Estrella Bertha**. Fomentando cultura de investigación en la Universidad del Ecuador. *Rev Fac Cien Med Quito* 2016; 41(1):3-8.
2. Estrella Ramiro, Paredes Yolanda, Salazar Fernando, Racines Marcia, **Bertha Estrella**. Síndrome metabólico y factores de riesgo cardiometabólico en adolescentes de Quito: descubrimiento de una epidemia oculta. *Rev Fac Cien Med Quito* 2016; 41(1):9-20.
3. Sempértegui Fernando, Egas Josefina, Maldonado Rocío, Paladines Mónica, **Estrella Bertha**, Fernando Salazar. Situación de zinc, gamma interferón y respuesta inmune en adultos con tuberculosis pulmonar. *Revista Eugenio Espejo* 2012; 2: 12-19.
4. Ramos Menéndez Yanira, Ramírez Carrera Carlos, Prado Bustamante Rodrigo, **Estrella Cahueñas Bertha**. Nivel sérico de interleucina 6 como marcador precoz de sepsis neonatal en recién nacidos con antecedente de ruptura prematura de membranas mayor a 24 horas. *Rev Ecuat Pediat (Quito)* 2008;9 (2):18-25.
5. Pillajo M, Agama M, Cobos O, **Estrella B**. Crecimiento de los recién nacidos durante su hospitalización en un servicio de neonatología *Rev Fac Cien Med (Quito)* 2008; 33: 9 - 16.
6. Aldás Y, Cevallos J, Coronado X, León L, **Estrella B**. Dosis efectiva de sacarosa para el control del dolor en neonatos sometidos a procedimientos dolorosos. *Rev Ecuat Pediat* 2007;8:5-13.
7. Ramiro Estrella, **Bertha Estrella**, Jorge Oviedo, Fernando Bossano, Ximena Narváez, María Teresa Reyes, Miguel Gutierrez. Relación entre rinitis, infecciones respiratorias altas y contaminación atmosférica de origen Vehicular. *Rev Ecuat Pediat (Quito)* 2000;1 (2):15-10.
8. Ramiro Estrella, **Bertha Estrella**, Ximena Narváez, et al. Respiratory diseases in school children according to environmental contamination by vehicular emissions. Fundación Natura. Quito- Ecuador, 2000

9. **Bertha Estrella**, Helder Peñaloza, Diana Cevallos. Primeros auxilios para niños de comunidades rurales. Universidad Central del Ecuador. Coordinación de Investigaciones, 1997.
10. L Escalante, JR Racines, M Vela, **B Estrella**, M Lascano, S Díaz, L Yerovi, W Ortiz, R Aranha, F Sempértégui. Identificación de Rotavirus por electroforesis: una experiencia local. *Microbiología e Infectología* 1:26-27, 1994
11. **Bertha Estrella**. Primeros Auxilios. En: Nutrición y Salud de niño Preescolar. Ministerio de Educación Pública. Programa Nacional El Ecuador Estudia. 1992.
12. **Bertha Estrella**. Accidentes y crecimiento. En: Nutrición y Salud de niño Preescolar. Ministerio de Educación Pública. Programa Nacional El Ecuador Estudia. 1992.
13. **Bertha Estrella**. Evitemos que los niños se enfermen. En: Nutrición y Salud de niño Preescolar. Ministerio de Educación Pública. Programa Nacional El Ecuador Estudia. 1992.
14. **Bertha Estrella**. Crecen bien los niños? Evaluación del Crecimiento. En: Nutrición y Salud de niño Preescolar. Ministerio de Educación Pública. Programa Nacional El Ecuador Estudia. 1992.
15. **Bertha Estrella**, Magdalena Montalvo, Lucia Valle. Alimentamos bien a los niños. En: Nutrición y Salud de niño Preescolar. Ministerio de Educación Pública. Programa Nacional El Ecuador Estudia. 1992.
16. Fernando Sempértégui, **Bertha Estrella**, Rodrigo Pozo (1991). Los Anticuerpos Monoclonales: Herramientas de Indagación Molecular. En: Los Secretos Moleculares de las Enfermedades Infecciosas (Estrella B, ed.) Editora Facultad de Ciencias Médicas. Quito. pp. 57-78.
17. **Bertha Estrella**, Fernando Sempértégui (1991). Las Investigaciones Moleculares en los Mecanismos Etiopatogénicos y en la Prevención de la Tuberculosis. En: Los Secretos Moleculares de las Enfermedades Infecciosas. (Estrella B, ed.). Editora Facultad de Ciencias Médicas. Quito. pp. 29-54.
18. **Bertha Estrella**, Fernando Sempértégui (1991). Avances Moleculares en el Conocimiento de la Malaria. En: Los Secretos Moleculares de las Enfermedades Infecciosas. (Estrella B, ed.). Editora Facultad de Ciencias Médicas. Quito. pp. 2-27.
19. **Bertha Estrella**, Rosario Moya, Alba Narváez, Milton Manotoa, Alfredo Molina, Nicolás Vivar, Fernando Sempértégui (1989). Inmunidad Celular en el Recién Nacido. En: Avances en Inmunología (Sempértégui F y Estrella B, eds.) Editora Facultad de Ciencias Médicas-CONACYT. Quito. pp.25-41.
20. Christina Vargas, Fernando Sempértégui, **Bertha Estrella**, José Yáñez, Fernando Salazar, José Sánchez, Bernarda Salas, Patricia Cueva, (1985). Influencia de la Situación Geográfica y Variables Sociales en el Crecimiento de los Niños de 0-12 años. En: El Crecimiento de

los Niños en la Provincia de Pichincha. (Sempértegui F, y Estrella B, eds.) Editora Facultad de Ciencias Médicas. Quito. pp.183-202.

21. Fernando Sempértegui, **Bertha Estrella**, Fernando Salazar, José Yáñez, Christina Vargas, Bernarda Salas, José Sánchez, Patricia Cueva (1985). Situación Nutricional de los Niños en las Areas Urbanas y Rurales. En: El Crecimiento de los Niños en la Provincia de Pichincha (Sempértegui F, y Estrella B, eds.). Editora Facultad de Ciencias Médicas. Quito. pp.165-179.
22. **Bertha Estrella**, Fernando Sempértegui, Christina Vargas, José Yáñez, Fernando Salazar, Patricia Cueva, José Sánchez, Bernarda Salas (1985). Crecimiento de los Niños de 0-12 años. En: El Crecimiento de los Niños en la Provincia de Pichincha. (Sempértegui F, y Estrella B, eds.). Editora Facultad de Ciencias Médicas. Quito. pp.71-161.

